CLINICAL STUDY PROTOCOL

STUDY CODE No.: CUSA-081-HEM-01

EUDRACT No. / IND No: 2019-002124-32 / 128551

NCT03594175

READY 1: A PHASE 3, RANDOMIZED, DOUBLE-BLIND, ACTIVE AND PLACEBO-CONTROLLED STUDY ON THE USE OF CUSA-081 FOR DYSFUNCTIONAL CENTRAL VENOUS ACCESS DEVICES (CVADs)

Version No.: 8.0
Date: 21 February 2023

Chiesi Farmaceutici S.p.A.
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GENERAL INFORMATION

SPONSOR:	Chiesi Farmaceutici S.p.A.* Via Palermo 26/A 43122 Parma - Italy + 39 0521 2791 *also reported as Chiesi throughout the text
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VERSION HISTORY

Version	Date	Change History
1.0	16 Jul 2018	First version of the protocol
2.0	17 Aug 2018	Clarification of inclusion and exclusion criteria, non-permitted concomitant medication, and use of other thrombolytics during treatment period
3.0	24 Sep 2018	Removed subjects less than 18 years of age from the study population
4.0	31 May 2019	Change of the Sponsor, protocol template updated, expansion of study to countries outside of the United States, updates to contact information, increased number of subjects enrolled to account for screen failures and early withdrawals, clarifications made to the eligibility criteria, and clarifications made regarding safety reporting
5.0	20 Nov 2020	Change in Sponsor Medical Expert. Revised inclusion and exclusion criteria. Clarifications on permitted and non-permitted concomitant medications related to catheter management. Addition of second manufacturer of placebo. Statement added for COVID-19. Revised study schedule for clarity including revision to language regarding urine pregnancy test. Revised language regarding determination of CVAD dysfunction and safety variables. Added definition for Adverse

		Events of Special Interest. Additional revisions for clarification.
6.0	18 Dec 2020	Revised inclusion criteria to reinclude subject age of 18 years or older as it was inadvertently removed from protocol version 5.0. Added language to clarify pregnancy test requirement. Added additional language to define menopause and provide clarification. Clarifications on permitted and non-permitted concomitant medications related to catheter management. Revised language regarding determination of CVAD withdrawal and infusion functions. Minor administrative changes.
7.0	30 Sep 2022	Revised to include a change of the protocol to an adaptive study design. A comparative interim analysis is planned to occur when approximately 416 patients have completed the study. This interim analysis is composed of two separate evaluations. 1) To test whether CUSA-081 is superior to placebo in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. 2) To perform a futility analysis by assessing the conditional power for the test of non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. The study design may change based on the outcomes of the interim analysis: • If CUSA-081 is demonstrated as superior to placebo at the interim analysis, then the placebo arm will be dropped, and the study will continue enrolling patients to receive either CUSA-081 or alteplase in a ratio of 9:6 CUSA-081: alteplase, as described in Section 3. • If CUSA-081 is not demonstrated as superior to placebo at the interim analysis, then the study will continue without modifications. • If the futility analysis finds that it is unlikely to demonstrate non-inferiority of CUSA-081 compared to alteplase, then the study will be terminated early. An independent and unblinded DMC will be established to review the data and provide a recommendation regarding the study design.
8.0	21FEB 2023	Revised to modify the interim analysis proposed in protocol v7.0 as a result of the interactions with the FDA. Modifications include the removal of the adaptive study design and the efficacy evaluation of the interim analysis that was introduced to test

		whether CUSA-081 is superior to placebo in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. After these modifications, only the interim futility analysis is foreseen.

PROTOCOL OUTLINE

Study title	READY 1: A Phase 3, Randomized, Double-Blind, Active and Placebo-Controlled Study on the use of CUSA-081 for Dysfunctional Central Venous Access Devices (CVADs)
Sponsor	Chiesi Farmaceutici S.p.A. - Via Palermo 26/A 43122 Parma - Italy
Name of the Product	CUSA-081 (reteplase)
Centre(s)	Approximately 100 sites
Indication	Restoration of function to central venous access devices (CVADs)
Study design	Multinational, multicenter, prospective, randomized, double-blind, parallel-group, active and placebo-controlled clinical trial
Study phase	Phase 3
Objectives	**Primary Objective:** To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following a single administration with a dwell time up to 90 minutes. Treatment success is defined as restoration of CVAD functionality measured as the ability to withdraw 3 mL of blood and infuse 5 mL of saline. **Secondary Objectives:** 1. To demonstrate the non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration with a dwell time up to 90 minutes. 2. To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following a single administration with a dwell time up to 60 minutes. 3. To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following up to two administrations with a dwell time up to 180 minutes. 4. To demonstrate the superiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration with a dwell time up to 90 minutes. 5. To evaluate the safety and tolerability of CUSA-081. 6. To evaluate the rate of recurrent catheter dysfunction defined as first re-occlusion within 30 days following administration of CUSA-081.
Treatment duration	Treatment configurations are summarized below:

Treatment Arm	**Syringe 1** (given at min 0)	**Syringe 2** (given, if needed, at min 90)
A	**CUSA-081**	**CUSA-081**
B	**placebo**	**placebo**
C	**alteplase**	**alteplase**

Test product dose/route/regimen	Treatment Arm A: One or two doses of CUSA-081 (reteplase), 0.70 mg (0.4 unit) / 2 mL, will be administered directly into the catheter lumen
Reference product dose/route/regimen	Treatment Arm B: One or two doses of placebo (normal saline) will be administered directly into the catheter lumen Treatment Arm C: One or two doses of alteplase, 2 mg in 2 mL, will be administered directly into the catheter lumen
Number of subjects	A minimum of 841 randomized subjects: Treatment Arm A (CUSA-081), 473 subjects Treatment Arm B (placebo), 53 subjects Treatment Arm C (alteplase), 315 subjects Subjects will be randomized in a 9:1:6 ratio of CUSA-081: placebo: alteplase.
Study population	Subjects who are 18 years of age or older who have dysfunctional CVADs
Inclusion/exclusion criteria	**Inclusion Criteria** Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study: 1. Inability to have 3mL of blood withdrawn from the selected study catheter; 2. A single or multi-lumen CVAD, implanted ports or peripherally inserted central catheters (PICCs) in place for > 24 hours and documented as previously being patent and functional; 3. Ability to designate one dysfunctional lumen of a multi-lumen catheter to be used throughout the study for both study drug instillation and assessment of CVAD function; 4. Male and non-pregnant female subjects 18 years or older (see note below); 5. Able to have fluids infused at the volume necessary to instill study drug into the CVAD (i.e., up to 2 mL); 6. Informed consent form (ICF) signed and dated indicating that the subject has been informed of and agreed with all pertinent aspects of the study and is willing to comply with all study requirements and procedures. NOTE: A urine pregnancy test is required for all females of childbearing potential. Women in natural or surgical post-menopause do not need to be tested for pregnancy. Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 consecutive months of lack of menstruation (amenorrhea) without any other obvious pathological or physiological cause. Surgical menopause is defined as the removal of both ovaries (bilateral oophorectomy) before the natural menopause. **Exclusion Criteria** The presence of any of the following will exclude a subject from study enrollment: 1. CVAD (any type) used for Hemodialysis;

	2. CVAD known to be dysfunctional for more than 48 hours; 3. Reasonable evidence of mechanical or non-thrombotic occlusion in the selected study catheter (e.g., catheter malposition or migration, sutures, kinks, or precipitates causing obstruction), radiographic assessment is not required; 4. Known or suspected catheter-related bloodstream infection (CRBSI); 5. Use of any intravenously administered fibrinolytic agent or anticoagulant (e.g., alteplase, tenecteplase, reteplase, urokinase or heparin) within 24 hours prior to the treatment period (first instillation of study drug). Use of subcutaneous LMWH, UFH or heparinoids for prophylaxis of thromboembolic events is allowed. Furthermore, the use of oral anticoagulants is allowed; 6. Known to be at high risk for bleeding events or embolic complications in the opinion of the Investigator, or has a known condition for which bleeding constitutes a significant hazard (e.g. recent stroke, recent intracranial or intraspinal surgery or serious head trauma, intracranial neoplasm, arteriovenous malformation or aneurysm, known bleeding diathesis); 7. Uncontrolled hypertension (systolic BP ≥160 or diastolic BP ≥110 mmHg) at screening; 8. Clinically unstable in the opinion of the site investigator; 9. Known to be pregnant or breastfeeding at screening; 10. Previously treated in this study (READY 1) or in study READY 2; 11. History of allergic reaction to reteplase, alteplase or vial ingredients (excipients or diluents); 12. Use of any investigational drug or experimental medical device within 28 days prior to treatment; non interventional observational studies participation is allowed. 13. Not mentally, socially or otherwise able to complete the trial assessments or not likely to survive beyond 30 days.
Study plan	A total of 2-3 visits may be performed during the study, as follows: • Screening (Baseline Visit) (Day 0 or Day 1) • Treatment Visit 1 (V1) (Day 1) • Follow-up Visit 2 (V2) (Day 30 ± 2 days) (Can be performed by telephone) An extra visit could be performed at Screening (Baseline Visit) for logistical reasons. Day 1 must be the following calendar day (for example, Monday and Tuesday; not Friday and Monday). Informed consent will be signed during this visit.
Most relevant allowed concomitant treatments	Catheter locking solutions (e.g. normal saline or low dose heparin, which requires a normal saline flush to clear the heparin from the IV line). The use of subcutaneous LMWH, UFH or heparinoids for prophylaxis of thromboembolic events is allowed. Furthermore, the use of oral anticoagulants is allowed.
Most relevant forbidden concomitant treatments	The use of any intravenously administered fibrinolytic agent or anticoagulant within 24 hours prior to the beginning of the treatment period (first instillation of study drug).
Efficacy variables (and/or pharmacokinetics variables)	**Primary Efficacy Variable:** Percentage of subjects who have treatment success following a single instillation of study drug (CUSA-081, placebo, or alteplase) with a dwell time up to 90 minutes. Treatment success is defined as the restoration of

	CVAD functionality measured as the ability to withdraw 3 mL of blood and infuse 5 mL of saline. **Secondary Efficacy Variables:** 1. Percentage of subjects who have treatment success following a single instillation of study drug with a total dwell time up to 30 and 60 minutes; 2. Percentage of subjects who have treatment success after up to two instillations of study drug with a total dwell time up to 120, 150 and 180 minutes; 3. The rate of recurrent catheter dysfunction defined as first re-occlusion within 30 days following treatment with study drug.
Safety variables	• Treatment Emergent Adverse Events (TEAEs), Adverse Drug Reactions (ADRs), Serious Treatment Emergent Adverse Events (SAEs), serious related TEAEs, TEAEs leading to study withdrawal, and TEAEs leading to death • Treatment Emergent Adverse Events of Special Interest (AESIs), as defined in Section 10.1
Sample size calculation	For the primary assessment of the superiority of CUSA-081 vs. placebo, a sample size of 450 in the active arm (CUSA-081), and 50 in the control arm (placebo), will provide >99% power to detect a difference of ≥35% using a two-sided test at significance level of 0.05. The expected placebo response rate is approximately 20%. For the assessment of the non-inferiority of CUSA-081 vs. alteplase, a sample size of 450 in the active arm (CUSA-081), and 300 in the control arm (alteplase) will provide 87% power at the 2.5% significance level (that is by using one-sided 97.5% confidence intervals with a non-inferiority margin of -10%), assuming an equal response rate of 75% in the control arm (alteplase) and in the active (CUSA-081) arm. A sample size of 450 in the active (CUSA-081) arm and 300 in the control arm (alteplase) will also provide 92% power to detect a difference of 10 % between the success rates using a two-sided test at significance level of 0.05. A minimum of 841 subjects will be randomized to achieve a minimum of 800 subjects completing the study.
Statistical methods	**Analysis Population:** • Full analysis set (FAS): All randomized subjects who receive at least one dose of study drug and with at least one available evaluation of efficacy after the baseline. Analyses using the FAS will be based on the treatment randomized. The FAS will be the primary analysis set for all efficacy analyses. • Safety set (SS): All randomized subjects who receive at least one dose of study drug. Subjects discontinued after dosing will be part of the SS. Analyses using the SS will be based on the actual treatment received. The SS will be the primary analysis set for all safety analyses. • Intent-To-Treat analysis set (ITT): All randomized subjects regardless if they received treatment with study drug. The ITT will be based on the randomized treatment allocation. The ITT will be used for sensitivity analysis for the primary efficacy analyses. • Per-protocol analysis set (PPS): all subjects from the ITT population without any major protocol deviation (i.e., wrong inclusions, incomplete dosing, non-permitted concomitant

medications). Exact definition of major protocol deviations will be discussed by the study team during the blind review of the data prior to the database lock. The PPS will be used for sensitivity analysis for the primary efficacy analyses and the non-inferiority analyses.

Efficacy:

The **Primary efficacy comparison** is to demonstrate the superiority in the rate of treatment success following a single administration of CUSA-081 compared to placebo with a dwell time up to 90 minutes. The percentage of subjects with treatment success and two-sided 95% Wald confidence intervals (CIs) will also be provided by treatment group. The between treatment comparisons will be tested using a 2-sample z test. The between treatment group difference and associated 95% Wald confidence intervals will be provided.

Similar analyses will be performed for the **secondary efficacy objectives**.

- The non-inferiority of CUSA-081 to alteplase in the rate of treatment success following a single administration of study treatment with a dwell time up to 90 minutes will be assessed based on the constructed 95% confidence interval for the difference in the rate of treatment success between CUSA-081 vs alteplase, with non-inferiority considered as demonstrated if the lower limit of the 95% confidence interval for the difference in rate of success is greater than -10%.
- The superiority in the rate of treatment success following a single administration of CUSA-081 compared to placebo with a dwell time up to 60 minutes will be tested using a 2-sample z test as described for the primary efficacy comparison.
- The superiority in the rate of treatment success following up to two administrations of CUSA-081 compared to placebo with a dwell time up to 180 minutes will be tested using a 2-sample z test as described for the primary efficacy comparison.
- The superiority in the rate of treatment success following a single administration of CUSA-081 compared to alteplase with a dwell time up to 90 minutes will be tested using a 2-sample z test as described for the primary efficacy comparison.

A closed testing procedure will be used to control the overall Type I error rate at less than 5%. The hypothesis testing will start with the primary efficacy objective. Then the secondary efficacy objectives (1 – 4) will be tested, in the order listed in the study objectives, until a p-value ≥ 0.05 is observed (or lower limit of the two-sided 95% CI $\leq$ -10% for the non-inferiority assessment).

The rate of recurrent catheter dysfunction defined as first reocclusion within 30 days following treatment with CUSA-081 will be analyzed using the Kaplan-Meier method and summarized with median, 25th and 75th percentiles, and 95% confidence intervals. The rate of recurrent catheter dysfunction within 30 days will be estimated. This analysis will be based on all subjects with treatment success following up to two administrations of study drug with a total dwell time up to 180 minutes.

	Safety: • The number and percentage of subjects experiencing TEAEs, ADRs, SAEs, serious related TEAEs, TEAEs leading to study withdrawal, TEAE leading to death, and AESIs, will be presented by treatment group and overall. Each of these groups of adverse events will also be summarized by System Organ Class and Preferred Term. • Safety variables will be presented as the number and percent of subjects with events and the number of events.
Interim Analysis	A futility analysis is planned to occur when approximately 416 patients have completed the study. The futility analysis will assess the conditional power for the test of non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. If the futility analysis finds that it is unlikely to demonstrate non-inferiority of CUSA-081 compared to alteplase, then the study will be terminated early. A separate independent and unblinded team will be established to perform the futility analysis and prepare the data for review ("Unblinded Statistical/Programming Team"). An independent and unblinded Data Monitoring Committee (DMC) will be established to review the data of the futility analysis and provide a recommendation to stop or continue the study. Details of the DMC, including roles, responsibilities, communication processes and frequency of meetings will be provided in a separate DMC Charter. The DMC will provide a recommendation regarding the continuation of the study as follows: • the study should continue without modification; • the study should be terminated prematurely. The DMC will also review the safety data at the time of the futility analysis. The study team and the other blinded team members will remain blinded throughout the futility analysis.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations are used in this study protocol.

Table 1: Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Events of Special Interest
AMI	Acute Myocardial Infarction
ATC	Anatomically Therapeutic Chemical
BP	Blood Pressure
cGCP	Current Good Clinical Practices
cGMP	Current Good Manufacturing Practices
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CRA	Clinical Research Associate
CRBSI	Catheter Related Bloodstream Infection
CRO	Contract Research Organization
CTA	Clinical Trial Agreement
CVAC	Central Venous Access Catheter
CVAD	Central Venous Access Device
CVC	Central Venous Catheter
DMC	Data Monitoring Committee
EC	Ethics Committee
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMPS	Investigational Medicinal Product Supplies
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intent to Treat analysis set
IUD	Intrauterine device
IUS	Intrauterine system
I.V.	Intravenous
IRT	Interactive Response Technology
LMWH	Low Molecular Weight Heparin
MedDRA	Medical Dictionary for Regulatory Activities
NI	Non-inferiority
PE	Pulmonary Embolism
PICC	Peripherally Inserted Central Catheter
PPS	Per-protocol analysis set
r-PA	Recombinant Plasminogen Activator
rt-PA	Recombinant Tissue Plasminogen Activator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMP	Safety Management Plan

SS	Safety Set
SUSAR	Suspected Unexpected Serious Adverse Reaction
STEMI	ST-Elevation Myocardial Infarction
SWFI	Sterile Water For Injection
t-PA	Tissue Plasminogen Activator
TEAE	Treatment Emergent Adverse Event
TFL	Tables, Figures, and Listings
UFH	Unfractionated Heparin

CONTENT

1. INTRODUCTION

1.1 Background Information

Central venous access devices (CVADs) are essential for managing chronic and acute conditions for which long-term medication is required, for example, the treatment of pain, cancer, infection, or to supply nutrition. In addition to the infusion of therapeutic agents, CVADs also allow for the withdrawal of blood and are therefore useful in reducing the need for repeated venipuncture.

Each year, as many as 25% of the approximately 7 million catheters that are placed in patients to deliver chemotherapy, nutritional support, antibiotics, or blood products become occluded (Brown, 2004; Richardson, 2007). Catheter occlusion can result in patient discomfort, deep vein thrombosis, hospitalization and possibly the need for invasive procedures, along with increased costs. Moreover, occluded catheters can result in delayed or missed therapies and often requires surgical replacement.

The majority of occlusions are caused by formation of a thrombus within or around the catheter. A fibrin sheath is one of the most common causes of thrombotic obstruction. It can occur within 24 hours after CVAD placement and usually develops within 2 weeks. The fibrin sheath does not usually affect catheter function but may cause a partial obstruction by creating a one-way valve over the catheter tip. Intraluminal clots account for 5–25% of all catheter occlusions and may cause complete catheter obstruction. Catheter-related venous thrombosis refers to a thrombus that develops in proximity to a CVAD. A mural thrombus is a blood clot that adheres to the vessel wall and can occlude the tip of the catheter but does not completely occlude the vein in which the catheter is positioned.

After ruling out mechanical dysfunction and medication or parenteral nutrition-related causes, the next step is to exclude thrombotic obstruction. Although diagnostic imaging techniques or linograms are available, a common practice is to treat suspected thrombus-related occlusions empirically with thrombolytics (fibrinolytics with thrombolytic agents) which allows catheter function to be restored efficiently and is a cost-effective alternative to replacing the catheter. In the United States, the current standard treatment for CVAD occlusions is intra-catheter instillation of alteplase 2 mg/2 mL (Baskin et al, 2009).

Fibrinolytic agents, sometimes referred to as plasminogen activators, are classified into two types: fibrin-specific and non–fibrin-specific agents. Fibrin-specific agents, which include alteplase (tPA), reteplase (recombinant plasminogen activator [r-PA]), and tenecteplase, produce limited plasminogen conversion in the absence of fibrin. Non–fibrin-specific agents (e.g., streptokinase) catalyze systemic fibrinolysis (Rivera-Bou, 2017).

Tissue plasminogen activator (tPA) is a naturally occurring fibrinolytic agent found in vascular endothelial cells and is involved in the balance between thrombolysis and thrombogenesis. It exhibits significant fibrin specificity and affinity. At the site of the thrombus, the binding of t-PA and plasminogen to the fibrin surface induces a conformational change that facilitates conversion of plasminogen to plasmin and dissolves the clot (Ouriel, 2004).

Alteplase has a plasma half-life of 4-6 minutes. It is FDA-approved under the brand name ACTIVASE® for the treatment of acute ischemic stroke (AIS), acute myocardial infarction (AMI) to reduce mortality and incidence of heart failure, and acute massive pulmonary embolism (PE) for lysis. At present, alteplase is the only thrombolytic agent approved in the US under the name CATHFLO® ACTIVASE® for the management of occluded CVADs (CATHFLO ACTIVASE USPI, 2017).

Reteplase is a recombinant tissue plasminogen activator (rt-PA) currently approved in the US (trade name: RETAVASE®), for treatment of acute ST-elevation myocardial infarction (STEMI) to reduce the risk of death and heart failure. Reteplase consists of the protease and Kringle 2 domains of human

tissue plasminogen activator (tPA), which are associated with fibrin specificity and low fibrin-binding affinity, respectively. Reteplase lacks the fibronectin-like finger domain of alteplase that promotes high fibrin binding affinity (Van Zonneveld et al, 1986). At high concentrations, reteplase does not compete with plasminogen for fibrin-binding sites, allowing plasminogen at the site of the clot to be transformed into clot-dissolving plasmin. These characteristics help explain why clots resolve faster in patients receiving reteplase than in those receiving alteplase (Rivera-Bou, 2017). Compared to alteplase, reteplase has a lower fibrin binding affinity, similar fibrin specificity, and longer half-life than alteplase (18 min, almost 4-fold greater than alteplase). These differences in interactions with fibrin may facilitate the in vivo clot penetration and faster clot lysis time observed with reteplase but it does not necessarily predict reperfusion rates (Fischer and Kohnert, 1997).

This lower fibrin affinity with reteplase has also been linked to a more pronounced attenuation of platelet aggregation in the early phase (2 hr) (Moser et al, 1999) and to a markedly greater degree of platelet activation in the late phase (24 hr) after thrombolytic therapy compared to alteplase (Gurbel et al, 1998).

1.2 Study Rationale

Chiesi is conducting a clinical development program to support the approval of CUSA-081 (reteplase) for the restoration of function to occluded central venous access devices not used in the hemodialysis setting. The Investigator's Brochure (IB) summarizes the data available from the medical literature regarding the use of reteplase for the restoration of function to CVADs as well as the known efficacy, safety, and immunogenicity results from the clinical program for the currently approved indication (STEMI) for reference.

The results of nine studies (7 in adults, 2 in pediatrics) from the medical literature provide supportive evidence that reteplase may be a safe and effective treatment option when used to restore functionality in occluded CVADs in the hemodialysis and no hemodialysis setting. Based on the data presented, it appears that a reteplase dose of 0.4 U, with a concentration of 0.2 U (0.35 mg)/ mL, instilled in a volume of ≤ 2 mL to fill the lumen of the CVAD is effective in adults, with a dwell time of 90 minutes and an assessment of patency at 30 minute intervals. The dose (0.4 U) of reteplase can be repeated one time if catheter function is not restored at 90 minutes after the initial instillation.

Overall success rates in the adult studies ranged from 67% to 100% and longer dwell times were generally associated with higher patency rates.

All these studies demonstrated that the safety profile of reteplase is predictable and similar in adult patients. The incidence and severity of AEs during these studies varied from none reported to bleeding from prior puncture site and minor complications. No instances of allergic reactions were noted. In Table 2, main efficacy and safety findings of reteplase in restoring patency of dysfunctional CVADs are reported.

Table 2: Summary of Available Literature of Reteplase Catheter Studies in Adults

Study	Catheter Use	Dose per Lumen (units/mg))	Dwell Time	Efficacy	Safety
Liu et al, 2004	Fluids/drugs	0.4/0.70 0.4/0.70 0.8/1.40 0.8/1.40	30 min 60 min 90 min 120 min	93/139 (66.9%) 123/139 (88.5%) 126/133 (94.7%) 126/133 (94.7%)	No treatment-associated AEs
Owens, 2002	Fluids/drugs and Dialysis	0.4/0.70	39.9±34.4 min	81/84 (96%)	No AEs noted
Castner, 2001	Dialysis	0.4/0.70	20-30 min	171/199 (85.9%)	No AEs were noted
Hilleman et al, 2003	Dialysis	0.5/0.87 2/3.48 3/5.22	33±10 hrs 32±7 hrs 32±7 hrs	38/45 (84%) 18/20 (90%) 18/20 (90%)	No AEs reported
Falk et al, 2004	Dialysis	0.4-0.8/0.70-1.40	30-60 min	44/50 (88%)	No AEs reported
Hyman et al, 2004	Dialysis	0.4-0.8/0.70-1.40	30-60 min	50/59 (85%)	No bleeding or allergic reactions noted
Wolf et al, 2000	Dialysis	5/8.70	60 min	8/8 (100%)	No evidence of hemorrhage, no blood transfusions required

1.3 Risk/Benefit Assessment

Two clinical studies evaluating efficacy and safety of reteplase in restoring patency of dysfunctional non-hemodialysis CVADs were conducted in a total of 166 subjects reporting high patency success rate after a single instillation.

In a Phase 2, open-label, single arm, prospective study conducted at the [redacted] from January 2001 through August 2002 reteplase was administered to 139 oncology patients (Liu et al, 2004). The primary efficacy outcome was the restoration of CVC function after a single administration of reteplase with a 30-minute dwell time. Catheter function was considered normal if blood could be withdrawn and solution could be infused without resistance or discomfort.

The 10 U vial of reteplase was reconstituted with 10 mL of SWFI to a concentration of 1 U/mL. The reconstituted reteplase solution was frozen in a 3-mL syringe containing 0.4 U/0.4 mL. After

thawing, the reteplase solution was diluted with 0.9% sodium chloride solution without bacteriostatic or fungistatic agents to the final concentration of 0.4 U/2 mL.

The intravenous drug therapy nurse instilled the ≤ 2 mL volume of 0.4 U of reteplase in each lumen of the catheter for 30 minutes. The reteplase dose was permitted to dwell for an additional 30 minutes if the catheter remained occluded. This procedure was repeated once if patency was not restored after 60 minutes. The success rate after the 30-minute dwell time was 66.9% (93/139 CVCs; 95% confidence interval [CI]: 0.59-0.74). After an additional 30-minute dwell time, 88.5% of the catheters were patent (123/139 CVCs; 95% CI: 0.82-0.93). Of the 16 CVCs that remained occluded, 10 were treated with a second dose of up to 0.4 U of reteplase, which was allowed to dwell for a total of 60 minutes (120 minutes cumulative time). Success rate after 120 minutes of cumulative time was 94.7% (126/133 CVCs; 95% CI: 0.90-0.98).

Both active IMPs, reteplase and alteplase, are tissue plasminogen activators, fibrinolytic agents already approved for the treatment of acute myocardial infarction and pulmonary embolism. Alteplase is approved for the treatment of occluded CVADs. In this phase III study, alteplase will be administered according to the prescribing information instructions. Reteplase will be administered intravenously (as per current in label indication) at a dose 1/25th the dose currently used for the approved indication (i.e. treatment of acute STEMI). Moreover, study visits, procedures and assessments are in accordance with well-established guidelines and clinical practice. Finally, both agents have been investigated in humans and have shown good safety and tolerability. In light of the above considerations no additional risk for enrolled subjects is thought to be associated to the participation into the study.

The study will focus on subjects with occluded CVADs. Previous data showed an optimal efficacy and safety profile of reteplase when administered in subjects with dysfunctional CVADs with the aim of restoring catheter patency. In vitro and preliminary clinical data, although with some limitation, seem to be suggestive of a possible faster onset of action when compared with alteplase, current standard of care in the treatment of dysfunctional CVAD for thrombosis.

The efficacy and safety endpoints are standard for evaluation of this drug class in the setting of dysfunctional CVADs management and will allow to determine the effect of IMP on clearance of CVADs.

Moreover, a futility analysis is planned to assess the conditional power for the test of non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. The aim of the futility analysis is to reduce the exposure to the investigational study drug, CUSA-081, in case the study is unlikely to demonstrate non-inferiority compared to alteplase.

This trial will be conducted in compliance with the Declaration of Helsinki (1964 and amendments) current ICH E6 Good Clinical Practices and all other applicable laws and regulations.

2. STUDY OBJECTIVES

2.1 Primary Objective(s)

To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following a single administration with a dwell time up to 90 minutes. Treatment success is defined as restoration of CVAD functionality measured as the ability to withdraw 3 mL of blood and infuse 5 mL of saline.

2.2 Secondary Objective(s)

The secondary objectives of the study are as follows:

1. To demonstrate the non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration with a dwell time up to 90 minutes;
2. To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following a single administration with a dwell time up to 60 minutes;
3. To demonstrate the superiority of CUSA-081 compared to placebo in the rate of treatment success following up to two administrations with a dwell time up to 180 minutes;
4. To demonstrate the superiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration with a dwell time up to 90 minutes;
5. To evaluate the safety and tolerability of CUSA-081;
6. To evaluate the rate of recurrent catheter dysfunction defined as first re-occlusion within 30 days following treatment with CUSA-081.

3. STUDY DESIGN

This is a Phase 3 multinational, multicenter, prospective, randomized, double-blind, parallel-group, active and placebo-controlled study examining the effect of instillations of CUSA-081 versus placebo or alteplase in subjects with dysfunctional non-hemodialysis CVADs. Restoration of CVAD function is defined as follows: the ability to both withdraw at least 3 mL of blood and infuse 5 mL of saline.

Since the goal is to acutely identify and treat a dysfunctional catheter, the treatment period consists of one visit and may take place on the same day as screening or on the following day. There will be a follow-up assessment performed on Day 30 (±2 days) after treatment with study drug. The assessment may be conducted by telephone interview.

Catheters with multiple lumens, ports and peripherally inserted central catheters (PICCs) will be allowed. If multiple lumens are dysfunctional, only one lumen will be designated by the Investigator for treatment in this study. The lumen chosen will be documented and indicated using a study lumen tag to ensure that the same lumen will be used for all study drug instillations and study assessments. No other thrombolytics may be used to treat other dysfunctional lumens during the treatment period (0 – 180 minutes).

Subjects will be screened according to the Study Inclusion and Exclusion criteria (sections 4.2 and 4.3) and will be candidates for randomization if all eligibility criteria are met. Subjects will be randomized in a 9:1:6 ratio of CUSA-081: placebo: alteplase (

Table 3).

Table 3: Study Treatment Arms

Treatment Arm	Syringe 1 (given at min 0, assessed at minutes 30, 60, 90)	Syringe 2 (given, if needed, at minutes 90, assessed at min 120, 150, 180)
A	**CUSA-081**	**CUSA-081**
B	**placebo**	**placebo**
C	**alteplase**	**alteplase**

NOTE: Refer to the Study Treatment Schema in

Figure 1 for more detail.

A futility analysis is planned to occur when approximately 416 patients have completed the study (see Section 12.3.7 for additional details). The futility analysis will assess the conditional power for the test of non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes.

If the futility analysis finds that it is unlikely to demonstrate non-inferiority of CUSA-081 compared to alteplase, then the study will be terminated early.

A separate independent and unblinded team will be established to perform the futility analysis and prepare the data for review ("Unblinded Statistical/Programming Team").

An independent and unblinded DMC will be established to review the data and provide a recommendation regarding the continuation of the study. Details of the DMC, including roles, responsibilities, communication processes, and frequency of meetings will be provided in a separate DMC Charter.

The DMC will provide a recommendation regarding the study continuation as follows:

- the study should continue without modification;
- the study should be terminated prematurely.

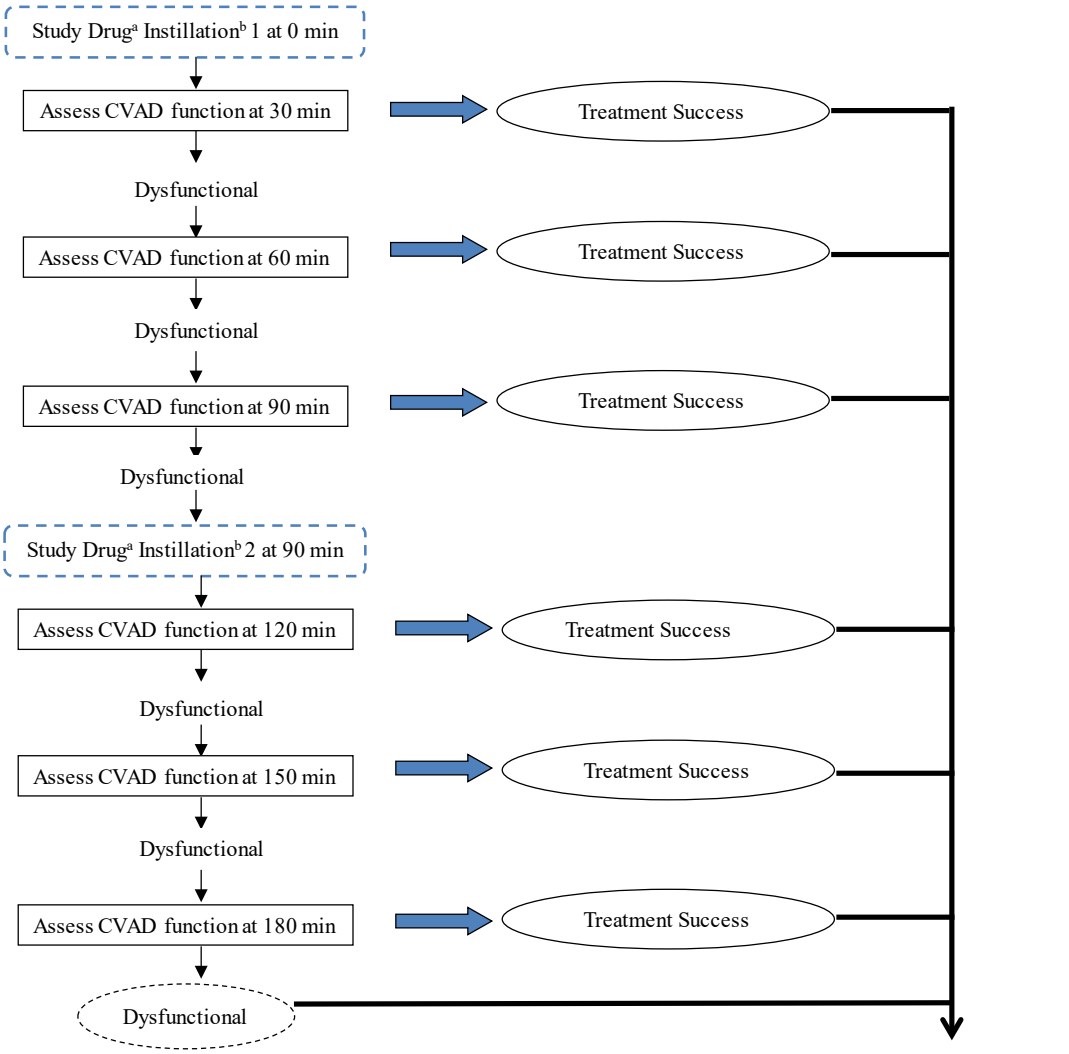
The study team and the other blinded team members will remain blinded throughout the futility analysis.

If the study is terminated prematurely, investigators, Competent Authorities and Ethics Committees (EC)/ Institutional Review Boards (IRBs) will be informed.

Safety will be monitored throughout the study. The DMC will also review safety data at the same time as the futility analysis.

The end of the trial is defined as the last follow-up contact of the last subject to receive study drug in the trial.

Figure 1: Treatment Schema



4. SUBJECT SELECTION CRITERIA

4.1 Subject Recruitment

A minimum of 841 subjects will be randomized in a 9:1:6 ratio of CUSA-081: placebo: alteplase in order to achieve a minimum of 800 subjects completing the study. A screen failure rate of approximately 5% is assumed, which implies that approximately 885 subjects should be enrolled in order to reach the required number of completed subjects.

4.2 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Inability to have 3mL of blood withdrawn from the selected study catheter;
2. A single or multi-lumen CVAD, implanted ports and peripherally inserted central catheters (PICCs) in place > 24 hours and documented as previously being patent and functional;
3. Ability to designate one dysfunctional lumen of a multi-lumen catheter to be used throughout the study for both study drug instillation and assessment of CVAD function;
4. Male and non-pregnant female subjects 18 years or older (see note below);
5. Able to have fluids infused at the volume necessary to instill study drug into the CVAD (i.e., up to 2 mL);
6. Informed consent form (ICF) signed and dated indicating that the subject has been informed of and agreed with all pertinent aspects of the study and is willing to comply with all study requirements and procedures.

NOTE: A urine pregnancy test is required for all females of childbearing potential. Women in natural or surgical menopause do not need to be tested for pregnancy. Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 consecutive months of lack of menstruation (amenorrhea) without any other obvious pathological or physiological cause. Surgical menopause is defined as the removal of both ovaries (bilateral oophorectomy) before the natural menopause.

4.3 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrolment:

1. CVAD (any type) used for hemodialysis;
2. CVAD known to be dysfunctional for more than 48 hours;
3. Reasonable evidence of mechanical or non-thrombotic occlusion in the selected study catheter (e.g., catheter malposition or migration, sutures, kinks, or precipitates causing obstruction), radiographic assessment is not required;
4. Known or suspected catheter related bloodstream infection;
5. Use of any intravenously administered fibrinolytic agent or anticoagulant (For example, but not limited to, alteplase, tenecteplase, reteplase, urokinase or heparin) within 24 hours prior to the treatment period (first instillation of study drug). Use of subcutaneous LMWH, UFH or heparinoids for prophylaxis of thromboembolic events is allowed. Furthermore, the use of oral anticoagulants is allowed;
6. Known to be at high risk for bleeding events or embolic complications in the opinion of the Investigator, or has a known condition for which bleeding constitutes a significant hazard (e.g. recent stroke, recent intracranial or intraspinal surgery or serious head trauma, intracranial neoplasm, arteriovenous malformation or aneurysm, known bleeding diathesis);

7. Uncontrolled hypertension (systolic BP ≥ 160 or diastolic BP ≥ 110 mmHg) at screening;
8. Clinically unstable in the opinion of the site Investigator;
9. Known to be pregnant or breastfeeding at screening;
10. Previously treated in this study (READY 1) or in study READY 2;
11. History of allergic reaction to reteplase, alteplase or vial ingredients (excipients or diluents);
12. Use of any investigational drug or experimental medical device within 28 days prior to treatment; non interventional observational studies participation is allowed.
13. Not mentally, socially or otherwise able to complete the trial assessment or not likely to survive beyond 30 days

4.4 Subject Withdrawals

Subjects may be discontinued from the study for any of the following reasons:

- An adverse event occurs that, in the opinion of the investigator, makes it unsafe for the subject to continue in the study. Should serious bleeding in a critical location (e.g., intracranial, gastrointestinal, retroperitoneal, pericardial) occur, treatment with study drug should be stopped and the study drug should be withdrawn from the catheter.
- The subject withdraws consent.
- The subject is lost to follow-up.
- The subject's safety is affected by violation of inclusion or exclusion criteria or use of not-permitted concomitant medication.
- The subject is unwilling or unable to adhere to the study requirements, i.e, non-compliance.
- The sponsor or the regulatory authorities or the Ethics Committee(s), for any reason, terminates the entire study, or terminates the study for this trial site or this particular subject.

When a subject discontinues the study, all efforts will be made to complete and report the observations during study participation.

If a subject is withdrawn/drops-out of the study after receiving the study drug, the subject study number and corresponding study drug should not be reassigned to another subject.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawals of subjects should be avoided.

However, should a subject discontinue the study, all efforts will be made to complete and report the observations as thoroughly as possible.

In case of withdrawal, the Investigator must fill in the “Study Termination” page in the CRF, reporting the main reason for withdrawal.

5. CONCOMITANT MEDICATIONS

5.1 Permitted Concomitant Medications Related to Catheter Management

The use of catheter locking solutions (e.g. normal saline, low dose heparin which requires a normal saline flush to clear the heparin from the IV line) is permitted.

The use of subcutaneous LMWH, UFH or heparinoids for prophylaxis of thromboembolic events is allowed. Furthermore, the use of oral anticoagulants is allowed.

5.2 Non-permitted Concomitant Medications to Catheter Management

The use of any intravenously administered fibrinolytic agent or anticoagulant within 24 hours prior to the beginning of the treatment period (first instillation of study drug) is not permitted.

No other fibrinolytic agent may be used to treat other dysfunctional lumens during the treatment period (0 – 180 minutes).

6. TREATMENT(S)

Chiesi will be responsible for supplying Study Drugs to the study sites. The study sites will be responsible for the safe storage of all Study Drugs assigned to this study, in a secure place with restricted access, and maintained within the appropriate ranges of temperature.

6.1 Appearance and Content

6.1.1 CUSA-081

Study drug will be prepared in 2 separate 10 mL syringes with a blinded label by an unblinded pharmacist in order to maintain the blind to study personnel for administration to the subject. All other study personnel at the study sites will be blinded. CUSA-081 information is described in Table 4.

6.1.2 Placebo

The placebo will be identical in appearance to the active drugs in order to keep the treatments blinded. The placebo will be prepared in 2 separate 10 mL syringes with a blinded label by an unblinded pharmacist in order to maintain the blind to study personnel for administration to the subject. The placebo information is described in Table 4.

6.1.3 Alteplase

Alteplase, the active comparator study drug, is being supplied as commercially available CATHFLO ACTIVASE. Study drug will be prepared in 2 separate 10 mL syringes with a blinded label by an unblinded pharmacist in order to maintain the blind to study personnel for administration to the subject. All other study personnel at the study sites will be blinded. Alteplase information is described in Table 4.

Table 4: Appearance and Content

Product Name	CUSA-081	Placebo	Alteplase
Active ingredient	Reteplase 10 U / vial	None	Alteplase 2 mg / vial
Excipients	Tranexamic acid, Phosphoric acid, Polysorbate 80, Sucrose, Dipotassium Hydrogen Phosphate	Normal Saline	L-arginine, Polysorbate 80, Phosphoric Acid
Presentation	Reconstituted and diluted RETAVASE provided in 2 syringes, each containing 0.4 U (0.70 mg) / 2 mL reteplase	2 syringes each containing 2 mL of normal saline	Reconstituted CATHFLO ACTIVASE provided in 2 syringes, each containing 2 mg / 2 mL alteplase

6.2 Dosage and Administration

6.2.1 Selection of Doses in the Study

The dose of 0.70 mg / 2 mL of reteplase was selected based on previous clinical experience and published literature in dysfunctional CVADs.

Most of the studies in the literature where reteplase was used to restore functionality in CVADs tested a dose of up to 0.4 U/2 mL per lumen (equivalent to 0.70 mg / 2 mL), with an additional dose after 30 to 60 minutes of dwell time if needed (Castner, 2001; Falk et al, 2004; Freyer et al, 2002; Freyer et al, 2003; Hilleman et al, 2003; Hyman et al, 2004; Liu et al, 2004; Owens , 2002; Terrill et al, 2003; Wolf et al, 2000).

This dosing was empirically based on a ratio of 1:5 between reteplase and alteplase dose (20 units : 100 mg) used for the treatment of AMI in the GUSTO III clinical program (GUSTO III, 1997), as well as the doses used in a study comparing the hemodynamic effects of reteplase and alteplase in massive pulmonary embolism (PE) (Tebbe et al, 1999). In those trials, little difference was noted between the two drugs as far as safety, mortality, stroke, intracranial hemorrhage, or clinical success rates.

Based on the dosing ratio in GUSTO III and the Pulmonary Embolism study, and the clinical success seen with 2 mg of alteplase in restoring functionality to CVADs (Haire et al, 1994; Ponec, 2001; Deitcher et al, 2002; Semba et al, 2002), the reteplase 0.4 units dose was the dose used most often in the studies found in the literature where reteplase was used to restore functionality in CVADs (Note: 0.4 units of reteplase to 2 mg of alteplase retains the ratio of 1:5). Higher doses were also utilized in various studies, but 0.4 units would seem to be a lower yet effective dose in adults. A single dose of the reteplase solution is instilled in a volume of ≤ 2 mL to fill the lumen of the CVAD and allowed to dwell for a minimum of 30 minutes (repeated once if catheter function is not restored) and subsequently removed by aspiration.

Therefore, in this study, a reteplase dose of 0.4U (0.7 mg) / 2mL will be used.

If catheter function is not restored at 90 minutes after one dose of reteplase, a second 0.4U/2 mL dose may be instilled.

6.2.2 Dosage

6.2.2.1 Treatment A: CUSA-081

RETAVASE (reteplase) for Injection 10 U / vial, reconstituted in Sterile Water for Injection (SWFI) and diluted to 0.4 U (0.70 mg) / 2 mL total dose (CUSA-081). One RETAVASE vial will be used to reconstitute up to two doses of CUSA-081.

- Subjects will receive the first instillation of study drug (CUSA-081) into the catheter via the single lumen designated for this study.
- If patency is not restored after 90 minutes following the first instillation, a second dose of study drug (CUSA-081) will be administered.

6.2.2.2 Treatment B: Placebo

Placebo will be 2 mL of Normal Saline (0.9% sodium chloride, USP). Normal saline was chosen instead of SWFI due to its isotonic property and to avoid risk of hemolysis with SWFI which may risk unblinding the study.

- Subjects randomized to Treatment Arm B will receive the first instillation of blinded study drug (placebo) into the catheter via the single lumen designated for this study.
- If patency is not restored after 90 minutes following the first instillation, a second dose of blinded study drug (placebo) will be administered.

6.2.2.3 Treatment C: Alteplase

Alteplase, 2 mg/ 2 mL single dose vials reconstituted in SWFI according to manufacturer's label.

- Subjects randomized to Treatment Arm C will receive the first instillation of blinded study drug (alteplase) into the catheter via the single lumen designated for this study.
- If patency is not restored after 90 minutes following the first instillation, a second dose of blinded study drug (alteplase) will be administered.

6.2.2.4 Randomized Treatment Period

To assure blinding of the study staff and subjects, the study pharmacist (unblinded) will prepare two 10 mL graduated syringes, each labelled appropriately, to be administered per protocol in sequential order for each subject after randomization. Preparation of syringes will be performed as follows:

Table 5: Study Treatment Arms

Treatment Arm	Syringe 1 (given at min 0)	Syringe 2 (given if needed, at min 90)
A	**CUSA-081**	**CUSA-081**
B	**Placebo**	**Placebo**
C	**Alteplase**	**Alteplase**

6.2.3 Preparation of Study Drug for Administration

6.2.3.1 CUSA-081

Aseptic technique will be used throughout the study drug preparation. Each RETAVASE vial of 10 units sterile lyophilized reteplase powder will be reconstituted and diluted with Sterile Water for Injection (SWFI) to reach the correct concentration of CUSA-081 for instillation for catheter clearance.

The final CUSA-081 concentration in each 10 mL graduated syringe will be 0.2 units/mL. Each syringe of CUSA-081 will contain 2 mL or 0.4 units of reteplase.

When reconstituted as directed, the CUSA-081 solution is required to be used (instilled) within 4 hours when stored at 2° to 30° C (36°F to 86°F).

Detailed instructions for the reconstitution and dilution of RETAVASE resulting in the CUSA-081 dosing syringes for administration are provided in the study Pharmacy Manual.

6.2.3.2 Placebo

Placebo syringes will be prepared by withdrawing 2 mL of Normal Saline (0.9% sodium chloride, USP) into two unused sterile 10 mL graduated syringes. Each syringe should be filled to deliver 2 mL of Normal Saline.

Detailed instructions for preparation of the Placebo dosing syringes for administration are included in the study Pharmacy Manual.

6.2.3.3 Alteplase

Alteplase, 2 mg/2 mL single dose vials will be reconstituted with SWFI according to the FDA approved package insert for CATHFLO ACTIVASE. Two syringes will be prepared, one for each dose.

The alteplase solution may be used for intra-catheter instillation within 4 hours following reconstitution when stored at 2-30°C (36-86°F).

Detailed instructions for the preparation of the alteplase dosing syringes for administration are provided in the study Pharmacy Manual.

6.3 Packaging

All investigational products will be prepared in accordance with current Good Manufacturing Practices (cGMP) as required by the current Good Clinical Practices (cGCP). Chiesi will supply all study drug Sterile Water for Injection (SWFI) and syringes.

6.3.1 Primary Packaging

Study drugs (CUSA-081, placebo and alteplase) will be supplied with the appropriate clinical labels in a unique kit for each treatment arm. A corresponding ancillary kit for each treatment arm that includes the ancillary supplies required for the reconstitution, dilution, and preparation of the study drug will also be provided. The contents of the study drug and ancillary kits are unblinded to the pharmacy study personnel.

Table 6: Study Drug Kit Components

Treatment Arm	Study Drug Kit Contents
A	One 10 U vial of reteplase
B	One 10 mL vial of normal saline
C	Two single dose vials of alteplase

6.3.2 Secondary Packaging

Study drug and ancillary kits will be packaged to be visually identical with identical labelling and storage requirements, in order to maintain the blind for any staff that are not unblinded.

The study drugs and ancillary kits will be packaged in separate boxes. Each kit will have a unique kit identification number on the label, traceable to the contents of the kits. Upon randomization of a subject in the IRT system, the unblinded pharmacist will receive the study drug and ancillary kit ID numbers corresponding to the treatment arm assigned to the subject.

6.4 Labeling

All the supplies will be labelled according to local law and regulatory requirements and will be compliant with Annex 13 to the Volume 4 of the GMP and 21 CRF part 312.6. All labels will be available in the local language.

For all applicable labels, the subject identification is expressed by the kit number. This number is assigned by the IRT System which allows full traceability of the essential details such as: site identification number, investigator's name, subject number, visit number and, randomization number.

6.5 Treatment Allocation

A balanced block randomization scheme will be prepared via a computerized system. Once the subject meets all inclusion and no exclusion criteria and has provided informed consent, the investigational pharmacy or designee at the study site will enroll the subject and request the study drug assignment using the Interactive Response Technology (IRT) at the time of randomization. Randomization of study drug must only occur on the day of study drug administration.

Assignment to double-blind treatment arms will be determined by the randomization schedule programmed into the IRT. Once a subject is randomized, the study site unblinded Pharmacist will be provided a study drug kit allocation and the appropriate ancillary kit allocation via the IRT. The unblinded Pharmacist will prepare the study drug and affix a blinded label to the syringes for dispensation to the blinded site personnel for administration to the subject.

6.6 Treatment Code

The Sponsor's clinical team will be blinded during the study as they will not have direct access to the randomization list.

In case of emergency, unblinding of the treatment code will be done through IRT. The treatment group will be disclosed, and confirmation will follow (by fax and/or notification email). The IRT will be designed to send a confirmation (by fax and/or notification email) to the site for every transaction performed by the Investigators.

When possible, the CRO's Medical Monitor should be consulted prior to unblinding the treatment code. The IRT will promptly notify the Sponsor, CRO's Medical Monitor and the Clinical Site

Monitor whenever a treatment code is unblinded; however, the blinded treatment code will remain intact in these notifications.

Users from Chiesi Global Pharmacovigilance will have their own credentials to unblind subjects in case of SUSARs to be reported to the competent Regulatory Authorities and Ethics Committees/IRB.

Subjects will be provided a card with the phone numbers of the Investigator or designee to be called in case of emergency.

For interim futility analysis purposes, (see Section 12.3.7 for details), treatment allocation codes will be unblinded only for patients included in the analysis, i.e. those who have completed the study or those who have prematurely discontinued from the study within the pre-determined cut-off date. Unblinded information will be available only to the Unblinded Statistical/Programming Team and to the independent DMC members and only following the interim database lock.

6.7 Treatment Compliance

Not applicable

6.8 Drug Storage

The unblinded pharmacist at the Investigator's site will be responsible for the safe storage of all medications assigned to this study, in a secure place with restricted access limited to those individuals authorized to dispense the study drug and maintained within the appropriate ranges of temperature and humidity.

All study drug kits will be stored at refrigerated temperatures at 2°C to 8°C (36°F to 46°F). The Pharmacy Manual contains additional information for storage and drug accountability. Ancillary kits will be stored at controlled room temperature 20°C to 25°C; excursions 15°C to 30°C (59°F to 86°F) permitted. The Pharmacy Manual contains additional information for storage and drug accountability.

For all Investigational Medicinal Product Supplies (IMPS) after reconstitution/preparation, they will have to be used (instilled) within 4 hours when stored at 2°C to 30°C (36 °F to 86 °F).

The Investigator or his/her designee is responsible for explaining the correct use of the study drugs to the site personnel, verifying that instructions are followed properly, maintaining accurate records of study drug dispensing, administration and collection, and returning all unopened and unused study drugs to the Sponsor or Sponsor's designee at the end of the study.

The unblinded pharmacist or unblinded designee must check the Min/Max temperatures once daily for adequate storage of refrigerated drug kits and ambient ancillary kits. The Min/Max temperatures must be recorded on a dedicated temperature tracking form. The temperature can also be checked/ controlled by the constant use of an automated temperature controller system. Any deviation from the temperature requirement for storage will be promptly reported and Sponsor shall assess if the affected study drugs can be used.

Note: The refrigerated study drug vials must never be warmed by artificial means.

6.9 Drug Accountability

The unblinded pharmacist at the Investigator's site is responsible for the management of all the study medication and ancillary kits to be used in the study. Study medication should be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense study medication.

An inventory will be maintained by the unblinded study pharmacist (or designee), to include a signed account of all the study medication(s) received, dispensed, administered and returned for each subject during the trial.

At the conclusion or termination of the study, the unblinded study pharmacist (or designee) shall conduct and document a final drug supply (used and unused) inventory. An explanation will be given for any discrepancies.

Prior sponsor authorization for destruction of unused clinical trial materials by the institution is expected. A certificate of destruction is required to be provided to the Sponsor.

6.10 Provision of Additional Care

At completion of subject's study participation, it is the Investigator's responsibility to prescribe standard of care for catheter clearance if patency has not been achieved.

Due to the double-blind design of the study, it is not possible for the investigator to ascertain if a randomized subject has received reteplase, alteplase, or placebo without breaking the blind. If CATHFLO® ACTIVASE®(alteplase) is prescribed as standard of care following documentation of treatment failure at 90 minutes after the second instillation of study drug, or upon Early Withdrawal, it is worth noting that according to the FDA-approved product label, studies have not been performed with administration of total doses greater than 4 mg (two 2 mg doses).

7. STUDY PLAN

Due to the short nature of the treatment period and the ability for the follow up visit to be conducted via telephone, no missing data or modifications are anticipated due to COVID-19. Additionally, no modifications to study procedures or study drug administration are anticipated. In the event that a subject withdraws from the study due to COVID-19, this reason will be documented.

7.1 Study Schedule

The study schedule (Table 7) summarizes the study assessments by visit and time point.

Table 7: Study Schedule

Assessment or Procedure	Screening and/or Treatment Visit[1] (Day 1)[2]								Visit 2 - Follow-Up Assessment[3]
	Screen Day 0 Or Day 1	Syringe 1 First Dose (at 0 min)				Syringe 2 Second Dose (at 90 min, if given)			Day 30 (±2 days) after Treatment[4]
		0 Min	30 Min	60 Min	90 Min	90 Min	120 Min	150 Min	180 Min	
Informed consent	X									
Inclusion/exclusion criteria	X									
Demographic data	X									
Medical history	X									
Body weight and height	X									
Blood pressure measurement and heart rate	X[2]									
Urine pregnancy test[5]	X									
Concomitant medications/procedures	X[1]									X[6]
CVAD history	X									
CVAD function assessment	X[1,2]		X[7, 8]	X[7, 8]	X[7, 8]		X[7, 8]	X[7, 8]	X[7, 8]	X[4,9]
Enrollment into IRT	X									
Study drug administration		X[8]				X[8]				
Adverse events	X[1, 10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]	X[10]

CVAD = central venous access device; d = day; IRT = interactive response technology, min = minutes.

[1] If at any time after the first instillation of study drug, a subject is withdrawn from the study (due to subject's wishes or at the Investigator's discretion), safety monitoring for that subject should still occur for up to 30 days (± 2 days) after the last withdrawal of study drug from the catheter (see adverse event section [AE] collection period in Section 10).

[2] Subjects will be screened for eligibility and treated over the course of one or two study visits. Randomization only occurs on day of treatment and cannot occur on screening visit if not the same as the treatment day. Therefore, the Screening Visit may occur on Day 0 or Day 1 (for example Monday and Tuesday; not Friday and Monday) and the Treatment Visit will occur on Day 1. Screening assessments will occur prior to the first dose of study medication. Blood pressure, heart rate, CVAD assessment and all other eligibility for inclusion criteria must be reassessed if screening and treatment are conducted on separate days.

[3] Subjects who are unable to be reached for follow-up assessment after discontinuing study drug will be categorized as lost to follow up from the study

[4] The Day 30 (± 2 days) follow-up assessment will occur via phone or may occur in person if the subject has a scheduled clinic visit. The follow-up period is calculated from the date of the randomization/treatment Day.

[5] A urine pregnancy test is required for all females of childbearing potential. Women in natural or surgical post-menopause do not need to be tested for pregnancy.

[6] Only concomitant medications and/or procedures ongoing at the time of consent, or stopped within 30 days prior to informed consent, and only new concomitant medications and/or procedures related to spontaneously reported SAEs/AESIs will be collected after randomization.

[7] Note that all catheter assessments are based on the dwell time from the time of study drug instillation (i.e., from "0 min"; NOT from the time of the prior assessment). All efforts should be made to conduct the assessment at the required time point. The window of ± 5 minutes is also from the time of study drug instillation and not time of the prior assessment. Assessments of catheter dysfunction are described in Sections 7.1.1 and 7.2.3. In the event a 2nd dose of study drug is needed, it should be administered within 15 min of withdrawal of the 1st dose from the CVAD.

[8] Once CVAD function has been restored (i.e. the ability to both withdraw at least 3 mL of blood and infuse 5 mL of saline solution), the subject will be deemed a Treatment Success and will exit the treatment algorithm (

Figure 1). Therefore, subsequent dosing and catheter function checks will not be performed during V1. At 180 minutes, or in the case of Early Withdrawal, if treatment success is not achieved, any subsequently administered standard of care and related outcome will be documented.

[9] Assessment of recurrent catheter dysfunction as described in Section 7.1.2. Information including, but not limited to, the need for other thrombolytic administration and/or catheter removal or replacement will be collected beyond study drug administration and up through Day 30 (± 2 days). Subjects will be requested to document the date of re-occlusion and any intervention or the date the catheter was removed in order to provide the data when contacted by site personnel.

[10] Collection of Aes and SAEs will begin from the time of Informed Consent signature. All AEs, TEAEs and SAEs on-going at the time the subject's study treatment ends should be evaluated up to 30 days after last administration of Study Drug. After this period, all unresolved TEAEs will be reported as "ongoing" in the CRF.

7.1.1 Visit 1

A screening visit will be conducted to identify eligible subjects to consent for the study. Screening will generally occur on the day of treatment (Day 1) but can also be performed on the day prior to treatment (Day 0) if needed for logistical reasons. Day 1 must be the following calendar day in this situation (for example Monday and Tuesday; not Friday and Monday).

Prior to any study assessments, examinations, or procedures being performed, the subject will sign the ICF.

It is imperative to confirm both eligibility and the subject's ability to undergo study drug instillations and assessments potentially lasting 3 to 4 hours. In the occasional circumstance where randomization needs to be rescheduled for the following calendar day, eligibility should be reconfirmed. The pharmacist or designee will access the IRT to obtain randomization allocation.

During the Screening Visit, the following procedures and assessments will occur:

- Written informed consent for each subject
- Review of the inclusion and exclusion criteria
- Collection of demographic data (birth date, sex, race/ethnicity)
- Medical history
- Weight and height
- Blood pressure measurement and heart rate
- A urine pregnancy test is requested for all females of childbearing potential. Women in physiological or surgical post-menopause do not need to be tested for pregnancy.
- AEs and concomitant medications/procedures to be assessed and recorded.
- CVAD history, including type, indication for insertion, and the number of hours the catheter has been known to be dysfunctional
- Determination of CVAD dysfunction, defined as the inability to withdraw 3 mL of blood (section 7.2.3), should be performed by a trained health care professional after the usual assessments, with repositioning and flushes, to confirm that an easily reversible or mechanical reason is not the suspected reason for poor catheter function.

Note: For multi-lumen catheters, the Investigator or designee will designate one lumen only to be used throughout the study for both study drug instillation and assessment of CVAD function. The lumen chosen will be documented and indicated using a study lumen tag to ensure that the same lumen will be used for all study drug instillations and study assessments.

- Subjects who qualify for the study, following completion of all screening assessments, will be administered study medication. **Note that all assessment time points / dwell times have a window of ± 5 minutes from the time of study drug instillation (0 min)**.
- After the first instillation of study drug, the CVAD will remain undisturbed for 30 minutes, at which time an assessment of function will be performed. Assessment of

function will be performed as described in section 7.2.3 by a trained (as deemed by the participating institution) health care professional. Restoration of CVAD function (i.e., treatment success) is defined as follows: the ability to both withdraw at least 3 mL of blood and infuse 5 mL of saline solution.

- If CVAD function has not been restored at **30** minutes after the first instillation of study drug, any aspirated or withdrawn study drug will be re-instilled, and the CVAD will remain undisturbed for an additional 30 minutes and an assessment of function will again be performed at 60 minutes after this first instillation of study drug.
- If CVAD function has not been restored at **60** minutes after the first instillation of study drug, any aspirated or withdrawn study drug will be re-instilled, and the CVAD will remain undisturbed for an additional 30 minutes and an assessment of function will again be performed at 90 minutes after this first instillation of study drug.
- If CVAD function has not been restored by **90** minutes after the first instillation of study drug, the first drug instillation will be withdrawn and discarded, and a second instillation of study drug will be administered within 15 minutes from the withdrawal of the 1st dose. Similarly, subsequent CVAD function will be assessed as described in section 7.2.3 (i.e., after 30 minutes, 60 minutes and then after 90 minutes from instillation of the 2nd dose, if needed).

Subjects will exit the treatment algorithm when treatment success is achieved at the specified time points, or after assessment of CVAD function 90 minutes following the second instillation of study drug (time point 180 min), whichever occurs first. Once a subject exits the treatment algorithm, assessments of CVAD function are completed for Day 1. In unsuccessful cases, further treatments for restoration of catheter functionality may be provided per the site's standard of care for CVAD. At 180 minutes, or in the case of Early Withdrawal, if treatment success is not achieved, any subsequently administered standard of care and related outcome will be documented.

Subjects will be monitored for AEs (including SAEs and AESIs) during the treatment period (Day 1) from the time the subject is administered the first dose of study drug until the withdrawal of study drug at V1, and for spontaneously reported SAEs and AESIs afterwards up until the Day 30 (±2 days) follow up assessment.

Concomitant medications and procedures will also be collected as part of the SAE reporting requirements.

At the end of Visit 1, subjects will be discharged with instructions on how to record and report the status of their CVAD during the 30-day follow-up period, including:

- Date of first recurrent catheter re-occlusion (for those with treatment success at V1),
- Date and type of any additional treatment they received for catheter dysfunction, and
- Date of catheter removal or replacement.

7.1.2 Visit 2

The Day 30 (±2 days) follow-up assessment will occur by phone or in person if the subject has a scheduled clinic visit. Spontaneously reported SAEs, AESIs, and related concomitant medications will be recorded and the status of the CVAD function will be determined by asking the subject to respond to the questions provided at the V1 discharge.

7.2 Investigations

7.2.1 Pregnancy Test

A urine pregnancy test is required for all females of childbearing potential at Visit 1. Women in natural or surgical post-menopause do not need to be tested for pregnancy at Visit 1.

7.2.2 Blood Pressure and Heart Rate

Blood pressure and heart rate will be measured at screening in supine position after 5-min rest. If blood pressure and heart rate are collected as part of Standard of Care on the same day of informed consent signing, then assessment taken prior to consent can be used. Blood pressure must be reassessed if screening and treatment are conducted on separate days.

7.2.3 Determination of CVAD Withdrawal and Infusion Functions

Withdrawal function will be determined by the following method:

- An empty 10mL graduated syringe will be attached to the catheter, forming an airtight seal. The syringe plunger will be pulled back to the 5 mL mark to attempt to withdraw blood. The CVAD is considered to have withdrawal function if at least 3 mL of blood (blood and any previously infused fluids) fills the syringe.

Infusion function will be determined by the following method:

- A 10-mL graduated syringe with 5 mL normal saline solution will be attached to the catheter to gently infuse the saline solution. The investigative site will be permitted to use saline from their own supply for the infusion function assessment. The CVAD is considered to have infusion function if the entire content of the syringe could be infused without significant resistance.

CVAD dysfunction, assessed for determining patient's eligibility, is defined as the inability to withdraw 3 mL of blood.

Restoration of CVAD function (patency), assessed for determining treatment efficacy, is defined by the two following actions:

- Ability to have 3 ml of blood (blood and any previously infused fluids) withdrawn
  AND
- Ability to gently infuse 5 ml of saline

Therefore, restoration of CVAD function (patency) is assessed by first attempting to withdraw 3 ml of blood and if successful, then attempting to gently infuse 5 ml of normal saline solution. To further clarify, if the 3 ml of blood (blood and any previously infused fluids) cannot be withdrawn, the CVAD is considered as dysfunctional, and further assessments are required as per protocol section 7.1.1 at the specified time points.

8. EFFICACY ASSESSMENTS

8.1 Primary Efficacy Variable

The primary efficacy variable is the percentage of subjects who have treatment success following a single instillation of study drug (CUSA-081, alteplase, or placebo) with a dwell

time up to 90 minutes. Treatment success is defined as the restoration of CVAD functionality measured as the ability to withdraw 3 mL of blood and infuse 5 mL of saline.

8.2 Secondary Efficacy Variables

The secondary variables of the study are as follows:

1. Percentage of subjects who have treatment success following a single instillation of study drug with a total dwell time up to 30 and 60 minutes;
2. Percentage of subjects who have treatment success after up to two instillations of study drug with a total dwell time up to 120, 150 and 180 minutes;
3. Rate of recurrent catheter dysfunction (defined as first re-occlusion) within 30 days following treatment with study drug.

9. SAFETY VARIABLES

The safety variables of the study are as follows:

- Treatment Emergent Adverse Events (TEAEs), Adverse Drug Reactions (ADRs), Serious Treatment Emergent Adverse Events (SAEs), serious related TEAEs, TEAEs leading to study withdrawal, and TEAEs leading to death
- Treatment Emergent Adverse Events of Special Interest (AESI), as defined in Section 10.1

10. ADVERSE EVENT REPORTING

10.1 Definitions

An **Adverse Event** is “any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment”.

An adverse event can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An **Adverse Drug Reaction** is an “untoward and unintended responses to an investigational medicinal product related to any dose administered”.

All adverse events judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression “reasonable causal relationship” means to convey in general that there are facts (evidence) or arguments meant to suggest a causal relationship.

The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

A. Serious Adverse Event (SAE)/Serious Adverse Drug Reaction is any untoward medical occurrence or effect that at any dose falls in one or more of the following categories:

- **Results in death**

Death is not an adverse event but an outcome. It is the cause of death that should be regarded as the adverse event. The only exception to this rule is “sudden death” where no cause has been

established; in this latter instance, "sudden death" should be regarded as the adverse event and "fatal" as its reason for being serious.

- **Is life-threatening**

Life-threatening refers to an event in which the subject was at risk of death at the time of the event (e.g., aplastic anemia, acute renal failure, and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.

- **Requires hospitalization or prolongation of existing hospitalization**

Hospitalization refers to a situation whereby an AE is associated with unplanned formal overnight admission into hospital, usually for purpose of investigating and/or treating the AE. Hospitalization for the treatment of a medical condition that occurs on an "elective" or "scheduled" basis or for a pre-existing condition that did not worsen during the study should not necessarily be regarded as an AE. Complications that occur during the hospitalization are AEs. If a complication prolongs hospitalization, the event is an SAE. Emergency room visits that do not result in a formal admission into hospital should be evaluated for one of the other seriousness criteria (e.g., life-threatening; persistent or significant disability or incapacity; medically significant).

- **Results in persistent or significant disability or incapacity.**

The term significant disability should be viewed as any situation whereby an AE has a clinically important effect on the subject's physical or psychological well-being to the extent that the subject is unable to function normally.

- **Is a congenital anomaly or birth defect**
- **Is a medically significant adverse event**

This criterion allows for any situations in which important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalization may jeopardize the subject's health or may require intervention to prevent one of the above outcomes.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether an event is serious because medically significant.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

B. **Non-Serious Adverse Event/Non-Serious Adverse Drug Reaction** is an adverse event or adverse drug reaction that does not meet the criteria listed above for a serious adverse event/serious adverse drug reaction.'

C. Adverse Events of Special Interest (AESI): Adverse Events of Special Interest (AESI) will be collected from the time the subject is administered the first dose of study drug on Day 1 until the withdrawal of study drug (V1), and if spontaneously reported afterwards up until the 30 days ± 2 days follow up assessment

I. Major bleeding (defined as severe blood loss [>5 mL/kg] or blood loss requiring transfusion or causing hypotension requiring use of inotropic agents),

II. Embolism
III. Thrombosis
IV. Catheter related blood stream infections (CRBSI).

10.2 Expectedness

An expected adverse reaction is an adverse reaction, the nature or severity of which is consistent with the applicable reference safety information (Investigator's Brochure for CUSA-081), otherwise it is considered unexpected.

Reports which add significant information on specificity or severity of a known, already documented serious adverse drug reaction constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure would be considered as "unexpected". Examples of such events are: (a) acute renal failure as a labelled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis

In the event an exacerbation is interpreted as due to lack of efficacy, it should not be classified as drug related.

10.3 Intensity of Adverse Event

Each Adverse Event must be rated on a 3-point scale of increasing intensity:

- **Mild:** The event causes a minor discomfort or does not interfere with daily activity of the subject or does not lead to either modification of test treatment dosage or establishment of a correcting treatment.
- **Moderate:** The event perturbs the usual activity of the subject and is of a sufficient severity to make the subject uncomfortable. The event leads to a diminution of dosage of the test treatment, or a temporary interruption of its administration or to the establishment of a correcting treatment.
- **Severe:** The event prevents any usual routine activity of the subject and causes severe discomfort. It may be of such severity to cause the definitive interruption of test treatment.

10.4 Causality Assessment

The following "binary" decision choice will be used by the Investigator to describe the causality assessment:

Reasonable possibility of a relatedness
No reasonable possibility of relatedness

The expression "reasonable possibility of relatedness" is meant to convey, in general, that there are facts (evidence) or arguments meant to suggest a causal relationship.

The Investigator will be asked to consider the following before reaching a decision on causality assessment:

- Time relationship between study drug intake and event's onset;
- Dechallenge (did the event abate after stopping drug?);
- Rechallenge (did the event reappear after reintroduction?);
- Medical history;
- Study treatment(s);

- Mechanism of action of the study drug;
- Class effects;
- Other treatments-concomitant or previous;
- Withdrawal of study treatment(s);
- Lack of efficacy/worsening of existing condition;
- Erroneous treatment with study medication (or concomitant);
- Protocol related process.

10.5 Action Taken with the Study Drug Due to an AE

- Dose not changed
- Drug permanently withdrawn
- Not applicable

10.6 Other Actions Taken

- Specific therapy/Medication
- Concomitant Procedure
- Not applicable

10.7 Outcome

Each Adverse Event must be rated by choosing among:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal
- Unknown

10.8 Recording Adverse Events

All Adverse Events occurring during the course of the study must be documented in the Adverse Event page of the electronic Case Report Form (eCRF). If the Adverse Event is considered serious or meets the definition of AESI, the Adverse Event Form in the eCRF must also be completed.

It is responsibility of the Investigator to collect all adverse events (both serious and non-serious) derived by spontaneous, unsolicited reports of subjects, by observation and by routine open questionings.

The recording period for Adverse Events is the period starting from the Informed Consent signature until the subject's study participation ends.

Clinically significant abnormalities detected at Visit 1 not due to a pre-existing condition or clinically significant changes at the following visits in the medical opinion of the investigator must be reported as adverse events in the eCRF.

If a clinically significant abnormal laboratory finding or other abnormal assessment meets the definition of an AE, then the AE eCRF page must be completed, as appropriate. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically

significant abnormal laboratory finding, should be reported on AE eCRF page. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded.

For pharmacovigilance purposes, all SAEs and AESIs should be followed-up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or until the subject is lost to follow-up. Follow-up may therefore continue after the subject has left the study. In this case, the follow-up will continue with no timelines for related SAEs and AESIs, while for unrelated SAEs and AESI the type and extent of follow-up undertaken will be determined for each individual case and will depend upon the nature (e.g. events with poor prognosis or which do not resolve), severity and medical significance of the event.

10.9 Reporting Serious Adverse Events and Adverse Events of Special Interest

The Investigator must report all Serious Adverse Events (SAEs) and all Adverse Events of Special Interest (AESIs) to the [redacted] Safety Contact listed below within 24 hours of awareness. The information must be sent by providing the completed SAE/AESI form. The [redacted] Safety Contact will report all information to Chiesi Global Pharmacovigilance, the Clinical Project Manager and the Clinical Research Physician.

Name and Title	E-mail
[redacted] Safety Contact	[redacted]
Chiesi Safety Contact	[redacted]

- The reporting period for SAEs from the investigator site is from the time of subject's signature of informed consent and until the subject's study participation ends. After this date, even if no active monitoring of subjects is required, SAEs occurring to a subject should be reported if the investigator becomes aware of them.
- Reporting of AESI is from the time the subject is administered the first dose of study drug on Day 1 until the 30 days ± 2 days follow up assessment.
- Up to the closure of the site, SAE reports should be reported to the [redacted] Safety Contact. New serious adverse events occurring after the site is closed should be reported directly to the Chiesi Safety Contact.
- The SAE and AESI information should also be completed simultaneously in the adverse event page of the case report form (CRF).

10.10 Reporting Serious Adverse Events to Regulatory Authorities/Ethics Committees/IRB

Chiesi or [redacted] will report serious unexpected adverse reactions to the regulatory authorities in compliance with the timelines and standards of reporting according to local regulations as well as to the Investigators, ethics committees and Central IRB, if applicable, by MedWatch/CIOMS form. The Investigator is responsible for local IRB reporting per Sponsor

instruction upon receipt of the SUSAR notification. Participating Investigators, ECs and IRBs will receive a blinded IND Safety Report, unless otherwise specified.

All SUSARs, which occur with the investigational medicinal products within or outside the concerned clinical trial, if required, will be reported in compliance with the timelines and standards for reporting SUSARs as set out in FDA 21 CFR part 312.32 and the EU Directive 2001/20/EC [Directive 2001/20/EC of the European parliament and of the council of 4/April/2001] and linked guidance [European Commission, Enterprise and Industry Directorate General: Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use, latest version]. The EMA and the concerned national health authority (if applicable) will be informed through Eudravigilance, while the Ethics Committees and the investigators by CIOMS I form or by periodic line- listings produced by Chiesi Global Pharmacovigilance. The EMA and the concerned national health authority (if applicable) will be informed through Eudravigilance, while the Ethics Committees and the investigators by CIOMS I form or by periodic line- listings produced by Chiesi Global Pharmacovigilance.

With regard to regulations in force for Pharmacovigilance, the Investigator must fulfill his/her obligation according to the law in force in his/her country.

10.11 General Notes

- In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the CRO Safety Contact by fax together with the Serious Adverse Event form, retaining a copy on site.
- If an autopsy is performed, copy of autopsy report should be actively sought by the Investigator and sent to the CRO Safety Contact as soon as available, retaining a copy on site.
- In case of pregnancy, the subject will be followed with due diligence until the outcome of the pregnancy is known and until the age of one year to detect any congenital anomaly or birth defect. The pregnancy must be reported by the investigator within 24 hours by fax/e-mail/via Monitor to the [redacted] Safety Contact using the paper Pregnancy Report Form. [redacted] Safety Contact will inform Chiesi of the pregnancy within one working day of being notified.

The first two pages of the Pregnancy Report Form should be completed by the investigator with all the available information and sent to the CRO Safety Contact. The third page will be completed as soon as the investigator has knowledge of the pregnancy outcome, together with a follow-up of the first two pages, if necessary (e.g. an update in the medications received during pregnancy by the mother). If it meets the criteria for immediate classification of a SAE (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital anomaly, birth defect) the Investigator should follow the procedure for reporting SAEs.

- If it is the partner, rather than the subject, who is found to be pregnant, the same procedure regarding pregnancy reporting is to be followed and the Pregnancy Report Form should be completed.

- If the pregnancy is discovered before taking any dose either of study drug medication, the subject will not be eligible for the study and the pregnancy does not need to be reported.
- Any ADR occurring with any marketed non-IMP and/or concomitant medication during the study must be reported by the Investigator to his/her concerned Health Authority according to the applicable laws. The Investigator is also recommended to report all ADRs to the relevant Marketing Authorisation Holders of the involved medicinal products. Additionally, conditions of use outside the marketing authorisation of the medicinal products (i.e. off label, overdose, misuse, abuse and medication errors) or from occupational exposure, as well as cases of suspected treatment interaction, pregnancy, breastfeeding exposure and lack of efficacy should be reported

11. DATA MANAGEMENT

An electronic CRF (eCRF) will be completed by the Investigator and/or his/her designee for all subjects including screen failures.

Front-end edit checks will run at the time of data collection and back-end edit checks will be used by the Data Manager to check for discrepancies and to ensure consistency and completeness of the data.

Medical history and Adverse Events will be coded using the MedDRA dictionary; medications will be coded using the WHO Drug dictionary and Anatomical Therapeutic Chemical classification (ATC).

Access to electronic systems used for data collection will be granted to the study personnel only after appropriate training.

After cleaning of the data, review meetings will be held to determine the occurrence of any protocol violations and to define the patient populations for the analysis. This process will occur for the first set of patients which will be involved in the interim analysis, and then for all subsequent patients that will be randomized up to study recruitment closure. Only completed and discontinued patients at the time of the cut-off will be included in the interim analysis.

Once all data of patients included in the interim futility analysis have been declared to be complete and accurate, the database will be locked ("interim database lock"), the randomization codes will be opened (only for the patients involved in the interim analysis) and shared only with the Unblinded Statistical/Programming Team and the independent DMC responsible for the conduction or interpretation of the interim analysis. Additional details on the interim analysis and involved parties are provided in Section 12.3.7.

A final database lock will occur once all data related to the patients enrolled in the study have been declared complete and accurate; the randomization list will be shared to the Sponsor and the final statistical analysis as planned in the SAP will be performed.

If the database is unlocked after the initial lock, the process must be carefully controlled and documented; updates to the study data must be authorized by Chiesi. This applies to the interim database lock for patients included in the interim futility analysis as well as for the final database lock including all patients.

At the study conclusion, a complete copy of the study data will be created for archival purposes at Chiesi. The investigators will receive copies of the patient data for retention at the investigational sites.

12. STATISTICAL METHODS

12.1 Sample Size

Hypotheses

The primary objective is to demonstrate superiority of CUSA-081 compared to placebo in the rate of treatment success (restoration of CVAD function) following a single administration with a dwell time up to 90 minutes. The primary hypotheses are as follows:

The null hypothesis H0: Rc-Rp=0
The alternative hypothesis H1: Rc-Rp>0

Rc=Rate of success of CUSA-081
Rp=Rate of success of placebo

The first secondary objective is to demonstrate non-inferiority of CUSA-081 vs alteplase in the rate of treatment success following a single administration with a dwell time up to 90 minutes. The hypotheses for the non-inferiority are as follows:

The null hypothesis H0: $Rc-Ra \leq -10$
The alternative hypothesis H1: $Rc-Ra > -10$

Rc=Rate of success of CUSA-081
Ra=Rate of success of alteplase

Additional hypotheses are constructed in a similar manner for secondary objectives to demonstrate superiority of CUSA-081 vs alteplase following a single administration with a dwell time up to 90 minutes, and to demonstrate superiority of CUSA-081 compared with placebo in the rate of treatment success at different time points, after one or two administrations. The order of the remaining secondary objectives is listed below:

2. Superiority of CUSA-081 compared to placebo in the rate of treatment success after a single administration with a dwell time up to 60 minutes;
3. Superiority of CUSA-081 compared to placebo in the rate of treatment success after up to two administrations with a dwell time up to 180 minutes;
4. Superiority of CUSA-081 compared to alteplase in the rate of treatment success after a single administration with a dwell time up to 90 minutes.

Significance Level

The comparisons of superiority will be performed at the two-sided 5% level of significance. The assessment of non-inferiority will be based on the 2.5% level of significance (using one-sided 97.5% confidence intervals) with a non-inferiority margin of -10%.

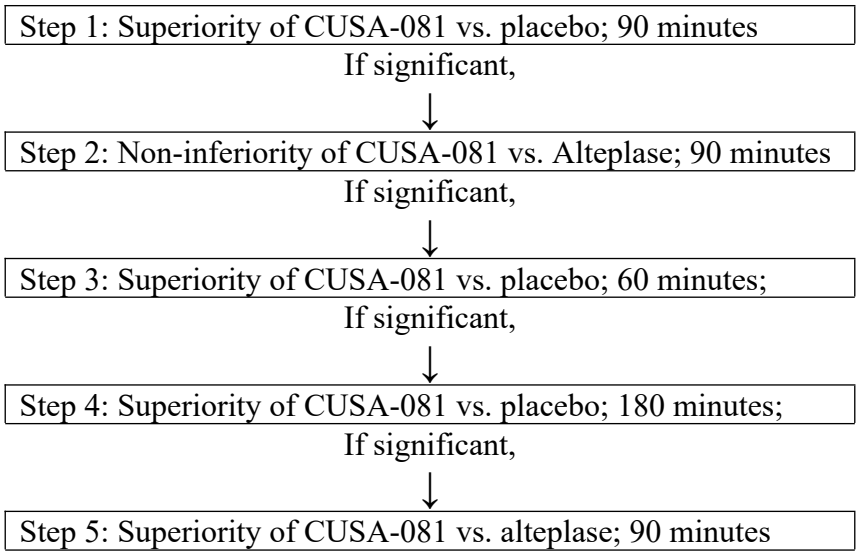
Multiplicity Adjustment

Multiplicity adjustment for testing of multiple hypotheses. A closed testing procedure will be used to control the overall type I error rate at <5%. The hypothesis testing will start with the primary objective, then the secondary efficacy objectives will be tested, in the order listed in

the study objectives and shown in the figure below, until a null hypothesis is not rejected (a two-sided p-value >0.05 is observed, or lower limit of the two-sided 95% CI ≤ -10% for the non-inferiority assessment).

Figure 2: Statistical Testing of Rate of Treatment Success in the READY-1 Trial

Treatment success is defined as restoration of CVAD functionality measured as the ability to withdraw 3 mL of blood and infuse 5 mL of saline.



Notes:

1. *All tests are performed at the 2-sided α=0.05 level of significance.*
2. *For tests at 60 and 90 minutes, the rate of treatment success is assessed as the percentage of subjects achieving treatment success following a single instillation of study drug and a dwell time up to 60 or 90 minutes, respectively.*
3. *For the test at 180 minutes, the rate of treatment success is assessed as the percentage of subjects achieving treatment success following up to two instillations of study drug and a dwell time up to 180 minutes, respectively.*

Multiplicity adjustment not planned for interim analysis futility analysis. No multiplicity adjustment will be incorporated for the futility analysis to assess the conditional power for the non-inferiority test of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes (the second step in the closed testing procedure). Of note, there will be no stopping of any arm for efficacy and no claims of efficacy made on the basis of the futility analysis. The recommendation of a threshold to stop the study for futility will be considered as "nonbinding," meaning the futility stopping criteria are guidelines to the DMC that may or may not be followed, depending on the totality of the available interim results.

Required Sample Size

For the primary assessment of the superiority of CUSA-081 vs. placebo, a sample size of 450 in the active arm (CUSA-081), and 50 in the control arm (placebo), will provide >99% power to detect a difference of ≥35% using a two-sided test at significance level of 0.05. The expected placebo response rate is approximately 20%.

For the assessment of the non-inferiority of CUSA-081 vs alteplase, a sample size of 450 in the active arm (CUSA-081), and 300 in the control arm (alteplase) will provide 87% power at the 2.5% significance level (that is by using one-sided 97.5% confidence intervals) with a non-inferiority margin of -10%, assuming an equal response rate of 75% in the control arm (alteplase), and the active (CUSA-081) arm. A sample size of 450 in the active arm (CUSA-081), and 300 in the control arm (alteplase) will also provide 92% power to detect a difference of 10% between the success rates using a two-sided test at significance level of 0.05.

A minimum of 841 subjects will be randomized in order to achieve a minimum of 800 subjects completing the study.

Statistical Consideration of Non-Inferiority Margin

The selection of the non-inferiority margin was based upon the following reasoning. The non-inferiority margin must be less than the smallest treatment difference between standard therapy and placebo (M1), and at least part of the treatment effect of the standard therapy must be preserved for the test drug (M2).

The NI margin of -10% between reteplase vs. alteplase was selected based on the following statistical consideration:

M1 = the smallest treatment effect of alteplase over placebo

M2 = a fraction 50% of M1 is the most commonly used value

Table 8: Historical Placebo Response

Study / Time point		Number of responders / Sample size	% Response	Lower 95% CI	Upper 95% CI
TROPICS 1 (Gabrail et al, 2010)	120 minutes	11/47	23%	11%	36%
COOL 1 (Cathflo PI, 2017)	120 minutes	12/74	16%	8%	25%

Placebo responses have been reported in hemodialysis setting and in the central venous catheter setting. We included the available placebo responses data from 2 studies in the central venous access setting (Table 8) with a definition of treatment success similar to the one used in this study and with a timepoint assessment of ≥ 90 minutes. Based on the data from these two studies, we estimated the placebo response using a weighted analysis based on

a random effect model (DerSimonian-Laird). The weighted placebo reponse is 19% with 95% CI of 12.9% to 26.8%.

Based on the CATHFLO ACTIVASE product label, the restoration of catheter function was assessed at 30 and 120 minutes after the first instillation of alteplase (rtPA). The response at 90 minutes was estimated based on linear interpolation. Table 9 shows the observed response rates at 30 minutes and 120 minutes and the estimated 95% CI at 90 minutes.

Table 9: Historical rtPA Response

Time point (COOL 2)		# of responders / Sample size	% Response	Lower 95%CI	Upper 95%CI
30 minutes	observed	516/995	51.9%	48.8%	55.0%
90 minutes	linear interpolation	670/995	67.3%	64.4%	70.3%
120 minutes	observed	747/995	75.1%	72.4%	77.8%

Based on the linear interpolation, the lower bound of the 95% CI of rtPA response at 90 minutes is 64.4%. The upper bound of the 95% CI for the weighted placebo response is estimated to be 26.8%. Therefore, the rtPA margin over placebo is at least 37.6% at 90 minutes. To preserve 50% of M1 in a NI trial, a NI margin of 18.8% (half of 37.6%) would be reasonable.

A more conservative -10% NI margin between reteplase and alteplase will be used for the 90 minutes endpoint in this trial, which should preserve >70% of M1.

12.2 Populations for Analysis

- **Full analysis set (FAS):** All randomized subjects who receive at least one dose of study drug, and with at least one available evaluation of efficacy after baseline. Analyses using the FAS set will be based on the treatment randomized. The FAS will be the primary analysis set for all efficacy analyses.

- **Safety set (SS):** All randomized subjects who receive at least one dose of study drug. Subjects discontinued after dosing will be part of the SS. Analyses based on the SS set will be based on the actual treatment received. The SS will be the primary analysis set for all safety analyses.

- **ITT analysis set (ITT):** All randomized subjects regardless if they received treatment with study drug. The ITT will be based on the randomized treatment allocation. The ITT will be used for sensitivity analysis for the primary efficacy analyses.

- **Per-protocol analysis set (PPS):** all subjects from the ITT set without any important protocol deviations. Important protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of key study data or that may significantly affect a subject's rights, safety, or well-being. Important protocol

deviations will be assigned in a blinded manner prior to database lock. The PPS will be used for sensitivity analysis for the primary efficacy analyses and the non-inferiority analyses.

12.3 Statistical Analysis

A detailed statistical analysis plan will be described in a separate document. The plan might be reviewed and updated as a result of the blind review of the data and will be finalized before breaking the blind.

12.3.1 Descriptive Statistics

General descriptive statistics for numeric variables will include the n (number of observed values), the mean, the standard deviation, the median, the minimum, and the maximum values. For categorical variables, the number and percent of subjects with a specific level of the variable will be presented.

12.3.2 Missing Data and Intercurrent Events

By design, all subjects exit the treatment algorithm once patency is achieved and no further assessments of catheter clearance are performed. Subjects are considered a treatment success at the time point at which patency was achieved and also at all subsequent time points during the treatment period.

Otherwise, if missing data is observed for assessment of treatment success and patency was not achieved prior to the time point with missing data, then the subject will be considered as a failure (worst case scenario) at the time point with missing data. The summary statistics for treatment success will display the derived data, including imputation for any missing data observed prior to treatment success. This approach will be followed for all the primary and key secondary analyses of treatment success. Protocol violations and any other intercurrent events will be ignored for analyses performed on the FAS, which target a treatment policy estimand. Patients with important protocol deviations will be excluded from analyses based on the PPS.

Further details on dealing with missing data will be described in the SAP. Other critical missing data, if any, will be discussed during the blinded review of the data prior to database lock.

12.3.3 Subject Demographics and Baseline Characteristics

Demographics and baseline variables will be summarized by treatment arm using descriptive statistics for the FAS set.

The following variables will be presented: age, gender, race, height, weight, medical history, concomitant diseases, medical history, CVAD history and function, and screening assessments.

12.3.4 Primary Efficacy Variables

The primary efficacy variable is the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. The percentage of subjects with treatment success and two-sided 95% Wald confidence intervals (CIs) will also be provided by treatment

group. The assessment of superiority vs. placebo will be performed using a 2-sample z test. A two-sided 95% confidence interval on the difference in rates of restoration of CVAD function between treatment groups will be constructed.

Assessment of non-inferiority will be based on the constructed 95% confidence interval for the difference in the rate of treatment success between CUSA-081 vs alteplase. The non-inferiority will be demonstrated (or null hypothesis will be rejected) when the lower limit of the 95% confidence interval for the difference in rate of success is greater than -10%. For the non-inferiority test, the primary validation of the assay is considered as the demonstration of superiority of CUSA-081 versus placebo in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes, which corresponds to the primary objective of the study and the first step in the closed testing procedure (Koch 2004). An additional check of assay sensitivity will also be performed as a test of the superiority of alteplase versus placebo in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes. The additional check of assay sensitivity will be performed at the same time as the non-inferiority assessment, using the same statistical methodology as for the other analyses of superiority, without adjustment for multiplicity.

12.3.5 Secondary Efficacy Variables

The number and percent of subjects with treatment success by treatment and time point will be summarized. The rate difference and associated 95% Wald confidence intervals between CUSA-081 vs. placebo and alteplase will be estimated. The between treatment comparisons will be performed using a 2-sample z test.
The rate of recurrent catheter dysfunction defined as first re-occlusion within 30 days following treatment will be analyzed using the Kaplan-Meier method and summarized with median, 25th and 75th percentiles, and 95% confidence intervals. The rate of recurrent catheter dysfunction will be estimated. This analysis will be based on all subjects with treatment success following up to two administrations of study drug with a total dwell time up to 180 minutes.

Additional efficacy analyses including demographics and baseline characteristics as covariate/subgroup may be explored.

12.3.6 Safety Variables

Safety analysis will be based on SS set.

Adverse Events
All adverse events starting from the time the subject is administered the first dose of study drug on Day 1 until the withdrawal of study drug will be classified as treatment emergent adverse event (TEAE). The number of subjects who experience at least one TEAE, ADR, SAE, serious related TEAE, TEAE leading to study withdrawal, TEAE leading to death, and AESI (as defined in Section 10.1), will be summarized. Post-treatment adverse events during the 30-day follow up period will be summarized. Summaries will be presented by actual treatment arm and by system organ class and preferred term as the number and percentage of subjects having at least one event and the total number of events.

For AESIs, the percentage of subjects who experienced AESI and associated exact binomial 95% confidence interval will be provided in the summary.

All adverse events will be listed.

Vital signs
Vital signs (systolic and diastolic blood pressure, heart rate) will be summarized by means of descriptive statistics. Mean, min, max and standard deviation of the observed values will be presented, in addition to being listed.

12.3.7 Interim analysis

A single interim analysis for futility will be performed to evaluate if the study should be stopped for futility. There will be no stopping rules for efficacy at the time of this interim analysis and no efficacy claims on the basis of the interim analysis. Therefore, no statistical adjustment will be done for the final analysis.

The interim futility analysis is planned to be performed when approximately 416 patients have completed the study. The timing of the interim futility analysis could be modified based on the progress of the recruitment and planning of other studies.

The interim futility analysis will assess the conditional power for the test of non-inferiority of CUSA-081 compared to alteplase in the rate of treatment success following a single administration of study drug with a dwell time up to 90 minutes.

Number and Timing of Interim Analyses
A single interim futility analysis will be conducted when approximately 416 patients have completed the study.

Statistical Evaluation of Futility
The conditional power for the test of the first secondary objective will be calculated on the basis of the observed data at the time of the interim analysis. The study could be terminated early if the conditional power at the interim futility analysis is lower than some pre-defined threshold (“conditional power threshold”).

The exact conditional power threshold will be defined in the DMC charter, to be finalized prior to the interim database lock and prior to any unblinding for the interim analysis. Of note, the futility stopping criteria will be considered as “nonbinding” (i.e., they are guidelines that may or may not be followed, depending on the totality of the available interim results). Aside from potentially halting the study for futility, there will be no additional changes to the study design on the basis of the futility analysis. Specifically, there will be no efficacy stopping rules related to the analysis, nor will there be any changes to the planned arms or allocation to the arms, and no formal hypothesis testing of the named study objectives. For this reason, the futility analysis does not cause inflation of type 1 error and no multiplicity adjustment will be conducted on the basis of the futility analysis.

Presentation of Results

The tables, figures, and listings (TFLs) produced for the interim analysis will include a designation as "Interim Analysis" next to the analysis population.

Roles of Unblinded Teams

A separate independent and unblinded team ("Unblinded Statistical/Programming Team") will be established to perform the futility analysis and prepare the TFLs. An independent and unblinded DMC will be established to review the data and provide a recommendation regarding the continuation of the study. The Sponsor's and CROs' study teams and the other blinded team members will remain blinded.

The Unblinded Statistical/Programming Team will be responsible for preparing the unblinded TFLs to be used for the interim analysis, with further details provided in the SAP. The unblinded Statistical/Programming Team may be part of the same CRO who is managing the data management and statistical activities for the study. If so, appropriate measures will be put in place to ensure that the CRO blinded team and all other members of blinded teams will not have access to any unblinded materials or information.

The DMC will be comprised of at minimum 1 statistician and 2 physicians who are experts in the field, all completely independent from the day-to-day activities of the study. The DMC will review the results of the interim futility analysis and provide a recommendation to Chiesi whether the study should continue without modification or whether the study should be terminated entirely. The DMC will also review safety data at the same time as the interim futility analysis. Review and discussion of unblinded materials will occur in a "closed session" meeting including only specifically designated unblinded participants. Aside from the recommendation to continue or halt the study, no results of the interim analysis will be shared by the DMC. No unblinded materials or information used by the DMC will be shared with blinded study team members. A separate DMC charter will be created to describe the specific details of the review by the DMC members.

Neither the Unblinded Statistical/Programming Team, nor the DMC will have access to any unblinded materials prior to the interim database lock described in Section 11.

13. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD APPROVAL

The protocol, the written informed consent form and any materials presented to the subject shall be submitted for review and approval to the IRB or IEC identified with this responsibility. Notification in writing of approval must come from the IRB or IEC chairman or secretary to the investigator, either as a letter or as a copy of the appropriate section of the IRB or IEC meeting minutes where this protocol and associated informed consent form were discussed, before the clinical trial commences at the study site. The study title and number must be clearly identified in the IRB approval documentation. If the investigator is an IRB or IEC member, the written approval must indicate such non-participation in the voting session.

The investigator will submit status reports to the IRB or IEC annually and as required by the governing body. The IRB or IEC must be notified by the investigator in writing of the interruption and/or completion of the study; the investigator must promptly report to the IRB or IEC all changes in research (protocol amendments) and will not make such changes without IRB or IEC approval, except where necessary to eliminate apparent immediate hazards to

human subjects. In cases where it is necessary to eliminate immediate hazards to subjects, the IRB or IEC must then be notified of the change as per local requirements.

The investigator is required to maintain an accurate and complete record of all written correspondence to and received from the IRB or IEC and must agree to share all such documents and reports with the Sponsor.

14. REGULATORY REQUIREMENTS

The study will be conducted in compliance with the protocol, CRO and/or Chiesi's standard operating procedures and/or guidelines, the United States FDA regulations, the ICH GCP guidelines, the Declaration of Helsinki, and other local regulations, as applicable. Selection of subjects will not start before the approval of the IRB/IEC.

15. INFORMED CONSENT

Written informed consent will be obtained from all subjects per IRB/IEC guidelines before any study-related procedures (including any pre-treatment procedures) are performed. The investigator has both ethical and legal responsibility to ensure that each subject being considered for inclusion in this study is given a full explanation of the protocol. This shall be documented on a written informed consent form, which shall be approved by the same IRB or IEC responsible for approval of this protocol. Each informed consent form shall include the elements required by ICH, Part E6, Section 4.8, and any applicable local regulations. The investigator agrees to obtain approval from the Sponsor of any written informed consent form used in the study, preferably prior to submission to the IRB or IEC.

Once the appropriate essential information has been provided to the subject and fully explained by the investigator (or a qualified designee) and it is felt that the subject understands the implications of participating, the subject and the investigator (or designee) shall sign and date the IRB- or IEC-approved written informed consent form. The subject shall be given a copy of the signed informed consent form, and the original shall be filed appropriately, according to the institution. A second copy may be filed in the subject's medical record, if allowed by the institution.

16. SOURCE DOCUMENTS/DATA

The Investigators must permit trial-related monitoring, audits, Ethics Committee/Institutional Review Board review or regulatory inspection, providing direct access to source data/documents.

16.1 Recording of Source Data

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

16.2 Direct Access to Source Document/Data

The Investigators or designee (when applicable) must permit trial-related monitoring, audits, Ethics Committee/Institutional Review Board review or regulatory inspection, providing direct access to source data/documents.

17. STUDY MONITORING

The Sponsor has ethical, legal and scientific obligations to carefully follow this study in accordance with established research principles and applicable regulations. The Investigator, as part of his/her responsibilities, is expected to cooperate with the Sponsor in ensuring that the study adheres to the protocol and GCP requirements.

As part of a concerted effort to fulfill these obligations, the Sponsor's monitor or designee will visit the center(s) during the study in accordance with the Monitoring Plan set forth for this trial. The Investigator will permit the Sponsor or designee to monitor the study as frequently as is deemed necessary and provide access to medical records/source documents to ensure that data are being recorded adequately, that data are verifiable, and that protocol adherence is satisfactory.

Monitoring will be performed by the CRO designated by Chiesi.

It is understood that the monitor(s) will contact and visit the Investigator/centre before the study, regularly throughout the study and after the study had been completed, and that they will be permitted to inspect the various study records: case reports form, Investigator study file and source data, provided that subject confidentiality is respected.

The purposes of these visits are:

- to assess the progress of the study;
- to review the compliance with the study protocol;
- to discuss any emergent problem;
- to check the CRFs for accuracy and completeness;
- to validate the contents of the CRFs against the source documents;
- to assess the status of drug storage, dispensing and retrieval.
- Prior to each monitoring visit, the Investigator or staff will record all data generated since the last visit on the case report forms. The Investigator and/or study staff will be expected to be available for at least a portion of the monitoring visit to answer questions and to provide any missing information.

18. QUALITY ASSURANCE

The R&D Quality Assurance Department of Chiesi may perform an audit at any time according to the Sponsor’s Standard Operating Procedures, in order to verify whether the study is being conducted in agreement with Good Clinical Practices and the protocol.

Regulatory authorities worldwide may also inspect the Investigator/site during or after the study. The Investigator should contact the Sponsor immediately if this occurs and must permit regulatory authority inspections.

18.1 Protocol Deviations

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the subject requires immediate intervention,

based on the judgment of the Investigator (or a responsible, appropriately trained professional designated by the investigator). In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator or designee must contact the CRO at the earliest possible time by telephone. This will allow an early joint decision regarding the subject's continuation in the study. The Investigator, Sponsor and the CRO will document this decision. The IRB/IEC will be informed of all protocol changes by the Investigator in accordance with the IRB/EC established procedure. No deviations from the protocol of any type will be made without complying with all the IRB/EC established procedures.

19. INSURANCE AND INDEMNITY

Chiesi holds and will maintain an adequate insurance policy covering damages arising out of Chiesi's sponsored clinical research studies.

Chiesi will indemnify the Investigator and hold him/her harmless for claims for damages arising out of the investigation, in excess of those covered by his/her own professional liability insurance, providing that the drug was administered under his/her or deputy's supervision and in strict accordance with accepted medical practice and with the study protocol.

The Investigator must notify Chiesi immediately upon notice of any claims or lawsuits.

20. CONFIDENTIALITY

All study documents are provided by the Sponsor in confidence to the Investigator and his/her appointed staff. None of this material may be disclosed to any party not directly involved in the study without written permission from Chiesi.

The Investigator must assure the subject's anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subject's study numbers, names, and addresses and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from Chiesi or designee.

21. PREMATURE TERMINATION OF THE STUDY

Both the Sponsor and the Investigator reserve the right to terminate the study at any time. Should this be necessary, the procedures for an early termination or temporary halt will be arranged after consultation by all involved parties. The Sponsor should submit a written notification to the Regulatory Authority concerned and Ethics Committee/Institutional Review Board providing the justification of premature ending or of the temporary halt.

22. CLINICAL STUDY REPORT

The clinical study report, including the statistical and clinical evaluations, shall be prepared and may be sent to the Principal Investigator for agreement and signature.

At the end of the trial a summary of the clinical study report will be provided to all Ethics Committees/Institutional Review Boards, to the Competent Authorities and to Investigators.

23. RECORD RETENTION

After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file.

Regulations require that essential documents must be retained for at least two years after the final marketing approval in an ICH region or until two years have elapsed since the formal interruption of the clinical development of the product under study.

It is the responsibility of the Chiesi to inform the Investigator of when these documents can be destroyed. The Investigator must contact Chiesi before destroying any trial-related documentation. In addition, all subjects' medical records and other source documentation will be kept for the maximum time permitted by the institution.

24. PUBLICATION OF RESULTS

Chiesi is entitled to publish and/or present any results of this study at scientific meetings, and to submit the clinical trial data to national and international Regulatory Authorities. Chiesi furthermore reserves the right to use such data for industrial purposes. Investigators will inform Chiesi before using the results of the study for publication or presentation and agree to provide the Sponsor with a copy of the proposed presentation. Data from individual study sites must not be published separately.

Negative as well as positive results should be published or otherwise made publicly available.

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APPENDIX 1 - Approval of the protocol by clinical investigator(s)

Study Title: READY 1: A Phase 3, Randomized, Double-Blind, Active and Placebo-Controlled Study on the use of CUSA-081 for Dysfunctional Central Venous Access Devices (CVADs)

Product: CUSA-081

Approval of Clinical Study Protocol by the Principal Investigator:

I have carefully read this protocol and I agree that it contains all the necessary information required to conduct the study and I agree to conduct it as described.

I understand that this trial will not be initiated without Ethics Committee/Institutional Review Board approvals and that the administrative requirements of the governing body of the institution will be fully complied with.

Informed written consent will be obtained from all participating subjects and appropriately documented, prior to their enrolment in the study.

The undersigned agrees that the trial will be carried out in conformity with the Declaration of Helsinki (as applicable, with attention being drawn to Section concerning freely given consent), ICH E6 Good Clinical Practices and with all the other local laws and regulations relevant to the use of new and approved therapeutic agents in subjects.

Principal Investigator's Name: _____

Centre No.: _____

_____ _____

Signature Date

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