Clinical Study Protocol

Protocol Title:	A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Preoperative Antithrombin Supplementation in Patients Undergoing High-Risk Cardiac Surgery with Cardiopulmonary Bypass
Investigational Product:	Thrombate III® (AntithrombinIII [Human])
Sponsor's Name and Address:	Grifols Therapeutics Inc. 79 T.W. Alexander Drive Research Triangle Park, North Carolina 27709 USA
Sponsor's Telephone Number:	[redacted]
Study Number/Protocol Version Number/Date:	GTI1307 / 4.0 (including Protocol Amendment 3) / 02 Apr 2015
Development Phase:	2b

The undersigned confirm that they agree to conduct the study under the conditions described in this protocol:

[redacted]

2 April 2015
Date:

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02 - APRIL - 2015
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Summary of Changes for Protocol Amendment 3

Protocol Version	Date of Finalization
4.0 including Amendment 3	02 Apr 2015
3.0 including Amendment 2	02 Sep 2014
2.0 including Amendment 1	27 Jan 2014
1.0 Original	29 Oct 2013

Version 4.0, 02 Apr 2015

A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Preoperative Antithrombin Supplementation in Patients Undergoing High-Risk Cardiac Surgery with Cardiopulmonary Bypass

The protocol for Study GTI1307 (Version 3.0 dated 02 Sep 2014) has been amended and reissued as Protocol Version 4.0, dated 02 Apr 2015.

AMENDMENT 3 – SUMMARY OF CHANGES

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
Global changes	**Global changes:** 1. Subjects undergoing complex/combined procedures will not be prescreened or screened for antithrombin (AT) levels. The inclusion requirement for AT level less than 80% has been removed for complex/combined procedures (coronary artery bypass graft [CABG+valve], double/triple valve repair/replacement, and ascending aorta/aortic arch surgeries). 2. Only subjects undergoing isolated CABG or single valve repair/replacements and not receiving preoperative heparin may be prescreened for AT levels (AT <80%). The inclusion criterion for isolated CABG or single valve repair replacement has been revised to allow inclusion based on either (a) AT level less than 80% OR (b) preoperative heparin administration (unfractionated heparin [UFH] for at least 12 hours; low-molecular-weight heparin [LMWH] for more than 5 days). 3. To accommodate the above protocol modifications which no longer require local measurement of AT level for study entry for all subjects, the study drug dose calculation has been simplified so that it is only based on the subject's weight in order to achieve a potentially therapeutic dose of blinded Antithrombin III (Human) (AT-III [Human]). **Rationale:** The rationale to support these modifications in eligibility criteria is based on previous observational and published studies that report post heart surgery cardiopulmonary bypass decreases in mean AT levels of approximately 30-35% from baseline, which can be as high as 45-50%. These prior studies were almost exclusively performed with low-risk patients undergoing isolated procedures with relatively short bypass runs. Our present study aims to enroll high-risk cardiac surgery patients defined as either undergoing combined/complex surgery in which higher bypass times and AT consumption are expected or patients undergoing less complex procedures who present with intrinsically low baseline AT levels or have been treated with preoperative heparin and have a high-risk of arriving to the ICU with exceptionally low AT levels. Therefore, patients who will present baseline AT levels ≥80% may still be at risk of reaching the ICU with low AT levels, which could put them at risk for adverse clinical outcomes. Therefore, for complex/combined cardiac procedures (Inclusion #4) this amendment removes the requirement for AT level of less than 80%. However, for isolated CABG or single valve repair/replacements in order to qualify for entry, patients will be required to either have an AT level less than 80% or have received preoperative heparin (delineated above). Detailed changes are described below including modification in study drug dose calculation which is now dependent solely on subject's weight and targeted to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels.		
Protocol Synopsis	**Study Centers Planned** ~~20~~	**Study Centers Planned** 35	To facilitate subject enrollment.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
Protocol Synopsis 3.1 Study Design and Plan	**Overall Study Description** This study will be a prospective, multicenter, randomized, double-blind, placebo-controlled study. Participating subjects will be 404 adult subjects undergoing cardiac surgery with CPB who will be randomized into 2 treatment groups preoperatively in a 1:1 ratio: AT-III (Human) treatment group and Placebo control group. ~~S~~subjects will be ~~prescreened for AT activity levels (AT <80%) and~~ stratified by their previous history of myocardial infarction (MI). Subjects randomized to AT-III (Human) treatment group will receive preoperative supplementation with AT-III (Human) designed to achieve ~~a 100%~~ AT level. Subjects randomized to Placebo control group will receive 0.9% Sodium Chloride (NaCl) for Injection, USP (United States Pharmacopeia).	**Overall Study Description** This study will be a prospective, multicenter, randomized, double-blind, placebo-controlled study. Participating subjects will be 404 adult subjects undergoing high-risk cardiac surgery with CPB who will be randomized into 2 treatment groups preoperatively in a 1:1 ratio: AT-III (Human) treatment group and Placebo control group. All subjects will be stratified by their previous history of myocardial infarction (MI). Subjects undergoing complex/combined procedures will not be prescreened or screened for AT levels using local lab values to assess eligibility. Only subjects undergoing isolated coronary artery bypass graft (CABG) or single valve repair/replacements and not receiving preoperative heparin may be prescreened locally for AT levels (AT <80%). Subjects randomized to AT-III (Human) treatment group will receive preoperative supplementation with AT-III (Human) designed to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels. Subjects randomized to Placebo control group will receive 0.9% Sodium Chloride (NaCl) for Injection, USP (United States Pharmacopeia).	See the rationale for the global changes.
Protocol Synopsis 3.2.1 Inclusion Criteria	**Diagnosis and Main Eligibility Criteria:** A subject must meet all the following inclusion criteria to be eligible for participation in this study: Inclusion Criteria: 4. Types of cardiac operations permitted: complex/combined procedures (~~coronary artery bypass graft [~~CABG+valve~~]~~), double/triple valve repair/replacement, ascending aorta/aortic arch surgeries. Isolated CABG or single valve repair/replacements are allowed only if ~~subject has~~	**Diagnosis and Main Eligibility Criteria:** A subject must meet all the following inclusion criteria to be eligible for participation in this study: Inclusion Criteria: 4. Types of cardiac operations permitted: - Complex/combined procedures (CABG+valve), double/triple valve repair/replacement, ascending aorta/aortic arch surgeries (without baseline AT level restriction or preoperative heparin requirement). OR - Isolated CABG or single valve repair/replacements are allowed only if either (a) AT level is less than 80% OR	See the rationale for the global changes.

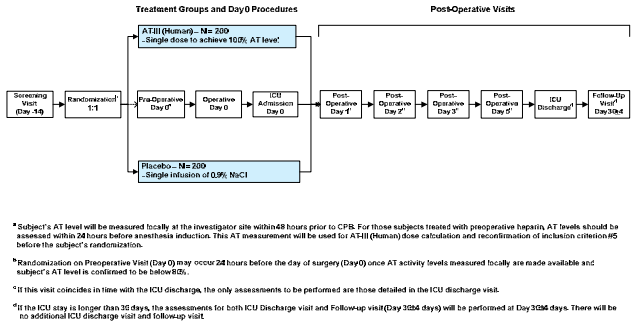
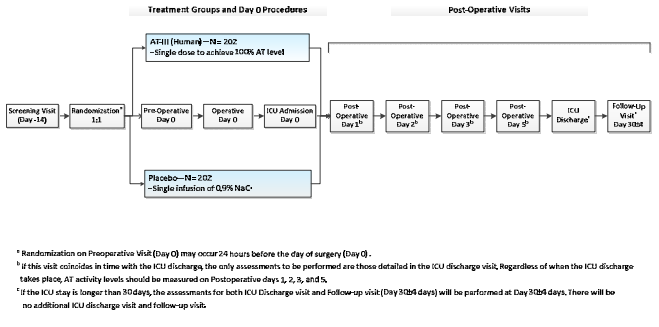
Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
	~~received preoperative heparin >2 days~~. 5. ~~Subject has a Prescreening/Screening and baseline local lab AT level of less than 80%.~~	(b) preoperative heparin is received (unfractionated heparin [UFH] for at least 12 hours; low-molecular-weight heparin [LMWH] for more than 5 days). 5. Not applicable – intentionally left blank for data management purposes (consistency in eCRF capture of eligibility criteria historically)	
Protocol Synopsis 3.3.2.1 Study Drug Doses	**Investigational Product, Dose, and Mode of Administration**: Every subject randomized to the AT-III (Human) treatment group will receive, immediately after anesthesia induction, a single dose of AT-III (Human) sufficient to achieve an AT level of ~~100%~~ according the following formula: ~~$AT-III\ dose\,(IU)\ required = \frac{(100 - actual\ AT\ level\ in\ subject) \times (subject\ weight\ in\ kg)}{1.4}$~~	**Investigational Product, Dose, and Mode of Administration**: Every subject randomized to the AT-III (Human) treatment group will receive, immediately after anesthesia induction, a single dose of AT-III (Human) sufficient to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels according to the following formula: $AT-III\ dose\,(IU)\ required = \frac{(20) \times (subject\ weight\ in\ kg)}{1.4}$ The only variable in the equation is the subject's body weight. For calculating AT-III (Human) dose, the subject's weight obtained during the Screening visit will be used.	Since the aim is to increase plasma AT levels by an absolute amount of 20% (percentage points) above the pretreatment value for all subjects, the equation for dose calculation does not need to include the subject's specific preoperative AT level because Target AT level – preoperative AT level is set at an absolute value of 20% (percentage points). Therefore the dose calculation equation in the original protocol and previous amendments is simplified by replacing the first parenthetical term by a value of 20.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
Protocol Synopsis 3.6.2.1 Prescreening Visit: (within Two Weeks Prior to Surgery)*	**Study Procedures:** The following procedures will be performed during the study: Prescreening Visit: (within Two Weeks Prior to Surgery)* * Must be performed prior to Screening visit, unless a subject's surgery is scheduled to occur in ≤ ~~24~~ hours. In the latter case, the Prescreening and Screening visit may be combined ~~and the only assessments to be performed are those detailed for the Screening visit~~. All determinations for a separate Prescreening visit must be recorded on specific Prescreening sheets.	**Study Procedures:** The following procedures will be performed during the study: Prescreening Visit: (within Two Weeks Prior to Surgery)* * Only applicable for subjects undergoing isolated CABG or single valve repair/replacements who will not be receiving preoperative heparin (see entry criteria) and whose eligibility will therefore be based on AT level ≤80%. If it is logistically feasible, Prescreening visit must be performed prior to Screening visit, unless a subject's surgery is scheduled to occur in ≤72 hours. In the latter case, the Prescreening and Screening visit may be combined. All determinations for a separate Prescreening visit must be recorded on specific Prescreening sheets. Note: The Prescreening visit is not required for subjects undergoing complex/combined procedures (CABG+valve, double/triple valve repair/replacements, ascending aorta/aortic arch surgeries).	See the rationale for the global changes.
Protocol Synopsis 3.6.2.2 Screening Visit: (within Two Weeks Prior to Surgery)*	**Study Procedures:** Screening Visit: (within Two Weeks Prior to Surgery)* • Medical case history and demographic data (age, gender, race, weight, height, body mass index [BMI], smoking habits, ejection fraction, previous MI, unstable angina, arrhythmia, cardiogenic shock, congestive heart failure, pre-existing renal failure or dialysis treatment, chronic obstructive pulmonary disease [COPD], previous stroke, percutaneous coronary interventions, previous pulmonary thromboembolism, previous IABP, diabetes mellitus requiring treatment, oral anticoagulation therapy, heparin therapy, peripheral vascular disease or systemic thromboembolism, hypertension, hypercholesterolemia, immunosuppressive treatment).	**Study Procedures:** Screening Visit: (within Two Weeks Prior to Surgery)* • All clinically relevant medical case history and demographic data (age, gender, race, weight, height, body mass index [BMI], smoking habits, ejection fraction, previous MI, unstable angina, arrhythmia, cardiogenic shock, congestive heart failure, pre-existing renal failure or dialysis treatment, chronic obstructive pulmonary disease [COPD], previous stroke, percutaneous coronary interventions, previous pulmonary thromboembolism, previous IABP, diabetes mellitus requiring treatment, oral anticoagulation therapy, heparin therapy, peripheral vascular disease or systemic thromboembolism, hypertension, hypercholesterolemia, immunosuppressive	See the rationale for the global changes.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
		treatment). Please include any relevant medical conditions as well as medical disorders requiring medication or that are currently active.	
	• AT activity levels (local lab)	• AT activity levels (local lab) (Only for subjects undergoing isolated CABG or single valve repair/replacements not receiving preoperative heparin [see entry criteria]).	
	* The Screening visit may occur on the same day of the Prescreening visit. ~~In this case, AT activity levels performed at the local lab will be assessed only once.~~ If a subject's surgery is scheduled to occur in ≤ ~~24~~ hours, the Prescreening and Screening visits may be combined~~. In this case, the only assessments to be performed are those detailed for the Screening visit.~~	* The Screening visit may occur on the same day of the Prescreening visit. If a subject's surgery is scheduled to occur in ≤72 hours, the Prescreening and Screening visits may be combined.	
Protocol Synopsis 3.6.2.3 Preoperative Visit (Before Anesthesia Induction): Day 0§	**Study Procedures:** Preoperative Visit (Before Anesthesia Induction): Day 0§ ~~• AT activity levels (local lab)*~~ • AT activity levels (central lab) • Randomization† • Confirmation of existing, and recording of any new events, or changes, in the medical and surgical history of the subject since the Screening visit. ~~The combined Screening and Preoperative visit requires documentation of the medical and surgical history for the last 12 months.~~ • Confirmation of existing and recording of any new medications, or changes in medications administered to the subject since the Screening visit. The combined Screening and Preoperative visit requires documentation of the lifetime history ~~for the use of topical hemostats and~~ bleeding abnormalities. In addition it requires documentation of medications that the subject is taking or has taken within the last month. ~~*Subject's AT level will be measured locally at the~~	**Study Procedures:** Preoperative Visit (Before Anesthesia Induction): Day 0§ • AT activity levels (central lab) • Randomization† • Confirmation of existing, and recording of any new events, or changes, in the medical and surgical history of the subject since the Screening visit. • Confirmation of existing and recording of any new medications, or changes in medications administered to the subject since the Screening visit. The combined Screening and Preoperative visit requires documentation of the lifetime history of bleeding abnormalities. In addition it requires documentation of medications that the subject is taking or has taken within the last month. § The Preoperative visit (Before Anesthesia induction: Day	See the rationale for the global changes.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
	~~investigator site within 48 hours prior to the CPB. For those subjects treated with preoperative heparin, AT levels should be assessed within 24 hours before anesthesia induction. This AT determination will be used for AT dose calculation (Section 3.3.2.1) and reconfirmation of inclusion criteria # 5 before the subject's randomization.~~ † Randomization on Preoperative visit (Day 0) may occur 24 hours before the day of surgery (Day 0) once~~ AT activity levels measured locally are made available and subject's AT level is confirmed to be below 80%.~~ § The Preoperative visit (Before Anesthesia induction: Day 0) may occur on the same day of the Screening visit. In this case, the only assessments to be performed are those detailed for the Screening visit AND the Randomization procedures detailed in Section 3.3.2.	0) may occur on the same day of the Screening visit <u>if the latter is to be performed within 48 hours of the surgery.</u> In this case, the only assessments to be performed are those detailed for the Screening visit AND the Randomization procedures detailed in Section 3.3.2. † Randomization on Preoperative visit (Day 0) may occur 24 hours before the day of surgery (Day 0) once <u>all eligibility criteria are confirmed.</u>	

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
Protocol Synopsis 3.5.1 Efficacy Variables	**Clinical Outcome Measures:** Efficacy Variables 2. **AT activity (levels)** determinations: a. AT activity levels will be measured for both AT-III (Human) treatment and Placebo control groups at the following time points: ◦ Prescreening visit (local lab) ◦ Screening visit (local lab) ◦ Screening visit ~~(central lab)~~ ◦ ~~Preoperatively (local lab)*~~ ◦ Preoperatively ~~(central lab)~~ ◦ Immediately after study drug administration ~~*Subject's AT level will be measured locally at the investigator site within 48 hours prior to the CPB. For those subjects treated with preoperative heparin, AT levels should be assessed within 24 hours before anesthesia induction. This AT determination will be used for AT dose calculation (Section 3.3.2.1) and reconfirmation of inclusion criteria # 5 before randomization.~~	**Clinical Outcome Measures:** Efficacy Variables 2. **AT activity (levels)** determinations: a. AT activity levels will be measured for both AT-III (Human) treatment and Placebo control groups at a central lab (unless specified at local lab) for the following time points: ◦ Prescreening visit (local lab)* ◦ Screening visit (local lab)*§ ◦ Screening visit ◦ Preoperatively ◦ Immediately before study drug administration ◦ Immediately after study drug administration * Only applicable for subjects undergoing isolated CABG or single valve repair/replacements (will be needed for eligibility only if subject will not be receiving preoperative heparin [see entry criteria]). § This AT (local lab) determination will not be required if the Prescreening visit is conducted.	See the rationale for the global changes.
1.1 Background	…Moreover, given that this study was performed with less complex procedures such as isolated CABG or single valve, compared to more complex/combined procedures such as double/triple valve repair/replacement, ascending aorta/aortic arch surgeries, with normal baseline AT values, many subjects in the control group reached the ICU with AT levels within the normal physiological range, suggesting that an AT supplementation may of more benefit in a higher risk patient population with lower baseline AT levels.	…Moreover, given that this study was performed with less complex procedures such as isolated CABG or single valve, compared to more complex/combined procedures such as double/triple valve repair/replacement, ascending aorta/aortic arch surgeries, with normal baseline AT values, many subjects in the control group reached the ICU with AT levels within the normal physiological range, suggesting that an AT supplementation may be of more benefit in a higher risk patient population (undergoing complex/combined cardiac surgeries with long bypass runs and/or high inflammatory response) prone to consume large amounts of AT or with lower baseline AT levels.	See the rationale for the global changes.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
Figure 3-1	 [a] Subject's AT level will be measured locally at the investigator site within 48 hours prior to CPB. For those subjects treated with preoperative heparin, AT levels should be assessed within 24 hours before anesthesia induction. This AT measurement will be used for ATIII (Human) dose calculation and reconfirmation of inclusion criterion #5 before the subject's randomization. [b] Randomization on Preoperative Visit (Day 0) may occur 24 hours before the day of surgery (Day 0) once AT activity levels measured locally are made available and subject's AT level is confirmed to be below 80%. [c] If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed in the ICU discharge visit. [d] If the ICU stay is longer than 30 days, the assessments for both ICU Discharge visit and Follow-up visit (Day 30±4 days) will be performed at Day 30±4 days. There will be no additional ICU discharge visit and follow-up visit.	 * Randomization on Preoperative Visit (Day 0) may occur 24 hours before the day of surgery (Day 0). [b] If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed in the ICU discharge visit. Regardless of when the ICU discharge takes place, AT activity levels should be measured on Postoperative days 1, 2, 3, and 5. [c] If the ICU stay is longer than 30 days, the assessments for both ICU Discharge visit and Follow-up visit (Day 30±4 days) will be performed at Day 30±4 days. There will be no additional ICU discharge visit and follow-up visit.	The figure is revised to reflect the relevant changes.
3.2 Selection of Study Population	Subjects undergoing high risk, non-emergency cardiac surgery with CPB will be studied. Subjects ~~will~~ be prescreened ~~according to baseline levels of~~ AT activity measured locally which should be below 80% ~~under all circumstances~~. Four hundred and four (404) subjects will be enrolled in this study.	Subjects undergoing high-risk, non-emergency cardiac surgery with CPB will be studied. Subjects undergoing isolated CABG or single valve repair/replacements may be prescreened for AT activity measured locally which should be below 80% unless treated with preoperative heparin (details below). Four hundred and four (404) subjects will be enrolled in this study.	See the rationale for the global changes.
3.3.2.2 Rationale for Selection of Doses in the Study	…These AT levels were associated with an overall worse postoperative outcome. To build on this study, a ~~preoperative AT target of 100%~~ and inclusion of more severe subjects are intended to achieve better efficacy and safety.	… These AT levels were associated with an overall worse postoperative outcome. To build on this study, a more conservative dosing scheme and the inclusion of more severe subjects (i.e., undergoing complex/combined cardiac surgeries or subjects undergoing isolated procedures with low preoperative AT levels or treated with heparin preoperatively) are intended to achieve better efficacy and safety.	See the rationale for the global changes.
3.6.3.2 Clinical Laboratory Tests	Laboratory panels include blood coagulation parameters and serum clinical chemistry as described below. Blood samples will be drawn at the following time points: • ~~Prescreening visit (local lab)~~ • Screening visit (local lab) • Screening visit (central lab) • Preoperatively ~~(local lab)*~~ Clinical chemistry panel: Creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride,	Laboratory panels include blood coagulation parameters and serum clinical chemistry as described below. Blood samples will be drawn at the following time points: • Screening visit (local lab) • Screening visit (central lab) • Preoperatively Clinical chemistry panel: Creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride,	See the rationale for the global changes.

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
	and calcium Blood samples will be drawn at the following time points: ~~• Prescreening visit (local lab)~~ • Screening visit (local lab) • Screening visit (central lab) • Preoperatively ~~(local lab)*~~ ~~* Subject's AT level will be measured locally at the investigator site within 48 hours prior to the CPB. For those subjects treated with preoperative heparin, AT levels should be assessed within 24 hours before anesthesia induction. This AT determination will be used for AT dose calculation (Section 3.3.2.1) and reconfirmation of inclusion criteria # 5 before randomization~~ The above listed laboratory panels will be performed by a central laboratory unless otherwise specified. Investigative site laboratories may also be used in some instances (e.g. in the case of Screening and Preoperative visit tests to determine subject's eligibility when testing results may be needed in a short timeframe). The Investigator will be required to classify laboratory results out of the normal range reported by the laboratory as clinically relevant or not according to his/her criteria. Results will be recorded in source documents and on the subject eCRF.	and calcium Blood samples will be drawn at the following time points: • Screening visit (local lab) • Screening visit (central lab) • Preoperatively The above listed laboratory panels will be performed by a central laboratory unless otherwise specified. Investigative site laboratories may also be used in some instances (e.g. in the case of Screening and Preoperative visit tests to determine subject's eligibility when testing results may be needed in a short timeframe). The Investigator will be required to classify laboratory results out of the normal range reported by the laboratory as clinically relevant or not according to his/her criteria. Results will be recorded in source documents and on the subject eCRF <u>when applicable</u>.	

Sections	Change From (deletion – strikethrough):	Change To (new text – underline):	Rationale:
3.7 Screen Failures	Prescreening and screening evaluations will be used to determine the eligibility of each subject for enrollment. Subjects found to have a baseline AT activity level ≥80% (local lab) at the Prescreening visit will not continue any further subject eligibility evaluations and will not be considered as screening failures. Subjects who fail to meet eligibility criteria during screening evaluations (during Screening visit) will be considered screen failures and will not be randomized and participate in the study.	Prescreening and screening evaluations will be used to determine the eligibility of each subject for enrollment. Subjects undergoing isolated CABG or single valve repair/replacements and not receiving preoperative heparin found to have a baseline AT activity level ≥80% (local lab) at the Prescreening visit will not continue any further subject eligibility evaluations and will not be considered as screening failures. If a subject undergoing isolated CABG or single valve repair/replacements will receive preoperative heparin (see entry criteria), all Screening assessments will be performed because the subject may meet eligibility criteria without AT level as prerequisite. Subjects who fail to meet eligibility criteria during screening evaluations (during Screening visit) will be considered screen failures and will not be randomized and participate in the study.	See the rationale for the global changes.
Appendix 1 Study Flow Chart and/or Schedule of Procedures		Study Flow Chart and/or Schedule of Procedures are revised to include all relevant changes described above.	See the rationale for the global changes.

Investigator Signature Page

Protocol Title:

A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study To Evaluate The Safety And Efficacy Of Preoperative Antithrombin Supplementation In Patients Undergoing High-Risk Cardiac Surgery With Cardiopulmonary Bypass

Protocol Number: GTI1307

Version Number: 4.0

Version Date: 02 Apr 2015

The undersigned confirms that he/she agrees to conduct the study under the conditions described in this protocol and comply with International Conference on Harmonization Good Clinical Practice (ICH GCP) and all applicable regulatory requirements:

_____	_____
INVESTIGATOR NAME (Please Print)	LOCATION
_____	_____
INVESTIGATOR SIGNATURE	DATE

PROTOCOL SYNOPSIS

Title of Study:

A Prospective, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Preoperative Antithrombin Supplementation in Patients Undergoing High-Risk Cardiac Surgery with Cardiopulmonary Bypass

Sponsor:

Grifols Therapeutics Inc.

Study Number:

GTI1307

Phase:

2b

Number of Subjects Planned:

404

Study Centers Planned:

35

Target Population:

Patients that undergo high-risk cardiac surgery with cardiopulmonary bypass (CPB)

Duration of Treatment:

Subject participation (since enrollment): Approximately 30 to 48 days (7 weeks).

Study Objectives:

Primary Efficacy Objective:

The primary objective of this clinical study is to compare the percentage of subjects with any component of a major morbidity composite in two treatment groups randomly allocated to receive preoperative supplementation of AT-III (Human) or Placebo.

Secondary Efficacy Objectives:

1. To compare postoperative antithrombin III (AT) levels at the Intensive Care Unit (ICU) admission between the AT-III (Human) treatment group and Placebo control group
2. To compare the following postoperative outcomes between the AT-III (Human) treatment group and Placebo control group:
   - Postoperative chest-drain blood loss in the first 12 and 24 hours after surgery
   - Transfusion requirements
   - Need for surgical reexploration

- Low cardiac output syndrome
- Myocardial infarction
- Stroke
- Acute kidney injury
- Arterial or venous thromboembolic events
- Infections
- Prolonged mechanical ventilation (>24 hours)
- All-cause postoperative mortality
- ICU stay duration
- Prolonged ICU stay (>6 days)
- Length of hospital stay

Safety Objectives:

Safety objectives include evaluation of AT-III (Human) for clinical safety including AEs and risks for bleeding, clinical laboratory testing, physical exam, and vital signs.

Overall Study Description:

This study will be a prospective, multicenter, randomized, double-blind, placebo-controlled study. Participating subjects will be 404 adult subjects undergoing high-risk cardiac surgery with CPB who will be randomized into 2 treatment groups preoperatively in a 1:1 ratio: AT-III (Human) treatment group and Placebo control group. All subjects will be stratified by their previous history of myocardial infarction (MI). Subjects undergoing complex/combined procedures will not be prescreened or screened for AT levels (local lab) to assess eligibility. Only subjects undergoing isolated coronary artery bypass graft (CABG) or single valve repair/replacements and not receiving preoperative heparin may be prescreened locally for AT levels (AT <80%).

Subjects randomized to AT-III (Human) treatment group will receive preoperative supplementation with AT-III (Human) designed to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels. Subjects randomized to Placebo control group will receive 0.9% Sodium Chloride (NaCl) for Injection, USP (United States Pharmacopeia).

The maximum study duration for a given subject will be approximately 7 weeks (48 days). There will be 2 weeks for prescreening, screening, and randomization into one of the 2 study groups (AT-III [Human] treatment or Placebo control). After the 2-week prescreening and screening period, the cardiac surgery procedure will take place with the preoperative administration of AT-III (Human) or Placebo supplementation. After surgery, the subject will be admitted to the ICU and be followed for approximately 1 month (30±4 days).

Both the primary efficacy endpoint (percentage of subjects with any component of the major morbidity composite) and the secondary endpoints will be evaluated for each subject after

each cardiac surgery procedure, at the ICU admission, ICU discharge, and during a follow-up period of approximately 1 month (30±4 days) (see protocol section 3.5.1).

Diagnosis and Main Eligibility Criteria:

A subject must meet all the following inclusion criteria to be eligible for participation in this study:

Inclusion Criteria:

1. Male or female.
2. At least 18 years of age.
3. Subject needs non-emergency cardiac surgery with cardiopulmonary bypass.
4. Types of cardiac operations permitted:
   - Complex/combined procedures (CABG+valve), double/triple valve repair/replacements, ascending aorta/aortic arch surgeries (without baseline AT level restriction or preoperative heparin requirement). OR
   - Isolated CABG or single valve repair/replacements are allowed only if either (a) AT level is less than 80% OR (b) preoperative heparin is received (unfractionated heparin [UFH] for at least 12 hours; low-molecular-weight heparin [LMWH] for more than 5 days).
5. Not applicable – intentionally left blank for data management purposes (consistency in eCRF capture of eligibility criteria historically).
6. Subject has signed informed consent form.
7. Subject is willing to comply with all aspects of the protocol, including blood sampling, for the total duration of the study.

Exclusion Criteria:

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study. All exclusion criteria involving hematology/coagulation and clinical chemistry determinations will be based on local lab results.

1. Subject needs emergency surgery.
2. Subject needs heart transplantation.
3. Use of minimally invasive surgery.
4. Previous cardiac operation.
5. Infective endocarditis.
6. Thromboembolic events, stroke, or ST-elevated myocardial infarction within 7 days of surgery.
7. Cardiogenic shock at the time of surgery.
8. Renal dysfunction: Creatinine levels >2 mg/dL or chronic dialysis.
9. Liver dysfunction: Aspartate transaminase (AST), alanine aminotransferase (ALT) increase ≥2-fold above the upper-limit of local lab normal ranges.
10. Treatment with Clopidogrel and Ticagrelor within 5 days before surgery, Prasugrel within 7 days before surgery, glycoprotein IIb/IIIa receptor blockers within 24 hours of surgery.
11. Treatment with new oral anticoagulants (Apixaban, Rivaroxaban, Dabigatran) within 48 hours before surgery.
12. Vitamin K antagonist therapy and an international normalized ratio >1.3 on the day of surgery.
13. Platelet count <120,000/μL.
14. History or suspicion of a congenital or acquired coagulation disorder.
15. History of anaphylactic reaction(s) to blood or blood components.
16. Allergies to excipients in study drug.
17. Refusal to receive allogenic transfusion of blood-derived products.
18. Received AT treatment within the last 3 months prior to Screening visit.
19. Subject is pregnant.
20. Subject has participated in any another investigational study within the last 3 months prior to Screening visit.

Investigational Product, Dose, and Mode of Administration:

This is a randomized, double-blind study.

Antithrombin III (Human): AT-III (Human):

AT-III (Human) is a sterile, nonpyrogenic, stable lyophilized preparation of purified human antithrombin III obtained from human plasma by a continuous purification process.

Each vial will be labeled with the antithrombin potency expressed in international units (IU) as defined by the World Health Organization (WHO).

Every subject randomized to the AT-III (Human) treatment group will receive, immediately after anesthesia induction, a single dose of AT-III (Human) sufficient to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels according to the following formula:

$$AT - III\ dose\,(IU)\ required\ = \frac{(20) \times (subject\ weight\ in\ kg)}{1.4}$$

The only variable in the equation is the subject's body weight. For calculating AT-III (Human) dose, the subject's weight obtained during the Screening visit will be used.

AT-III (Human) will be infused intravenously.

Placebo:

Placebo used in this study will be 0.9% Sodium Chloride (NaCl) for Injection, USP and will be infused intravenously.

Study Procedures:

The following procedures will be performed during the study:

Prescreening Visit: (within Two Weeks Prior to Surgery)*

- Signed informed consent for Prescreening
- AT activity levels (local lab)

* Only applicable for subjects undergoing isolated CABG or single valve repair/replacements who will not be receiving preoperative heparin (see entry criteria) and whose eligibility will therefore be based on AT level <80%. If it is logistically feasible, Prescreening visit must be performed prior to Screening visit, unless a subject's surgery is scheduled to occur in ≤72 hours. In the latter case, the Prescreening and Screening visit may be combined. All determinations for a separate Prescreening visit must be recorded on specific Prescreening sheets.

Note: The Prescreening visit is not required for subjects undergoing complex/combined procedures (CABG+valve, double/triple valve repair/replacements, ascending aorta/aortic arch surgeries).

Screening Visit: (within Two Weeks Prior to Surgery)*

- Signed informed consent
- Allocation of Screening Number
- Inclusion/Exclusion criteria
- Addition of subject's data into Screening Log
- All clinically relevant medical case history and demographic data (age, gender, race, weight, height, body mass index [BMI], smoking habits, ejection fraction, previous MI, unstable angina, arrhythmia, cardiogenic shock, congestive heart failure, pre-existing renal failure or dialysis treatment, chronic obstructive pulmonary disease [COPD], previous stroke, percutaneous coronary interventions, previous pulmonary thromboembolism, previous IABP, diabetes mellitus requiring treatment, oral anticoagulation therapy, heparin therapy, peripheral vascular disease or systemic thromboembolism, hypertension, hypercholesterolemia, immunosuppressive treatment). Please include any relevant medical conditions as well as medical disorders requiring medication or that are currently active.
- Physical examination
- Vital signs (temperature [T], blood pressure [BP], heart rate [HR], respiratory rate [RR])
- AT activity levels (local lab) (Only for subjects undergoing isolated CABG or single valve repair/replacements not receiving preoperative heparin [see entry criteria])
- AT activity levels (central lab)
- Hematology and coagulation (hemoglobin [Hb], hematocrit [Hct], red blood cell [RBC], white blood cell [WBC], platelets [PLT], activated partial thromboplastin time [aPTT], prothrombin time [PT], International Normalized Ratio [INR], fibrinogen, thrombin-antithrombin complex [TAT], Prothrombin fragment 1+2 [F1+2])
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, blood urea nitrogen [BUN], lactate dehydrogenase [LDH], glucose, sodium, potassium, chloride, and calcium)
- Serum pregnancy test (for women of childbearing potential)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).
- Documentation of medications that the subject is taking or has taken within the last month (including transfusions of blood or any blood-derived product)
- Adverse events (AEs)

* The Screening visit may occur on the same day of the Prescreening visit. If a subject's surgery is scheduled to occur in ≤72 hours, the Prescreening and Screening visits may be combined.

Preoperative Visit (Before Anesthesia Induction): Day 0[§]

- Review of inclusion/exclusion criteria to confirm subject eligibility

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels (central lab)
- Randomization†
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).
- Confirmation of existing, and recording of any new events, or changes, in the medical and surgical history of the subject since the Screening visit.
- Confirmation of existing and recording of any new medications, or changes in medications administered to the subject since the Screening visit. The combined Screening and Preoperative visit requires documentation of the lifetime history of bleeding abnormalities. In addition it requires documentation of medications that the subject is taking or has taken within the last month.
- Adverse events

§ The Preoperative visit (Before Anesthesia induction: Day 0) may occur on the same day of the Screening visit if the latter is to be performed within 48 hours of the surgery. In this case, the only assessments to be performed are those detailed for the Screening visit AND the Randomization procedures detailed in Section 3.3.2.

† Randomization on Preoperative visit (Day 0) may occur 24 hours before the day of surgery (Day 0) once all eligibility criteria are confirmed.

<u>Operative Visit (After Anesthesia Induction – Before ICU Admission): Day 0</u>

- Type of surgical procedure
- Duration of CPB and aortic clamp
- Preoperative heparin dose response*
- Dose of heparin (initial, subsequent pre-CPB, during CPB), ACT, and dose of protamine* (* see protocol Section 3.6.2.4 for detail)
- Study drug infusion
- Minimum hematocrit during CBP (local lab)
- Transfusion requirements
- AT activity levels immediately before and after infusion of AT-III (Human) or Placebo
- Concomitant medications
- Adverse events

ICU Admission: Day 0

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Chest-drain blood loss in the first 12 and 24 hours postoperatively
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, and postoperative mortality)
- Concomitant medications
- Adverse events
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).

Postoperative Day 1, Day 2, Day 3, and Day 5

- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Chest-drain blood loss in the first 12 and 24 hours postoperatively
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, and postoperative mortality)
- Concomitant medications
- Adverse events

If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed below in the ICU discharge visit. Regardless of when the ICU discharge takes place, AT activity levels should be measured on Postoperative days 1, 2, 3, and 5.

ICU Discharge Visit (Maximum One Month after Admission)

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Concomitant medications
- Adverse events
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, ICU stay duration, prolonged ICU stay, and postoperative mortality)

Follow-Up Visit (Day 30±4 Days after ICU Admission)*

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).
- Concomitant medications
- Adverse events
- Clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, postoperative mortality, and overall hospital stay duration)

* A phone call follow-up visit will be performed for those subjects unable to return to the investigational site during the scheduled time frame. The occurrence of any AEs since the last study visit and clinical outcomes will be investigated.

Clinical Outcome Measures:

Efficacy Variables

1. Percentage of subjects with any component of a **major morbidity composite** defined as

a composite of any one or more of the following events (up to Day 30±4 days):

a. Postoperative **mortality** (defined as all deaths occurring within 30 days of the operation or occurring during the primary hospitalization)

b. **Stroke** (defined as a clinical diagnosis of focal or global neurological deficit of abrupt onset caused by a disturbance in cerebral blood supply)

c. **Acute kidney injury** (defined as an increase of serum creatinine levels to >2.0 mg/dL and twice the baseline level or a new requirement for dialysis postoperatively)

d. **Surgical reexploration** (defined as having to return to the operating room because of bleeding, tamponade, graft occlusion or any other cardiac reason)

e. **Arterial or venous thromboembolic event** (consisting of perioperative MI, mesenteric infarction, peripheral thromboembolism, acute coronary graft thrombosis, intracardiac thrombosis, deep vein thrombosis [DVT], and pulmonary embolism)

f. **Prolonged mechanical ventilation** (defined as mechanical ventilation >24 hours)

g. **Infection** (defined as presence of deep sternal-wound infection and/or bloodstream infections).

2. **AT activity (levels)** determinations:

a. AT activity levels will be measured for both AT-III (Human) treatment and Placebo control groups at a central lab (unless specified at local lab) for the following time points:

- Prescreening visit (local lab)*
- Screening visit (local lab)* §
- Screening visit
- Preoperatively
- Immediately before study drug administration
- Immediately after study drug administration
- Postoperatively at ICU admission
- Postoperatively Day 1
- Postoperatively Day 2
- Postoperatively Day 3
- Postoperatively Day 5
- ICU discharge
- 1-month follow-up visit

* Only applicable for subjects undergoing isolated CABG or single valve repair/replacements (will be needed for eligibility only if subject will not be receiving preoperative heparin [see entry criteria]).

§ This AT (local lab) determination will not be required if the Prescreening visit is conducted.

b. Percentage of subjects with AT levels of 58% or higher at the ICU admission will be calculated for both treatment groups and compared.

All AT measurements will be performed on a central venous catheter blood sample if the central venous catheter is already in place, through a calibration curve prepared by serial dilution of the human normal plasma pool and expressed as a percentage of activity. If a central venous catheter is not already in place, peripheral blood sample will be used for measuring AT levels.

3. **Chest-drain blood loss** (mL) will be measured within the first 12 and 24 hours of chest closure.

   Chest-drain blood loss in the first 12 and 24 hours is defined as the amount of blood collected in the cardiotomy reservoir from chest closure through the following 12 and 24 hours measured.

4. **Transfusion requirements** will be measured as the number of units of Fresh frozen plasma (FFP), RBCs, PLTs, cryoprecipitate/fibrinogen, prothrombin complex concentrates (PCCs), from the day of ICU admission and throughout the follow-up period.
5. Percentage of subjects needing **surgical reexploration** defined as having to return to the operating room because of bleeding, tamponade, graft occlusion or any other cardiac reason will be considered from chest closure to the end of the follow-up period.
6. Percentage of subjects with **low cardiac syndrome** defined as the need for major inotropic support or intra-aortic balloon pump will be considered from the day of ICU admission and throughout the follow-up period.
7. Percentage of subjects with postoperative **myocardial infarction** defined through enzymatic criteria plus new Q-waves at the electrocardiogram (ECG) will be considered from the day of ICU admission and throughout the follow-up period.
8. Percentage of subjects with postoperative **stroke** defined as a clinical diagnosis of focal or global neurological deficit of abrupt onset caused by a disturbance in cerebral blood supply will be considered from the day of ICU admission and throughout the follow-up period.
9. Percentage of subjects with **acute kidney injury** defined as an increase of serum creatinine levels to >2.0 mg/dL and twice the baseline levels or new requirement for dialysis postoperatively, will be considered from the day of ICU admission and throughout the follow-up period. A new requirement for postoperative dialysis may include hemodialysis, peritoneal dialysis, and any form of ultrafiltration.
10. Percentage of subjects with **arterial or venous thromboembolic events** defined as perioperative MI, stroke, mesenteric infarction, peripheral thromboembolism, acute coronary graft thrombosis, intracardiac thrombosis, DVT, and pulmonary embolism, will be considered from the day of ICU admission and throughout the follow-up period.

11. Percentage of subjects with **infections** defined as presence of deep sternal-wound infections involving muscle, bone or mediastinum or bloodstream infections from the day of ICU admission and throughout the follow-up period.

    The diagnosis of infection must have at least one of the following conditions:

    a. Wound opened with excision of tissue or reexploration of the mediastinum
    b. Positive culture
    c. Treatment with antibiotics

12. Percentage of subjects with **prolonged mechanical ventilation** defined as the need for mechanical ventilation >24 hours from the day of ICU admission and throughout the follow-up period.
13. Percentage of subjects with **all-cause postoperative mortality** defined as death during the same hospitalization as surgery or after discharge and throughout the follow-up period.
14. **ICU stay** duration (days) will be measured from the ICU admission. If duration is greater than the follow-up period, >1 month will be recorded.
15. Percentage of subjects with **prolonged ICU stay** defined as >6 days will be considered from ICU admission during a maximum of 34 days.
16. **Length of hospital stay** (days) in both groups defined as the discharge date minus the surgery date plus 1 day, during a maximum of 34 days after ICU admission. If this time interval is greater than the follow-up period, >1 month will be recorded.

Safety Variables:

- Adverse events, serious adverse events (SAEs), suspected adverse drug reactions (ADRs), and discontinuations due to AEs.
- Risk for bleeding (will be assessed by monitoring Hct levels, urine dipstick tests, and any clinical AEs for bleeding).
- Physical examination and vital signs.
- Clinical laboratory parameters: Hematology (red blood cell count, white blood cell count, hematocrit, hemoglobin, and platelets, etc.) and clinical chemistry (creatinine, total bilirubin, ALT, and AST, etc.).

Statistical and Analytical Methods:

Study Populations:

- Intention-to-Treat population (ITT) will include all subjects who are randomized.
- Per-protocol (PP) population will include all ITT subjects who have no major protocol deviations.

- Safety population will include all subjects who receive any amount of study drug.

Demographics:

Demographic and baseline characteristics will be summarized by treatment group using ITT population. For quantitative variables, mean, standard deviation (SD), median, and minimum/maximum will be provided. For qualitative variables, the frequency and percentage will be provided.

Primary Efficacy Analyses:

The primary efficacy variable is the percentage of subjects with any component of the composite of major morbidity. The primary efficacy variable will be analyzed with Cochran Mantel Haenszel test adjusting for history of MI using ITT population. The sensitivity analyses using PP populations will also be provided.

Secondary Efficacy Analyses:

Postoperative AT levels at the ICU admission will be summarized by treatment group. The analysis of covariance (ANCOVA) will be used to compare the treatment difference. ANCOVA model will include the AT level as dependent variable, treatment as fixed effect and baseline AT level as covariate. The similar approach will also be used to analyze the AT levels in other time points.

Postoperative chest-drain blood loss in the first 12 and 24 hours after surgery will be summarized by treatment group and will be analyzed using group t-test.

For transfusion requirements, Number of units of RBC, Number of units of FFP, and Number of units of platelets will be summarized by treatment group and analyzed using group t-test.

The number and percentage of subjects with each following procedure or morbidity will be separately summarized by treatment group and will be analyzed using Chi-square test (or Fisher Exact test as appropriate)

- Need for surgical reexploration
- Low cardiac output syndrome
- Myocardial infarction
- Stroke
- Acute kidney injury
- Arterial or venous thromboembolic events
- Infections
- Prolonged mechanical ventilation (>24 hours)
- All-cause postoperative mortality
- Prolonged ICU stay (>6 days)

Duration of ICU stay and the length of hospital stay will be summarized by treatment group. , Non-parametric will be used to compare the treatment differences and Hodges-Lehmann estimation will be provided.

Safety Analysis

The safety analysis will be based on safety population.

The incidence of AEs, SAEs, suspected ADRs, and AEs by severity will be summarized by treatment, system organ class and preferred term using descriptive statistics. Subjects with deaths, SAEs, and AEs leading to premature discontinuation from the study will be listed.

The Investigator verbatim term and the MedDRA coded term (system organ class and preferred term) will be shown simultaneously on the data listings of all AEs.

Clinical laboratory variables will be summarized by treatment using summary statistics at each time point and on the change from baseline. Shift tables will be provided to summarize values that fall outside the normal ranges.

Vital signs will be summarized by treatment using summary statistics at each time point and on the change from baseline.

Physical exam data will be provided in data listings.

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LIST OF APPENDICES

GLOSSARY AND ABBREVIATIONS

ACT	Activated clotting time
ADR	Adverse drug reaction
AE	Adverse event
AKI	Acute kidney injury
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
aPTT	Activated partial thromboplastin time
AR	Adverse reaction
AST	Aspartate transaminase
AT	Antithrombin III
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
CABG	Coronary artery bypass graft
CK	Creatine phosphokinase
CK-MB	Creatine phosphokinase MB isoenzyme
COPD	Chronic obstructive pulmonary disease
CPB	Cardiopulmonary bypass
CRO	Clinical research organization
CVP	Central venous pressure
DBP	Diastolic blood pressure
dL	Deciliters
DVT	Deep vein thrombosis
ECG	Electrocardiogram
eCRF	electronic case report form
F1+2	Prothrombin fragment 1+2
FFP	Fresh frozen plasma
GCP	Good Clinical Practice
Hb	Hemoglobin
Hct	hematocrit

HR	Heart rate
IABP	Intra-aortic balloon pump
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICU	Intensive care unit
LMWH	Low-molecular-weight heparin
IND	Investigational new drug
INR	International Normalized Ratio
IRB	Institutional Review Board
ITT	Intention to treat population
IU	International unit
IV	Intravenous
IVR	In vivo recovery
L	Liter
LBBB	Left bundle branch block
LMWH	Low-molecular weight heparin
MACE	Major adverse cardiac event
mg	Milligram
MI	Myocardial infarction
mL	Milliliter
mmol	millimole
mmHg	Millimeters of mercury
NaCl	Sodium chloride
NAT	Nucleic acid testing
PF	Prothrombin fragment
PLT	Platelets
PP	Per protocol population
PRN	Pro re nata
PT	Prothrombin time
RBC	Red blood cell

rFVIIa	Recombinant activated factor VII
RR	Respiratory rate
SAE	Serious adverse event
SBP	Systolic blood pressure
SD	Standard deviation
SERPIN	SERine Protease Inhibitor
STS-SCA	Society of Thoracic Surgeons and Society of Cardiovascular Anesthesiologists
SWFI	Sterile water for injection
T	Temperature
TAT	Thrombin-antithrombin complex
TRALI	Transfusion associated acute lung injury
UFH	Unfractionated heparin
USP	United States Pharmacopeia
USA	United States of America
WBC	White blood cell
WHO	World Health Organization

1 INTRODUCTION

In addition to the information provided below, please also refer to the Investigator's Brochure (IB) and any additional data supplied by the sponsor.

1.1 Background

Cardiac surgery interventions with cardiopulmonary bypass (CPB) lead to a systemic activation of the hemostatic and inflammatory systems compromising the equilibrium of pro- and anticoagulant factors. Excessive hemostatic activation due to exposure of blood to non-endothelial extracorporeal surfaces, re-transfusion of shed pericardial blood, enhanced fibrinolysis, and hemodilution often leads to a profound coagulopathological state predisposing patients to suffer both hemorrhagic and thrombotic complications during the perioperative and postoperative periods.

Despite the administration of large amounts of heparin and antifibrinolytics, systemic thrombin generation is still prominent throughout and after CPB. (1, 2, 3) Bursts of thrombin and fibrin formation are observed immediately after CPB initiation and after reperfusion of the heart and lungs. (4) Moreover, continuous thrombin generation can be detected several days after surgery. Overall, these findings have been postulated to be related with the occurrence of postoperative ischemic events such as myocardial infarctions, graft occlusions, ischemic stroke, and venous thromboembolism in addition to the procedure itself. (1) Likewise, microvascular activation triggered by inflammatory mediators has also been suggested to contribute to organ function deterioration in the immediate postoperative period due to microvascular thrombosis. (5, 6)

Antithrombin (AT), an alpha 2-glycoprotein with a molecular weight of 58,000, is the major plasma inhibitor of thrombin. AT is produced by hepatocytes and belongs to the SERine Protease Inhibitor (SERPIN) superfamily and is composed by 432 amino acids. (7, 8) Inactivation of thrombin occurs by the formation of a covalent bond resulting in an inactive 1:1 stoichiometric complex making the thrombin active site inaccessible to its usual substrates. This bond involves an active serine of thrombin and an arginine reactive site on AT. (9) In the absence of heparin, complex formation occurs at a relatively slow rate. However, when heparin or heparin sulfate on the endothelial wall is present, it binds to AT and dramatically accelerates the rate of complex formation. (10, 11) In addition to thrombin inhibition, AT is also capable of inactivating several other key coagulation proteases including factors IXa, Xa, XIa, and XIIa, and plasmin as well as displaying an array of anti-inflammatory effects. (12, 13, 14, 15)

During cardiac operations with CPB the endogenous anticoagulant AT is diluted and extensively consumed, therefore AT activity at admission to the intensive care unit (ICU) has been reported to be significantly decreased. (16, 17, 18, 19, 20) Normal plasma AT concentrations range between 12.5-15 mg/dL, which translates to a normal range of the functional AT assay between 80% and 120%. (21) After cardiac surgery with CPB a decrease of AT activity of 40-60% is common and absolute values of AT activity at admission to the

ICU in the range of 50% are frequently encountered. (22) Such low AT levels are similar to those observed in patients with congenital AT deficiency in which spontaneous venous thromboembolic events are described. (23, 24) Moreover, a consistent rate of coronary y patients reach the operation theatre with sub-normal AT values (70%±15%, $p<0.05$) due to the preoperative use of unfractionated or low-molecular weight heparin (LMWH), advanced age or having suffered a recent myocardial infarction. (25, 26, 27, 28) Patients presenting ng with low preoperative AT levels often demonstrate reduced heparin responsiveness, sometimes leading to a heparin resistance pattern, defined as the inability to achieve a target clotting time result after a standard dose of unfractionated heparin. (25, 29, 30, 31, 32, 33)

The safety and efficacy of the administration of purified AT concentrates for the management of heparin resistance has been consistently demonstrated in both small studies and large phase III trials and is currently recommended by the Society of Thoracic Surgeons and Society of Cardiovascular Anesthesiologists (STS-SCA) Guidelines on Blood Conservation Clinical Practice over the use of fresh frozen plasma. (34, 35, 36) The rationale behind this preference is based on the safer profile of AT concentrates with respect to infectious disease transmission, avoidance of hemodilution and transfusion-related complications such as volume overload and transfusion-related acute lung injury (TRALI). (37)

The association of low AT levels and morbidity has been established in inherited AT deficiency and in certain situations of acquired AT deficiency such as sepsis. These observations triggered the hypothesis that lowered postoperative AT levels could influence the occurrence of postoperative complications after CPB. To this end, two large observational studies showed that patients with postoperative AT levels below 60% had greater risk of several negative outcomes during the ICU and postoperative period. (38, 39) Specifically, the retrospective observational study by Ranucci et al. demonstrated that low AT levels at the arrival to the ICU after cardiac operations was independently associated with prolonged ICU stay, surgical reexploration, adverse neurologic outcome, and thromboembolic events. Moreover, ICU-AT activity levels <58% was shown to be predictive for prolonged ICU stay (>5 days) with a sensitivity of 67% and a specificity of 83%. In this study, supplementary exogenous AT was not provided to any subjects to evaluate if the use of ICU-AT levels is a predictable marker for poor postoperative outcomes. (38) These findings were later confirmed by Paparella et al. which found that ICU-AT levels below 63.7% was independently associated with prolonged mechanical ventilation, need of inotropic support, postoperative bleeding, and blood product transfusion. (39) However, another observational study failed to find a clear temporal correlation between baseline AT activity levels and the occurrence of postoperative major adverse cardiac events (MACE). (28)

In the light of these observations, a phase II randomized-controlled study was carried out to investigate the effects of preoperative AT supplementation in maintaining postoperative AT levels within normal physiologic range, avoidance of heparin resistance, and limiting postoperative morbidity. (40) Patients in the treatment group were supplemented with a single dose of a plasma-derived AT concentrate immediately after anesthesia induction aimed to achieve a target activity level of 120% whereas control patients were given the

same standard of care without the administration of AT. Preoperative supplementation of AT yielded significantly higher AT activity levels compared to untreated patients not only at the ICU admission, but throughout the entire postoperative stay until ICU discharge. Moreover, 25 patients in the control group (26%) presented postoperative AT activity levels below 58% whereas no patients in the treatment group (0%) reached the ICU with AT levels below this threshold. In addition, patients in the AT arm had a significantly lower rate of heparin resistance and a trend towards fewer thromboembolic events. However, a higher postoperative bleeding rate (8 mL/hour) was observed in the treatment group, but was judged by the investigators not to be clinically significant. Conversely, due to the lack of statistical power, differences in postoperative outcomes were unattainable between study groups.

Post-hoc analysis of the abovementioned study confirmed previous observations that low postoperative AT levels (<58%) are a consistent predictor of prolonged ICU stay (>7 days) in untreated patients. Further, patients presenting ICU-AT levels below this threshold suffered overall worse postoperative outcome than patients that reached the ICU with AT levels above 58% regardless of their treatment suggesting that prophylactic measures to avoid such low AT levels after CPB are warranted. On the other hand, a similar pattern was observed in patients reaching the ICU with unexpectedly high AT activity levels. The latter were more prone to suffer bleeding-related postoperative complications suggesting that AT overcorrection should also be avoided and that a preoperative AT target of 120% may be excessive. Moreover, given that this study was performed with less complex procedures such as isolated CABG or single valve, compared to more complex/combined procedures such as double/triple valve repair/replacement, ascending aorta/aortic arch surgeries, with normal baseline AT values, many subjects in the control group reached the ICU with AT levels within the normal physiological range, suggesting that an AT supplementation may be of more benefit in a higher risk patient population (undergoing complex/combined cardiac surgeries with long bypass runs and/or high inflammatory response) prone to consume large amounts of AT or with lower baseline AT levels.

Overall these findings still do not clarify if a pharmacological supplementation of AT aimed to avoid excessively low and excessively high postoperative AT levels is an effective therapeutic strategy for the prevention of negative postoperative outcomes in patients undergoing cardiac surgery with cardiopulmonary bypass.

1.2 Rationale for Conducting the Study

Cardiac surgery with CPB causes a massive consumptive coagulopathy and thrombotic complications in a similar way that disseminated intravascular coagulation does. Therefore, suppressing excessive hemostatic activation is a plausible therapeutic strategy that may impact postoperative morbidity.

Several studies have consistently reported the association of low postoperative AT levels with poor clinical outcomes (28, 38, 39) and that preoperative AT supplementation is able to o maintain AT levels within a normal range throughout the postoperative period. (40, 41) Conversely, past studies have also highlighted the potential anticoagulant action of purified AT. (31, 34) Likewise, a dose dependent trend towards higher bleeding rates and a trend

towards an increased incidence of postoperative complications (namely bleeding related) in AT treated patients reaching the ICU with exceptionally high AT levels has been reported. (40, 41) The latter observations generate the hypothesis that an AT supplementation strategy aimed to avoid both excessively low (<58%) and excessively high (>100%) postoperative AT levels may represent an effective therapeutic goal to ameliorate the occurrence of bleeding and thrombotic events, respectively. However, these assumptions have been derived from post-hoc analysis and small studies performed with a limited number of patients. (40, 41)

Thus, this clinical study has been designed to evaluate the safety and efficacy of a preoperative treatment with AT supplementation (Antithrombin III [Human]) in subjects who undergo high-risk cardiac surgery with CPB targeting AT levels in such a way to avoid both excessively low and excessively high postoperative AT activity levels and to assess if management of AT levels in this range may decrease negative clinical outcomes during the ICU and hospital stay. The present clinical trial is intended to assess if directed preoperative AT supplementation will show favorable trends in decreasing the incidence of a composite of major morbidity when compared to placebo.

Antithrombin III (Human) with the trade name of THROMBATE III® is marketed in several countries for replacement therapy in subjects with hereditary deficiency of AT during pregnancy or surgery, or if at risk of thromboembolism. For simplicity, Antithrombin (Human) is abbreviated as AT-III (Human) in this protocol. Please refer to AT-III (Human) IB for additional detail.

2 STUDY OBJECTIVES

2.1 Primary Efficacy Objective

The primary objective of this clinical study is to compare the percentage of subjects with any component of a major morbidity composite between 2 groups of subjects randomly allocated to receive preoperative supplementation of AT-III (Human) or Placebo.

2.2 Secondary Efficacy Objectives

The secondary objectives of this clinical study are the following:

1. To compare postoperative AT levels at the ICU admission between the AT-III (Human) treatment group and Placebo control group
2. To compare the following postoperative outcomes between the AT-III (Human) treatment group and Placebo control group:
    - Postoperative chest-drain blood loss in the first 12 and 24 hours after surgery
    - Transfusion requirements
    - Need for surgical reexploration
    - Low cardiac output syndrome

- Myocardial infarction
- Stroke
- Acute kidney injury
- Arterial or venous thromboembolic events
- Infections
- Prolonged mechanical ventilation (>24 hours)
- All-cause postoperative mortality
- ICU stay duration
- Prolonged ICU stay (>6 days)
- Length of hospital stay

2.3 Safety Objectives

Safety objectives include evaluation of AT-III (Human) for clinical safety including adverse events (AEs), risks for bleeding, clinical laboratory testing, physical exam, and vital signs.

3 INVESTIGATIONAL PLAN

3.1 Study Design and Plan

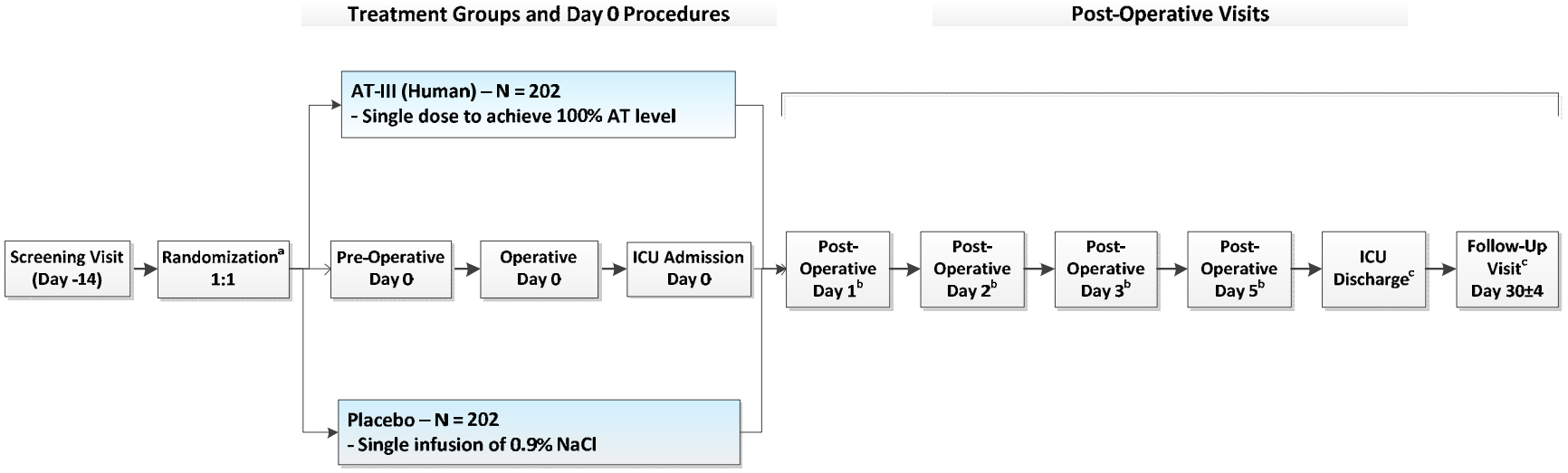
This study will be a prospective, multicenter, randomized, double-blind, placebo-controlled study. Participating subjects will be 404 adult subjects undergoing cardiac surgery with CPB who will be randomized into two treatment groups preoperatively in a 1:1 ratio: AT-III (Human) treatment group and Placebo control group. All subjects will be stratified by their previous history of MI. Subjects undergoing complex/combined procedures will not be prescreened or screened for AT levels using local lab values to assess eligibility. Only subjects undergoing isolated CABG or single valve repair/replacements and not receiving preoperative heparin may be prescreened locally for AT levels (must be AT <80%).

Subjects randomized to AT-III (Human) treatment group will receive preoperative supplementation with AT-III (Human) designed to achieve an absolute increase (Δ) of 20% (percentage points) above pretreatment AT levels. Subjects randomized to Placebo control group will receive 0.9% Sodium Chloride (NaCl) for Injection, USP (United States Pharmacopeia).

The maximum study duration for a given subject will be approximately 7 weeks (48 days). There will be 2 weeks for prescreening and screening, and randomization into one of the two study groups (AT-III [Human] treatment or Placebo control). After the 2-week prescreening and screening period, the cardiac surgery procedure will take place with the preoperative administration of AT-III (Human) or Placebo supplementation. After surgery, the subject will be admitted to the ICU and be followed for approximately 1 month (30±4 days).

Both the primary efficacy endpoint (percentage of subjects with any component of the major morbidity composite) and the secondary endpoints will be evaluated for each subject after each cardiac surgery procedure, at the ICU admission, ICU discharge, and during a follow-up period of approximately 1 month (30±4 days) (see Section 3.5.1).

The overall study schema is shown in Figure 3-1.



[a] Randomization on Preoperative Visit (Day 0) may occur 24 hours before the day of surgery (Day 0).

[b] If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed in the ICU discharge visit. Regardless of when the ICU discharge takes place, AT activity levels should be measured on Postoperative days 1, 2, 3, and 5.

[c] If the ICU stay is longer than 30 days, the assessments for both ICU Discharge visit and Follow-up visit (Day 30±4 days) will be performed at Day 30±4 days. There will be no additional ICU discharge visit and follow-up visit.

Figure 3-1 Overall Study Schema

3.2 Selection of Study Population

Subjects undergoing high-risk, non-emergency cardiac surgery with CPB will be studied. Subjects undergoing isolated CABG or single valve repair/replacements maybe prescreened for AT activity measured locally which should be below 80% unless treated with preoperative heparin (details below). Four hundred and four (404) subjects will be enrolled in this study.

3.2.1 Inclusion Criteria

A subject must meet all the following inclusion criteria to be eligible for participation in this study:

1. Male or female.
2. At least 18 years of age.
3. Subject needs non-emergency cardiac surgery with cardiopulmonary bypass.
4. Types of cardiac operations permitted:
   - Complex/combined procedures (CABG+valve), double/triple valve repair/replacements, ascending aorta/aortic arch surgeries (without baseline AT level restriction or preoperative heparin requirement). OR
   - Isolated CABG or single valve repair/replacements are allowed only if either (a) AT level is less than 80% OR (b) preoperative heparin is received (unfractionated heparin [UFH] for at least 12 hours; low-molecular-weight heparin [LMWH] for more than 5 days).
5. Not applicable – intentionally left blank for data management purposes (consistency in eCRF capture of eligibility criteria historically).
6. Subject has signed informed consent form.
7. Subject is willing to comply with all aspects of the protocol, including blood sampling, for the total duration of the study.

3.2.2 Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study. All exclusion criteria involving hematology/coagulation and clinical chemistry determinations will be based on local lab results.

1. Subject needs emergency surgery.
2. Subject needs heart transplantation.
3. Use of minimally invasive surgery.
4. Previous cardiac operation.
5. Infective endocarditis.

6. Thromboembolic events, stroke, or ST-elevated myocardial infarction within 7 days of surgery.
7. Cardiogenic shock at the time of surgery.
8. Renal dysfunction: Creatinine levels >2 mg/dL or chronic dialysis.
9. Liver dysfunction: AST, ALT increase ≥2-fold above the upper-limit of local lab normal ranges.
10. Treatment with Clopidogrel and Ticagrelor within 5 days before surgery, Prasugrel within 7 days before surgery, glycoprotein IIb/IIIa receptor blockers within 24 hours of surgery.
11. Treatment with new oral anticoagulants (Apixaban, Rivaroxaban, Dabigatran) within 48 hours before surgery.
12. Vitamin K antagonist therapy and an international normalized ratio >1.3 on the day of surgery.
13. Platelet count <120,000/μL.
14. History or suspicion of a congenital or acquired coagulation disorder.
15. History of anaphylactic reaction(s) to blood or blood components.
16. Allergies to excipients in the study drug.
17. Refusal to receive allogenic transfusion of blood-derived products.
18. Received AT treatment within the last 3 months prior to Screening visit.
19. Subject is pregnant.
20. Subject has participated in any another investigational study within the last 3 months prior to Screening visit.

3.3 Treatments

3.3.1 Treatments to Be Administered

3.3.1.1 AT-III (Human)

Detailed information of AT-III (Human) is also available in the IB.

AT-III (Human), a freeze-dried preparation for intravenous (IV) administration, has AT as the active ingredient. It is obtained from human plasma by a continuous purification process, which is performed in an authority-regulated plant in Clayton, North Carolina. Antithrombin is the main inhibitor of blood coagulation.

AT-III (Human) is prepared from pooled units of human plasma from normal donors by modifications and refinements of the cold ethanol method of Cohn. When reconstituted with sterile water for injection (SWFI), USP, AT-III (Human) has a pH of 6.0–7.5, a sodium content of 110–210 mEq/L, a chloride content of 110–210 mEq/L, an alanine content of

0.075–0.125 M, and a heparin content of not more than 0.1 IU heparin/IU ATIII. AT-III (Human) contains no preservative and must be administered by the IV route.

AT-III (Human) preparation will be reconstituted in 10 mL SWFI, which will be transferred into the vial containing the concentrate.

Usually the solution is clear or slightly opalescent. Do not use solutions that are cloudy or have deposits. Infusion of AT-III (Human) should be started within 3 hours of study drug reconstitution. It should be kept at room temperature not to exceed and precautions must be taken to avoid microbiological contamination. It should not be stored in the refrigerator or mixed with other drugs. Leftover product must never be kept for later use.

3.3.1.2 Placebo

Placebo used in this study will be 0.9% Sodium Chloride (NaCl) for Injection, USP.

3.3.1.3 Labeling of Investigational Products

Investigational products will be labeled according to the requirements of local law and legislation. Label text will be approved according to agreed Grifols procedures, and a copy of the labels will be made available to the study site.

Each vial of AT-III (Human) will be labeled with the antithrombin potency expressed in international units (IU) as defined by the World Health Organization (WHO).

3.3.1.4 Storage of Investigational Products

The investigational product should be stored at temperatures not to exceed 25°C (77°F). Do not freeze. The investigational product must be stored in a secure area accessible only to the unblinded pharmacist, or designee, until dispensed for the study subject.

3.3.1.5 Accountability for Investigational Products

Investigational product is to be used only for the study in accordance with the directions given in this protocol. The Investigator, or designee such as the study pharmacist, is responsible for the distribution of the investigational product in accordance with directions given in the protocol and pharmacy manual.

The Investigator is responsible for maintaining accurate records of the investigational product for his/her site. Investigational product inventory/dispensing documentation verifying the receipt, dispensing, destruction, or return must be maintained and kept current by the unblinded pharmacist. The inventory must be made available for inspection by the monitor. Investigational product supplies (including placebo) must be accounted for by the monitor and inventory/dispensing logs must be verified by the monitor prior to Investigational product return or destruction. Written documentation of any used and unused

inventory is required. At the end of the study, a copy of the inventory/dispensing log(s) will be retrieved by the monitor and returned to Grifols Therapeutics Inc.

Any unused investigational product will be accounted for and returned to the Sponsor's distributor. When required by local policies, unused supplies may be destroyed at the study site. The Investigator will ensure that such disposition does not expose subjects to risks from the investigational product. The Investigator and the Sponsor's representative will maintain records of any such alternate disposition to permit accurate drug accountability.

3.3.2 Rationale for Selection of Doses/Timing of Investigational Products in the Study

3.3.2.1 Study Drug Doses

Every subject allocated in the AT-III (Human) treatment group will receive, immediately after anesthesia induction, a single dose of AT-III (Human) sufficient to achieve an absolute increase (Δ) of 20% (percentage points)above pretreatment AT levels according to the formula:

$$AT - III\ dose\ (IU)\ required\ = \frac{(20) \times (subject\ \ weight\ \ in\ kg)}{1.4}$$

The only variable in the equation is the subject's body weight. For calculating AT-III (Human) dose, subject's weight obtained during the Screening visit will be used.

AT-III (Human) or Placebo (0.9% NaCl) will be prepared according to the volume calculated by unblinded pharmacist or designee and will be infused intravenously.

AT-III (Human) will not be administered postoperatively unless the Investigator decides otherwise for clinical reasons (if this is the case, the subject will be excluded from the efficacy analysis).

After the operation, all subjects will be admitted to the ICU and will be followed for approximately 1 month regardless of when the ICU discharge takes place (see Section 3.6.2).

3.3.2.2 Rationale for Selection of Doses in the Study

The goal for dose selection is to avoid both low and high postoperative AT levels in treated subjects. In a previous study using another AT product for the same indication (40), the preoperative AT target of 120% was explored. The results suggested that dosing of subjects with a preoperative AT target of 120% resulted in a large number of AT treated subjects reaching the ICU with high postoperative AT levels (>100%). These AT levels were associated with an overall worse postoperative outcome. To build on this study, a more conservative dosing scheme and the inclusion of more severe subjects (i.e., undergoing complex/combined cardiac surgeries or subjects undergoing isolated procedures with low

preoperative AT levels or treated with heparin preoperatively) are intended to achieve better efficacy and safety.

3.3.3 Method of Assigning Subjects to Treatment Groups

3.3.3.1 Subject Numbering

Within each study site, subjects in the study will receive a consecutive subject number. Subject numbers are generated beginning with the study center number (3 digits, assigned by the sponsor) followed consecutively with a unique number for each subject (4 digits, including leading zeros). For example, if the Investigator's center number is 301, subject number will be 3010001, 3010002, 3010003, etc., in consecutive order. Subject numbers, once assigned, will not be reused at any center.

3.3.3.2 Randomization

Subjects will be randomized into one of two treatment groups: AT-III (Human) treatment group and Placebo control group. Subjects will be stratified by their previous history of myocardial infarction (MI). Unblinded pharmacist or designee will obtain the treatment assignment based on a computer-generated randomization schedule. The randomization number will be assigned at Preoperative visit (Day 0) after all enrollment criteria are met.

3.3.3.3 Blinding

To minimize the risk of unblinding, all subjects will receive the same total volume for all treatments with no visible differences in the external appearance of treatments, infusion bags will be covered with a non-transparent sleeve, and opaque IV tubing will be utilized.

The unblinded study pharmacist, or designee, will prepare investigational product (i.e., AT-III [Human] and placebo) at each site. Monitoring of activated clotting time (ACT) for subject safety can potentially unblind an investigator if performed immediately before AND immediately after IP administration. Thus, to avoid potential unblinding, investigators should restrain from monitoring ACT values immediately before AND immediately after IP administration. Individual ACT determinations before OR after IP administration is not considered as potentially unblinding and is therefore permitted. However, if ACT monitoring is done prior to IP administration care must be taken to ensure blinded study personnel are not unblinded. Grifols Clinical Trial Materials personnel and Grifols Pharmacovigilance personnel will have access or be privy to treatment assignments.

Emergency unblinding instructions will be located in the Pharmacy Manual (to be used only if required).

Investigators must not draw any blood samples for AT levels outside the requirements of the protocol. Results from protocol-specified AT, TAT, and F1+2 measurements will not be provided to the Investigator or blinded sponsor personnel in order to ensure maintenance of the treatment blind.

3.3.3.4 Administration and Timing of Investigational Products for Each Subject

The dose of AT-III (Human) will be slowly administered, at a maximum rate of 0.1 mL/kg/min (minute), intravenously. The initial rate will be 0.05 mL/kg/min during the first 5 minutes, accelerating to 0.1 mL/kg/min if tolerated. Intolerance (the inability to provide the subject one full dose) of an infusion due to allergic reactions and anaphylactic symptoms may require decreasing or stopping the infusion rate of the product. In these instances, the following algorithm will be employed:

Severe reaction: Stop infusion. If symptoms subside, it will be at the physician's discretion whether to restart the infusion. The infusion may be restarted under careful nursing monitoring at one-half the previous rate. Appropriate concomitant medication may be given as directed by the physician based on the clinical evaluation.

Moderate reaction: Stop the infusion until all symptoms resolve. If resolution occurs in less than 30 minutes, the infusion may be restarted under careful nursing monitoring at one-half the previous infusion rate. Appropriate concomitant medication may be given as directed by the physician based on the clinical evaluation.

3.3.3.5 Treatment Compliance

Treatment compliance will be measured only in terms of the subject receiving an infusion of AT-III (Human) from the study personnel. No other measure of treatment compliance will be employed.

Reasons for any deviation from the administration of less than 100% of the investigational product dose as prepared by the pharmacist, or designee, must be recorded in the electronic case report form (eCRF) and in the subject's medical records.

3.4 Prior and Concomitant Therapy

Concomitant medications and those taken within the last month (including transfusions of blood or any blood-derived product) prior to the Screening visit must be recorded in the eCRF, including the trade or generic names of the medication, the dose, the route of administration, and the duration of the medication (frequency).

3.4.1 Prior to Study Treatment

Subjects may be on heparin or low-molecular weight heparin, and vitamin K antagonists if international normalized ratio (INR) <1.3 on the day of surgery. Prior treatment with Clopidogrel and Ticagrelor must be discontinued at least 5 days before surgery. Prior treatment with Prasugrel must be discontinued at least 7 days before surgery. Glycoprotein IIb/IIIa receptor blockers must be discontinued at least 24 hours before surgery.

3.4.2 Concomitant Medications during the Study

Concomitant administration of heparin, low-molecular weight heparin, coumadin, tranexamic acid, aminocaproic acid, desmopressin, and recombinant activated factor VII (rFVIIa) may be used in order to avoid thrombotic risk.

Details of any concomitant medication must be recorded on the eCRF except for anesthesia related products, fluid replacement therapies, and electrolyte supplementation.

In addition, products given at different doses on the same day to manage and maintain vital functions (e.g. insulin, furosemide) will only be recorded once on the eCRF and dosage will be reported as PRN (pro re nata).

In the event that an AE or suspected adverse drug reaction (ADR) should occur, complete details of all concomitant medication will be reported to the sponsor.

3.4.3 Prohibited Concomitant Medications during the Study

Tirofiban, abciximab, and eptifibatide may not be used during this clinical trial.

3.5 Study Variables

3.5.1 Efficacy Variables

1. Percentage of subjects with any component of a **major morbidity composite** defined as a composite of any one or more of the following events (up to Day 30±4 days):
    a. Postoperative **mortality** (defined as all deaths occurring within 30 days of the operation or occurring during the primary hospitalization).
    b. **Stroke** (defined as a clinical diagnosis of focal or global neurological deficit of abrupt onset caused by a disturbance in cerebral blood supply).
    c. **Acute kidney injury** (defined as an increase of serum creatinine levels to >2.0 mg/dL and twice the baseline level or a new requirement for dialysis postoperatively).
    d. **Surgical reexploration** (defined as having to return to the operating room because of bleeding, tamponade, graft occlusion or any other cardiac reason).
    e. **Arterial or venous thromboembolic event** (consisting of perioperative MI, mesenteric infarction, peripheral thromboembolism, acute coronary graft thrombosis, intracardiac thrombosis, deep vein thrombosis [DVT], and pulmonary embolism).
    f. **Prolonged mechanical ventilation** (defined as mechanical ventilation >24 hours).
    g. **Infection** (defined as presence of deep sternal-wound infection and/or bloodstream infections).

2. **AT activity (levels)** determinations:

   a. AT activity levels will be measured for both AT-III (Human) treatment and Placebo control groups at a central lab (unless specified at local lab) for the following time points:

      - Prescreening visit (local lab)*
      - Screening visit (local lab)* §
      - Screening visit
      - Preoperatively
      - Immediately before study drug administration
      - Immediately after study drug administration
      - Postoperatively at ICU admission
      - Postoperatively Day 1
      - Postoperatively Day 2
      - Postoperatively Day 3
      - Postoperatively Day 5
      - ICU discharge
      - 1-month follow-up visit

      * Only applicable for subjects undergoing isolated CABG or single valve repair/replacements (will be needed for eligibility only if subject not receiving preoperative heparin [see entry criteria]).

      § This AT (local lab) determination will not be required if the Prescreening visit is conducted.

   b. Percentage of subjects with AT levels of 58% or higher at the ICU admission will be calculated for both treatment groups and compared.

   All AT measurements will be performed on a central venous catheter blood sample, if the central venous catheter is already in place, through a calibration curve prepared by serial dilution of the human normal plasma pool and expressed as a percentage of activity. If a central venous catheter is not already in place, peripheral blood sample will be used for measuring AT levels.

3. **Chest-drain blood loss** (mL) will be measured within the first 12 and 24 hours of chest closure.

   Chest-drain blood loss in the first 12 and 24 hours is defined as the amount of blood collected in the cardiotomy reservoir from chest closure through the following 12 and 24 hours measured.

4. **Transfusion requirements** will be measured as the number of units of Fresh frozen plasma (FFP), red blood cells (RBCs), platelets (PLTs), cryoprecipitate/fibrinogen, prothrombin complex concentrates (PCCs), from the day of ICU admission and throughout the follow-up period.

A specific perioperative transfusion algorithm will be applied:

Standardized Transfusion Algorithm

Red blood cells:

Administration of RBCs should be performed one unit at a time after which the hemoglobin (Hb) levels should be reanalyzed to check the suitability for the next unit.

- Hb <6.0 g/dL (≤3.72 mmol/L [millimoles/liter]): Transfusion is mandatory
- Hb = 6.0-8.0 g/dL (3.72-4.96 mmol/L): Transfusion is optional
- Hb = (8.0-10.0 g/dL). Transfusion is acceptable only if clinically justified*
- Hb >10.0 g/dL: Transfusion is not permitted.

  * One or more of the following clinical justifications must be provided:

- Central venous saturation (Swan Ganz / Central Venous Pressure (CVP) <60% with an arterial oxygen saturation of at least 90%.
- Cardiac Index (CI) <2.0 unresponsive to inotropic or mechanical support (catecholamine, intra-aortic balloon pump [IABP], etc.).
- Signs / symptoms of organ ischemia, such as: change in ST-segment or left ventricular wall motion abnormalities (LV).

Coagulation products:

Only if subject is bleeding 4 mL/kg/hour or more over at least 30 minutes or 1.5 mL/kg/hour or more over 2 consecutive hours.

- Fresh frozen plasma (10-15 mL/kg) / Prothrombin complex concentrates: If subject is actively bleeding and presents an INR ≥1.5.
- Platelets: 1 apheresis unit or 4-6 pooled units if the PLT count is <100,000/μL.
- Cryoprecipitate / Fibrinogen concentrates: Administration of 8-10 units of cryoprecipitate or 2 grams of fibrinogen concentrate if the concentration of fibrinogen ≤1.5 g/L.

Shed blood collected during surgery can be retransfused according to institutional practice. Retransfusion of chest-drain collected blood is not permitted.

5. Percentage of subjects needing **surgical reexploration** defined as having to return to the operating room because of bleeding, tamponade, graft occlusion or any other cardiac reason will be considered from chest closure to the end of the follow-up period.
6. Percentage of subjects with **low cardiac syndrome** defined as the need for major inotropic support or intra-aortic balloon pump will be considered from the day of ICU admission and throughout the follow-up period.
7. Percentage of subjects with postoperative **myocardial infarction** defined through enzymatic criteria plus new Q-waves at the electrocardiogram (ECG) will be considered from the day of ICU admission and throughout the follow-up period.

A perioperative MI is diagnosed by the following criteria:

(0-24 hours postoperation):

The CK-MB (or CK if MB not available) must be greater than or equal to 5 times the upper limit of normal with or without new Q waves present in two or more contiguous ECG leads. No symptoms required.

(>24 hours postoperation): Documented by at least one of the following criteria:

a. Evolutionary ST-segment elevations
b. Development of new Q-waves in two or more contiguous ECG leads
c. New or presumably new left bundle branch block (LBBB) pattern on the ECG
d. Creatine phosphokinase-MB isoenzyme (CK-MB) (or CK if MB not available) ≥3 times the upper limit of normal

8. Percentage of subjects with postoperative **stroke** defined as a clinical diagnosis of focal or global neurological deficit of abrupt onset caused by a disturbance in cerebral blood supply will be considered from the day of ICU admission and throughout the follow-up period.
9. Percentage of subjects with **acute kidney injury** defined as an increase of serum creatinine levels to >2.0 mg/dL and twice the baseline levels or new requirement for dialysis postoperatively, will be considered from the day of ICU admission and throughout the follow-up period. A new requirement for postoperative dialysis may include hemodialysis, peritoneal dialysis, and any form of ultrafiltration.
10. Percentage of subjects with **arterial or venous thromboembolic events** defined as perioperative MI, stroke, mesenteric infarction, peripheral thromboembolism, acute coronary graft thrombosis, intracardiac thrombosis, deep vein thrombosis [DVT], and pulmonary embolism, will be considered from the day of ICU admission and throughout the follow-up period.

11. Percentage of subjects with **infections** defined as presence of deep sternal-wound infections involving muscle, bone or mediastinum or bloodstream infections from the day of ICU admission and throughout the follow-up period.

    The diagnosis of infection must have at least one of the following conditions:

    a. Wound opened with excision of tissue or reexploration of the mediastinum
    b. Positive culture
    c. Treatment with antibiotics

12. Percentage of subjects with **prolonged mechanical ventilation** defined as the need for mechanical ventilation >24 hours from the day of ICU admission and throughout the follow-up period.

13. Percentage of subjects with **all-cause postoperative mortality** defined as death during the same hospitalization as surgery or after discharge and throughout the follow-up period.

14. **ICU stay** duration (days) will be measured from the ICU admission. If duration is greater than the follow-up period, >1 month will be recorded.

15. Percentage of subjects with **prolonged ICU stay** defined as >6 days will be considered from ICU admission during a maximum of 34 days.

16. **Length of hospital stay** (days) in both groups defined as the discharge date minus the surgery date plus 1 day, during a maximum of 34 days after ICU admission. If this time interval is greater than the follow-up period, >1 month will be recorded.

3.5.2 Safety Variables

The following safety variables will be assessed in this study:

- Adverse events, serious adverse events (SAEs), and discontinuations due to AEs.
- Risk for bleeding (will be assessed by monitoring hematocrit levels, urine dipstick tests, and any clinical adverse events for bleeding).
- Physical examination and vital signs.
- Clinical laboratory parameters: Hematology (red blood cell count, white blood cell count, hematocrit, hemoglobin, and platelets, etc.) and clinical chemistry (creatinine, total bilirubin, ALT, and AST, etc.).
- Viral retention samples:

  Blood samples for viral nucleic acid testing (NAT) and viral serology testing will be collected throughout the 30-day follow-up but will be tested only if the subject exhibits clinical signs and symptoms of viral infection during the one-month follow-up period.

3.6 Assessments

3.6.1 Assessment Periods

The expected duration of a study subject's participation will be approximately 7 weeks (30 to 48 days).

The study will consist of the following periods:

- Prescreening and Screening period: within 2 weeks prior to surgery.
- Preoperative period: from the Screening visit to the day of surgery, before anesthesia induction.
- Operative period: from the anesthesia induction to ICU admission.
- ICU admission.
- Postoperative follow-up period: from ICU admission to approximately 1 month (30 ± 4 days) regardless of when the ICU discharge takes place.

3.6.2 Observations and Measurements

The following is a description of the procedures/assessments to take place at each study visit. See the Study Flow Chart and/or Schedule Procedure in Appendix 1.

3.6.2.1 Prescreening Visit: (within Two Weeks Prior to Surgery)*

- Signed informed consent for Prescreening
- AT activity levels (local lab)

* Only applicable for subjects undergoing isolated CABG or single valve repair/replacements who will not be receiving preoperative heparin (see entry criteria) and whose eligibility will therefore be based on AT level <80%. If it is logistically feasible, Prescreening visit must be performed prior to Screening visit, unless a subject's surgery is scheduled to occur in ≤72 hours. In the latter case, the Prescreening and Screening visit may be combined. All determinations for a separate Prescreening visit must be recorded on specific Prescreening sheets.

Note: The Prescreening visit is not required for subjects undergoing complex/combined procedures (CABG+valve, double/triple valve repair/replacements, ascending aorta/aortic arch surgeries).

3.6.2.2 Screening Visit: (within Two Weeks Prior to Surgery)*

- Signed informed consent
- Allocation of Screening Number
- Inclusion/Exclusion criteria

- Addition of subject's data into Screening Log
- All clinically relevant medical case history and demographic data (age, gender, race, weight, height, body mass index [BMI], smoking habits, ejection fraction, previous MI, unstable angina, arrhythmia, cardiogenic shock, congestive heart failure, pre-existing renal failure or dialysis treatment, chronic obstructive pulmonary disease [COPD], previous stroke, percutaneous coronary interventions, previous pulmonary thromboembolism, previous IABP, diabetes mellitus requiring treatment, oral anticoagulation therapy, heparin therapy, peripheral vascular disease or systemic thromboembolism, hypertension, hypercholesterolemia, immunosuppressive treatment). Please include any relevant medical conditions as well as medical disorders requiring medication or that are currently active.
- Physical examination
- Vital signs (temperature [T], blood pressure [BP], heart rate [HR], respiratory rate [RR])
- AT activity levels (local lab) (Only for subjects undergoing isolated CABG or single valve repair/replacements and not receiving preoperative heparin [see entry criteria])
- AT activity levels (central lab)
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, lactate dehydrogenase [LDH], glucose, sodium, potassium, chloride and calcium)
- Serum pregnancy test (for women of childbearing potential)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).
- Documentation of medications that the subject is taking or has taken within the last month (including transfusions of blood or any blood-derived product)
- Adverse events

* The Screening visit may occur on the same day of the Prescreening visit. If a subject's surgery is scheduled to occur in ≤72 hours, the Prescreening and Screening visits may be combined.

3.6.2.3 Preoperative Visit (Before Anesthesia Induction): Day 0[§]

- Review of inclusion/exclusion criteria to confirm subject eligibility
- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels (central lab)
- Randomization[†]
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)

- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70°C and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).
- Confirmation of existing, and recording of any new events, or changes, in the medical and surgical history of the subject since the Screening visit.
- Confirmation of existing and recording of any new medications, or changes in medications administered to the subject since the Screening visit. The combined Screening and Preoperative visit requires documentation of the lifetime history of bleeding abnormalities. In addition, it requires documentation of medications that the subject is taking or has taken within the last month.
- Adverse events

§ The Preoperative visit (Before Anesthesia induction: Day 0) may occur on the same day of the Screening visit if the latter is to be performed within 48 hours of the surgery. In this case, the only assessments to be performed are those detailed for the Screening visit AND the Randomization procedures detailed in Section 3.3.2.

† Randomization on Preoperative visit (Day 0) may occur 24 hours before the day of surgery (Day 0) once all eligibility criteria are confirmed.

3.6.2.4 Operative Visit (After Anesthesia Induction – Before ICU Admission): Day 0

- Type of surgical procedure
- Duration of CPB and aortic clamp
- Preoperative heparin dose response*
- Dose of heparin (initial, subsequent pre-CPB, during CPB), ACT, and dose of protamine*
- Study drug infusion
- Minimum hematocrit during CBP (local lab)
- Transfusion requirements
- AT activity levels immediately before and after infusion of AT-III (Human) or Placebo
- Concomitant medications
- Adverse events

* A specific perioperative anticoagulation management protocol below will be applied to all participating subjects:

Anticoagulation management protocol:

- An initial heparin loading dose of 400 IU/kg will be administered aimed to achieve a pre-CPB ACT value of 480 seconds. Heparin responsiveness will be determined according to institutional practice (i.e., use of heparin dose response method).
- Failure to achieve the desired ACT after an IV administration of 400 IU/kg of heparin will be managed by a maximum of three (3) separate heparin administrations of 100 IU/kg each. If the target ACT is still not obtained additional measures will be performed according to institutional practice (e.g. additional heparin, FFP, AT). If AT is administered the subject will be excluded from the final analysis.
- At the end of CPB, heparin will be reversed with protamine at a 0.5:1 ratio of the total heparin dose. Additional protamine may be infused according to local standards.
- Postoperatively, 15 mg of protamine will be administered as an infusion every hour (± 15 minutes) during the first 5 hours postoperation.

3.6.2.5 ICU Admission: Day 0

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Chest-drain blood loss in the first 12 and 24 hours postoperatively
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, and postoperative mortality)
- Concomitant medications
- Adverse events
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results).

3.6.2.6 Postoperative Day 1:

- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)

- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Chest-drain blood loss in the first 12 and 24 hours postoperatively
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, and postoperative mortality)
- Concomitant medications
- Adverse events

If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed below in the ICU discharge visit. Regardless of when the ICU discharge takes place AT activity levels should be measured on Postoperative days 1, 2, 3, and 5.

3.6.2.7 Postoperative Day 2:

- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, and postoperative mortality)
- Concomitant medications
- Adverse events

If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed below in the ICU discharge visit. Regardless of when the ICU discharge takes place AT activity levels should be measured on Postoperative days 1, 2, 3, and 5.

3.6.2.8 Postoperative Day 3:

- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)

- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, and postoperative mortality)
- Concomitant medications
- Adverse events

If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed below in the ICU discharge visit. Regardless of when the ICU discharge takes place AT activity levels should be measured on Postoperative days 1, 2, 3 and 5.

3.6.2.9 Postoperative Day 5:

- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, and postoperative mortality)
- Concomitant medications
- Adverse events

If this visit coincides in time with the ICU discharge, the only assessments to be performed are those detailed below in the ICU discharge visit. Regardless of when the ICU discharge takes place AT activity levels should be measured on Postoperative days 1, 2, 3 and 5.

3.6.2.10 ICU Discharge Visit (Maximum One Month after Admission)

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- Clinical chemistry (creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium)
- Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, ICU stay duration, prolonged ICU stay, and postoperative mortality)

- Concomitant medications
- Adverse events

If the ICU stay is longer than 1 month, the assessments for both ICU Discharge visit and Follow-up Day 30±4 days visit will be performed at Day 30±4 days. There will be no additional ICU Discharge visit and follow-up visits.

3.6.2.11 Follow-Up Visit (Day 30±4 Days after ICU Admission)*

- Physical examination
- Vital signs (T, BP, HR, RR)
- AT activity levels
- Hematology and coagulation (Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, fibrinogen, TAT, F1+2)
- An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results)
- Clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, prolonged mechanical ventilation, postoperative mortality, and overall hospital stay duration)
- Concomitant medications
- Adverse events

*A phone call follow-up visit will be performed for those subjects unable to return to the investigational site during the scheduled time frame. The occurrence of any adverse events since the last study visit and clinical outcomes will be investigated.

3.6.3 Description of Procedures and Laboratory Tests

3.6.3.1 Surgical Procedures

During the operative period and in addition to the routine monitoring of the subject, the following data will be recorded on the eCRF:

- Type of surgical procedure: Isolated CABG, isolated aortic valve replacement, aortic valve replacement + CABG, isolated mitral valve replacement, mitral valve replacement + CABG, isolated mitral valve repair, mitral valve repair + CABG, ascending aorta/aortic arch surgeries, etc.)
- Duration of CPB and aortic clamp
- Preoperative heparin dose response

- Dose of heparin (initial, subsequent pre-CPB, during CPB), ACT, and dose of protamine
- Minimum hematocrit during CPB (local lab)
- Adverse events

3.6.3.2 Clinical Laboratory Tests

Laboratory panels include blood coagulation parameters and serum clinical chemistry as described below.

Hematological and coagulation parameters: Hb, Hct, RBC, WBC, PLT, aPTT, PT, INR, Fibrinogen, TAT, F1+2.

Blood samples will be drawn at the following time points:

- Screening visit (local lab)
- Screening visit (central lab)
- Preoperatively
- ICU admission (Day 0)
- Postoperative Day 1
- Postoperative Day 2
- Postoperative Day 3
- Postoperative Day 5
- ICU discharge
- End of the follow-up period (Day 30±4 days)

Clinical chemistry panel: Creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride and calcium

Blood samples will be drawn at the following time points:

- Screening visit (local lab)
- Screening visit (central lab)
- Preoperatively
- ICU admission (Day 0)
- Postoperative Day 1
- Postoperative Day 2
- Postoperative Day 3
- Postoperative Day 5
- ICU discharge

The above listed laboratory panels will be performed by a central laboratory unless otherwise specified. Investigative site laboratories may also be used in some instances (e.g. in the case of Screening and Preoperative visit tests to determine subject's eligibility when testing results may be needed in a short timeframe). The Investigator will be required to classify laboratory results out of the normal range reported by the laboratory as clinically relevant or not according to his/her criteria. Results will be recorded in source documents and on the subject eCRF when applicable.

Laboratory results out of the normal range judged by the Investigator as clinically relevant will be considered AEs.

3.6.4 Drug Concentration Measurements

All subjects will have blood samples collected for measurement of serum AT concentration at select study visits as specified in Section 3.6.2 and Appendix 1. However, it should be pointed out that AT levels reflect both endogenous AT and infused AT-III (Human) exogenously.

All samples for measurement of serum AT concentration will be analyzed using an antigenic content assay that is validated according to current regulatory and industry guidelines and expectations. To maintain the study blind, no unblinded AT concentration result will be reported to the study sites or to blinded Grifols personnel until after closure of the study database.

3.7 Screen Failures

Prescreening and screening evaluations will be used to determine the eligibility of each subject for enrollment. Subjects undergoing isolated CABG or single valve repair/replacements and not receiving preoperative heparin found to have a baseline AT activity level ≥80% (local lab) at the Prescreening visit will not continue any further subject eligibility evaluations and will not be considered as screening failures.

If a subject undergoing isolated CABG or single valve repair/replacements will receive preoperative heparin (see entry criteria), all Screening assessments will be performed because the subject may meet eligibility criteria without AT level as prerequisite. Subjects who fail to meet eligibility criteria during screening evaluations (during Screening visit) will be considered screen failures and will not be randomized and participate in the study.

3.8 Removal of Subjects

Subjects may withdraw or be withdrawn from the study for the following reasons:

1. At their own request or at the request of their legally acceptable representative.
2. If, in the Investigator's opinion, continuation in the study would be detrimental to the subject's well-being.

3. At the specific request of the sponsor.

Subject participation in the study may be terminated early under the following circumstances:

1. The subject withdraws his/her informed consent to participate in the clinical trial.
2. The subject does not meet all inclusion criteria and is deemed a screen failure.
3. The subject meets any of the exclusion criteria and is deemed a screen failure.
4. The subject is not able to adhere to the main protocol requirements (major protocol violations).
5. The occurrence of an AE, which in the Investigator's opinion requires the withdrawal of the subject from the clinical trial.
6. The subject is lost to follow-up.
7. Subject's death.
8. Subjects with an occurrence of a concomitant disease, or any medical condition which, either because of its severity or duration or necessary change in treatment, contravenes the condition of the study or puts the subject at unnecessary risk or harm per Investigator discretion.
9. Subjects who develop an infection with HAV, HBV, HCV, B19V, or HIV during the study. A subject should be withdrawn if, in the opinion of his/her physician, he/she develops any of these viral infections.
10. Any event, which in the opinion of the Investigator impedes the subject's participation in the study.

If the reason for early discontinuation is an AE, in so far as is possible, the subject will be followed-up until the event resolves, or has been stabilized, and no further change is expected.

In all cases, the reason for withdrawal must be recorded in the eCRF and in the subject's records.

A subject may leave the study at any time for any reason and will be permitted to do so without penalty. Grifols Therapeutics, Inc. will be notified by the Investigator if a subject terminates or is terminated from the study early. The reason for early termination will be clearly documented in the eCRF and reasonable efforts to perform study completion procedures will be made when appropriate. The Investigator can withdraw a subject from the clinical trial at any time.

For subjects who are considered screen failures because they do not meet all inclusion criteria or they meet any of the exclusion criteria, it is not necessary to perform additional study completion procedures other than recording all the study data gathered until the

moment when the subjects is deemed to be a screen failure, including the reasons for the screen failure.

For subjects who are not screen failures and withdraw from the clinical trial before the beginning of the surgical procedure, study completion procedures consisting of a physical assessment, recording of vital signs (heart rate, respiration rate, systolic and diastolic blood pressure and temperature) and concomitant medications, and assessment of AEs will be performed during their last visit at the site, if possible.

For enrolled subjects who early discontinue the clinical trial, study procedures and assessments scheduled for the Follow-up visit will be performed.

For subjects who withdraw their informed consent to participate in the clinical trial, it is not necessary to perform additional study completion procedures other than recording all the study data gathered until the moment when the subjects withdraw their informed consent.

3.9 Subject Replacement

Subjects withdrawn from the study because of safety reasons will not be replaced. Enrolled subjects (i.e. randomized subjects) who early discontinue the clinical trial cannot be replaced.

3.10 Follow-up of Subjects Withdrawn from Study

Subjects who receive any amount of investigational product and discontinue early from the study will be requested to return for the follow-up visit (Day 30±4 days) procedures as close as practical to 30 days after their last administration of the investigational product.

3.11 Premature Termination of Study/Closure of Center

The sponsor, Institutional Review Board/Ethics Committee (IRB/EC), and/or regulatory authorities have the right to close this study or a study center, and the Investigator/Sponsor has the right to close a center, at any time, although this should occur only after consultation between involved parties. The IRB/EC must be informed. Should the study/center be closed prematurely, all study materials (except documentation that has to remain stored at site) must be returned to the sponsor. The Investigator will retain all other documents until notification given by the sponsor for destruction.

A study center can be closed for the following reasons:

- Lack of enrollment
- Non-compliance with the requirements of the study protocol
- Non-compliance with ICH GCP

4 ASSESSMENT OF SAFETY

4.1 Specification of Safety Parameters

Safety of AT-III (Human) will be evaluated in this study. Safety endpoints will include:

1. Adverse events including serious adverse events (SAEs), suspected ADRs, and discontinuations due to AEs.
2. Risk for bleeding (will be assessed by monitoring Hct levels, urine dipstick tests, and any clinical AEs for bleeding).
3. Clinical laboratory panels.
4. Physical examination.
5. Vital signs.

Also see Section 3.5.2.

4.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Safety will be assessed throughout the clinical trial to all subjects who has received any amount of the investigational product (AT-III [Human] or Placebo). See Section 5.1.4.

4.2.1 Adverse Events

Adverse events (includes adverse reactions [AR]) occurring at any time between signing of the subject's ICF and the last day of the subject's participation in the clinical trial will be reported and recorded on the appropriate subject's eCRF entry.

It is the Investigator's responsibility to ensure that all AEs are appropriately recorded.

Adverse events will be elicited by spontaneous reporting by the study individual or by a non-leading inquiry or direct observation by the study staff.

4.2.2 Physical Examinations

A medically certified individual will conduct a physical examination at the screening visit, within 48 hours prior to the first AT-III (Human) infusion, at ICU admission, at ICU discharge, and at the follow-up visits. The findings of each examination will be recorded on the eCRF. Abnormal findings judged as clinically relevant by the Investigator will be considered AEs.

4.2.3 Vital Signs

Vital signs will be measured by a medically certified individual or a nurse according to his/her standard clinical practice. Vital signs will be measured at the Screening visit, preoperatively, at ICU admission, and during the follow-up period.

The following vital signs will be assessed:

- T
- BP (systolic blood pressure [SBP]) and diastolic blood pressure [DBP]),
- HR
- RR

Vital signs will be routinely monitored by the study staff as detailed in Section 3.6.2 and Appendix 1. The Investigator will be required to classify vital signs abnormalities as clinically relevant or not according to his/her criteria. Results will be recorded in source documents and on the subject eCRF. Vital signs abnormalities judged by the Investigator as clinically relevant will be considered AEs.

4.2.4 Clinical Chemistry and Hematology

All clinical laboratory data for renal (creatinine, BUN), hepatic (ALT, AST, AP and TB) and haematological parameters (CBC including differential leukocyte count) will be listed for each clinical trial subjects.

The Investigator will be required to classify laboratory results out of the normal range reported by the laboratory as clinically relevant or not according to his/her criteria.

Laboratory results out of the normal range judged by the Investigator as clinically relevant will be considered AEs.

4.3 Procedures for Eliciting Reports of and for Recording and Reporting Adverse Event and Intercurrent Illnesses

4.3.1 Adverse Events

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

Any AE that occurs at any time between signature of the informed consent and last day of the subject's participation in the clinical safety period of the study (Follow-up visit Day 30 ± 4 days) must be reported on the AE eCRF entry.

4.3.2 Causality of Adverse Event

The investigator will be asked to assess the causal relationship of the AE to the study treatments according to the following classification based on Karch FE et al (42):

- **Definite**: An event that follows a reasonable temporal sequence from administration of the treatment or in which the treatment level has been established in body fluids or tissues; that follows a known response pattern to the suspected treatment; and that is confirmed by improvement on stopping the treatment (dechallenge), and reappearance of the event on repeated exposure (rechallenge).
- **Probable**: An event that follows a reasonable temporal sequence from administration of the treatment; that follows a known response pattern to the suspected treatment; that is confirmed by dechallenge; and that could not be reasonably explained by the known characteristics of the subject's clinical state.
- **Possible**: An event that follows a reasonable temporal sequence from administration of the treatment; that follows a known response pattern to the suspected treatment; but that could have been produced by the subject's clinical state or other modes of therapy administered to the subject.
- **Doubtful/Unlikely**: An event that follows a reasonable temporal sequence from administration of the treatment; that does not follow a known response pattern to the suspected treatment; but that could not be reasonably explained by the known characteristics of the subject's clinical state.
- **Unrelated**: Any event that does not meet the criteria above.

The operational tool to decide the AE causal relationship is based on algorithms by Karch-Lasagna and Naranjo (43, 44).

When an AE is classified, assessing casual relationship by the investigator, as definitive, probable, possible, or doubtful/unlikely, the event will be defined as suspected ADR. When the causal relationship is labeled "Unrelated", then it will be considered that the AE is not imputable to the study treatment and it is not a suspected ADR. For definition of AR, see Section 4.3.3.

Any AE experienced by the subject prior to the first blinded investigational product infusion on Day 0 will be labeled as Unrelated to the study drug.

In addition, when a causal relationship between the study treatment and the AE cannot be ruled out by the Investigator, it means that the AE cannot be labeled "Unrelated"; then, the Investigator will be asked to evaluate whether the AE is attributable only to the study

treatment or also to the surgical technique employed. Potential causal relationships between an AE and the surgical technique will be evaluated.

4.3.3 Suspected Adverse Drug Reaction and Adverse Reaction

All noxious and unintended responses to a medicinal product or device related to any dose should be considered suspected ADRs. The phrase "responses to a medicinal product or device" means that a causal relationship between a medicinal product or device and an AE is at least a reasonable possibility, that is, the relationship cannot be ruled out. In the framework of this study, a suspected ADR with a causal relationship of "definite" will be named as AR. Adverse reactions are a subset of suspected ADR.

The sponsor is responsible for assessing the suspected ADR expectedness during the clinical trial.

4.3.4 Severity (Grade) of the Adverse Event

For any AE or SAE, the severity (grade) of an event must be assessed. The following guidance should be used to determine event severity:

- **Mild**: usually transient in nature and generally not interfering with normal activities. Subjects have transient symptoms; no treatment required other than non-prescription drugs.
- **Moderate**: sufficiently discomforting to interfere with (but may not overtly prevent) normal activities. Subject was symptomatic and required treatment, but had no sequela.
- **Severe**: prevents normal activities. Subject was incapable of work or usual activity; treatment with prescription medication was required and/or sequela were produced

Adverse event and suspected ADR severity (intensity) gradation must be distinguished from AE and suspected ADR seriousness gradation, which is defined according to event consequence. For example, a headache could be mild, moderate, or severe but unusually is serious in all these cases.

The investigator will be responsible for assessing the AE or suspected ADR severity (intensity) during the clinical trial, taking into account currently criteria detailed in this section.

4.3.5 Serious Adverse Event

An SAE is an AE or a suspected ADR, occurring at any dose that fulfils one or more of the following:

- results in **death**

- is immediately life-threatening (life-threatening in the definition of SAE refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- requires in-subject **hospitalization** or prolongation of existing hospitalization*
- results in persistent or significant **disability** or incapacity
- is a **congenital anomaly** or birth defect
- is an important medical event: important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening, or result in death, or hospitalization, but from medical and scientific judgment may jeopardize the subject and/or may require medical or surgical intervention to prevent one of the other outcomes listed above. 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 hospitalization. Development of cancer or drug dependency or drug abuse will normally be considered serious by this criterion.

*Hospitalization is to be considered only hospital stay for equal or more than 24 hours. The following hospitalizations should not be reported as SAEs:

- Hospitalization or prolongation of hospitalization needed for procedures required by the clinical trial protocol.
- Hospitalization or prolongation of hospitalization as part of a routine procedure followed by the center.
- Hospitalization for a survey visit, annual physicals, or social reasons.
- Elective (pre-planned) hospitalizations for a pre-existing condition that had not worsened from Baseline (e.g. elective or scheduled surgery arranged prior to start of the study).
- Admissions not associated with an AE (e.g. social hospitalization for purposes of respite care).

A distinction should be drawn between serious and severe AEs. The term "severe" is used to describe the severity of a specific event; the event itself, however, may be of relative minor medical significance (such as severe headache). This is not the same as "serious", which is defined on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) is a medical term while severity is a subjective term.

According to the medical criteria, an AE, or a suspected ADR, can be classified as serious, although it does not fulfill the conditions fixed in this section, if it is considered important from a medical point of view. The investigator is responsible for assessing the AE, or suspected ADR, seriousness during the clinical trial, taking into account currently criteria fixed in this section.

4.3.6 Expectedness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR is considered "unexpected" if the nature, seriousness, severity, or outcome of the reaction(s) is not consistent with the reference information. The expectedness of an AR shall be determined by the sponsor according to the reference document (ie, IB).

Events not listed for the particular drug under investigation in the IB are considered "unexpected" and those listed are considered "expected." When new AE information is received, it is the sponsor's responsibility to determine whether the event is "unexpected" for investigational new drug (IND) safety reporting purposes.

4.3.7 Adverse Event Documentation

All AEs and SAEs occurring after the subject has signed the **informed consent form (ICF)** must be fully recorded in the subject's eCRF or SAE form. For example, a laboratory test abnormality considered clinically relevant, eg, causing the subject to withdraw from the study, requiring treatment, causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE. Each event must be adequately supported by documentation as it appears in the subject's medical or case file.

At each visit, AEs will be elicited by asking the individual a non-leading question such as "Do you feel different in way since the last visit?". Moreover, AE will also be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms, and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible. It is responsibility of the Investigator to ensure that AEs are appropriately recorded.

The following variables must to be recorded on the AE eCRF entry:

1. the verbatim term (a diagnosis is preferred)
2. date/time of onset
3. date/time of resolution
4. intensity (mild, moderate, severe)
5. causality (unrelated, doubtful/unlikely, possible, probable, definite)*
6. seriousness (yes, no)
7. action taken (with regard to study drug)
8. other action (to treat the event)
9. outcome and sequel (follow-up on AE)

*Causality assessment will be only made when the AE occurs after the subject has received one or another study treatment. AE occurring before subject's exposure to study treatments will be always labeled as "Unrelated".

For AEs that occur during infusions, the infusion rate in effect at the time of onset of the AE, the time of onset of the AE and the time of AE change materially in intensity and/or resolve will be captured the eCRF entry.

In addition to the Investigator's own description of the AEs, each AE will be encoded by the sponsor (or designee, eg, CRO) according to the MedDRA® dictionary of medical codes.

A pregnancy not verified before randomization but occurring during the course of the study will be not considered an AE, unless a relation to the study drug is suspected. In any case, must be completed a Pregnancy Report Form and send as soon as possible to the Sponsor, and the study treatment must be discontinued. A copy of the form should be filled at the study site for follow-up until the end of the pregnancy.

4.4 Reporting of Serious Adverse Events or Pregnancy

4.4.1 Reporting Serious Adverse Event

All SAEs must be expeditiously reported, whether or not considered attributable to the study drugs. A SAE Report Form should be used to expeditiously report the SAE to the receiver appointed.

When the Investigator becomes aware of an SAE, she/he must submit by email a complete, signed, and dated SAE Report Form **within 24 hours** to the Sponsor.

After the initial report, all relevant follow-up information for the SAE including the outcome, must be also supplied to the Sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form or by other appropriate means, such as data clarification form issued by the Sponsor or CRO. In addition, the Sponsor or CRO may request additional information and/or reports.

All SAE Report Forms must be reported to:

Grifols Global Drug Safety

[redacted]

[redacted]

Serious adverse events will be assessed by the Sponsor or designee for expectedness assuming that all subjects have been treated with AT-III. If the event is considered serious, suspected potentially related to the study drug and unexpected (SUSAR), then the treatment allocation will be unblinded. The following possibilities resulting from the unblinding will be considered:

1. If the study drug administered to the subject is AT-III, the case will be reported in accordance to local regulations.
2. If the study drug administered to the subject is Placebo, the suspected ADR will be reassessed for expectedness according to the reference safety information and:
    a. If the suspected ADR is still considered unexpected, it will be reported in accordance with applicable requirements and guidelines.
    b. If the suspected ADR is considered expected, it will not be reportable on an expedited basis, unless specifically requested by local regulations.

4.4.2 Reporting Pregnancy

While pregnancy itself is not a true "AE," pregnancy occurring in a clinical study must be followed, to collect information regarding the experiences of gestation and pregnancy with investigational product exposure. The Investigator must report any pregnancy that occurs in a study subject subsequent to informed consent until 28 days after the last dose of investigational product. Any female subject who becomes pregnant during the study will be discontinued from investigational product treatment and will be followed for pregnancy outcome.

For any pregnancy with an outcome of live birth, the newborn infant will be followed until one month of age. Any anomalies, complications, abnormal outcomes, or birth defect observed in the child must be reported as an SAE within 24 hours of the Investigator or study personnel's first knowledge.

5 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

5.1 Statistical and Analytical Plans

Unless otherwise specified, descriptive statistics will include the number of observations, mean, standard deviation (SD), median, minimum and maximum values for the continuous/quantitative data, or frequency counts and percentages for categorical/qualitative data.

Data handling and evaluation procedures will be described in the Statistical Analysis Plan.

5.1.1 Subject Population(s) for Analysis

Intention-to-Treat population (ITT) will include all subjects who are randomized.

Per-protocol (PP) population will include all ITT subjects who have no major protocol deviations.

Safety population will include all subjects who receive any amount of study drug.

5.1.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized by treatment group using ITT population. For quantitative variables, mean, SD, median, and minimum/maximum will be provided. For qualitative variables, the frequency and percentage will be provided.

5.1.3 Efficacy Analysis

5.1.3.1 Primary Efficacy Analyses

The primary efficacy variable is the percentage of subjects with any component of the composite of major morbidity. The primary efficacy variable will be analyzed with Cochran Mantel Haenszel test adjusting for history of MI using ITT population. The sensitivity analyses using PP populations will also be provided.

5.1.3.2 Secondary Efficacy Analyses

Postoperative AT levels at the ICU admission will be summarized by treatment group. The analysis of covariance (ANCOVA) will be used to compare the treatment difference. ANCOVA model will include the AT level as dependent variable, treatment as fixed effect and baseline AT level as covariate. The similar approach will also be used to analyze the AT levels in other time points.

Postoperative chest-drain blood loss in the first 12 and 24 hours after surgery will be summarized by treatment group and will be analyzed using group t-test.

For transfusion requirements, Number of units of RBC, Number of units of FFP, and Number of units of platelets will be summarized by treatment group and analyzed using group t-test.

The number and percentage of subjects with each following procedure or morbidity will be separately summarized by treatment group and will be analyzed using Chi-square test (or Fisher Exact test as appropriate)

- Need for surgical reexploration
- Low cardiac output syndrome
- Myocardial infarction
- Stroke
- Acute kidney injury
- Arterial or venous thromboembolic events
- Infections
- Prolonged mechanical ventilation (>24 hours)

- All-cause postoperative mortality
- Prolonged ICU stay (>6 days)

Duration of ICU stay and the length of hospital stay will be summarized by treatment group. Non-parametric will be used to compare the treatment differences and Hodges-Lehmann estimation will be provided.

5.1.4 Safety Analysis

The safety analysis will be based on safety population.

The incidence of AEs, SAEs, suspected ADRs, and AEs by severity will be summarized by treatment, system organ class and preferred term using descriptive statistics. Subjects with deaths, SAEs, and AEs leading to premature discontinuation from the study will be listed.

The Investigator verbatim term and the MedDRA coded term (system organ class and preferred term) will be shown simultaneously on the data listings of all AEs.

Clinical laboratory variables will be summarized by treatment using summary statistics at each time point and on the change from baseline. Shift tables will be provided to summarize values that fall outside the normal ranges.

Vital signs will be summarized by treatment using summary statistics at each time point and on the change from baseline.

Physical exam data will be provided in data listings.

5.2 Determination of Sample Size

Assuming that percentage of subjects with major morbidity composite is 22.0% in Placebo group and 14.3% in AT-III treatment group (ie, AT-III treatment group has a 35% relative reduction comparing to the Placebo control group), 192 subjects per treatment arm (total 384 subjects) are needed to have 70% power to detect a trend at alpha = 0.15. Considering 5% dropout rate, it is estimated that 404 subjects (202 subjects per treatment arm) need to be randomized.

6 ADMINISTRATIVE

6.1 Investigators, Other Study Personnel and External Committees

Information regarding additional key personnel involved in the conduct of the study, including names and contact details of participating investigators, monitors, clinical laboratories, technical departments and/or institutions, as well as information on members of

additional study committees, will be found in the study files of the sponsor and at the Investigator sites within the study reference manual/file.

6.2 Data Quality

Monitoring and auditing procedures defined/agreed by the sponsor will be followed, in order to comply with ICH GCP guidelines. Each center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, ICH GCP and legal aspects. The on-site verification of the eCRF for completeness and clarity will include cross checking with source documents, and clarification of administrative matters. Query verification of data will be described in the Data Management Plan.

6.3 Documentation

The study data will be recorded and kept current in the eCRF by the site study personnel directly responsible for the information. Entries made in the eCRF must be verifiable against source documents, or have been directly entered into the eCRF, in which case the entry in the eCRF will be considered the source data.

The data in the eCRF will be monitored at the site by Grifols Therapeutics Inc. representatives at regular intervals and reviewed for completeness and compared with the source documents. Examples of source documents include individual subject medical records, which are separate from the eCRFs.

All AEs and SAEs must be recorded. All SAEs must be recorded on the SAE form. The SAE form must be kept in site records with a copy provided to the designated person as detailed in the study file.

6.3.1 Record Retention

At study completion, all study data will be transferred to Grifols Therapeutics Inc. according to ICH GCP guidelines, local laws, regulations, and Grifols Therapeutics Inc. requirements. The study file and all source data should be retained until notification is given by the sponsor for destruction.

An Investigator is required by ICH GCP guidelines to retain the study files. If an Investigator moves, withdraws from an investigation, or retires, the responsibility for maintaining the records may be transferred to another person (*eg,* other Investigator). Grifols Therapeutics Inc. must be notified in writing of the person responsible for record retention and the notification will be retained in the sponsor study file and the Investigator site file.

6.3.2 Access to Information for Monitoring

The data will be recorded and kept current in CRF/eCRFs by the study site personnel directly responsible for the information and reviewed for completeness by the monitor. Grifols Therapeutics Inc. personnel or designee can review the records.

In accordance with ICH GCP guidelines, the monitor must have direct access to the Investigator's source documentation in order to verify the data recorded in the eCRFs for consistency and to verify adherence to the protocol, and the completeness, consistency, and accuracy of data entered. "Source documentation" includes individual subject files, separate from the eCRFs, which should be maintained and include visit dates, laboratory results, concomitant treatment, vital signs, medical history, examinations, AEs, investigational product dispensing logs, and other notes as appropriate. The Investigator agrees to cooperate with the monitor to ensure that any problems noted during the course of these monitoring visits are resolved.

6.3.3 Access to Information for Audits or Inspections

Representatives of regulatory authorities or of Grifols Therapeutics Inc. or designee may conduct audits or inspections or audits of the Investigator study site. If the Investigator is notified of an audit or inspection by a regulatory authority, the Investigator agrees to notify the Grifols Therapeutics Inc. Medical Monitor immediately. The Investigator agrees to provide to representatives of a Regulatory Agency or Grifols Therapeutics Inc. access to records, facilities, and personnel for the effective conduct of an audit or inspection.

7 ETHICAL AND LEGAL ASPECTS

7.1 Institutional Review Board/Ethics Committee

Documented approval from appropriate IRBs/ECs will be obtained for all participating centers/countries prior to study start, according to ICH GCP guidelines, local laws, regulations, and organizations. When necessary, an extension, amendment or renewal of the IRBs/ECs approval must be obtained and also forwarded to the sponsor. The IRBs/ECs must supply to the sponsor, upon request, a list of the IRBs/ECs members involved in the vote and a statement to confirm that the IRBs/ECs is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

7.2 Ethical Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and Investigator abide by ICH GCP guidelines. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the sponsor representatives and/or an inspection by Regulatory Authority representatives at any time. The Investigator must agree to the audit or inspection of study-related records by the sponsor representatives and/or

Regulatory Authority representatives, and must allow direct access to source documents to the sponsor and/or Regulatory Authority representatives.

Modifications to the study protocol will not be implemented by either the sponsor or the Investigator without agreement by both parties. However, the Investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/EC/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IRB/EC/Sponsor. Any deviations from the protocol must be fully explained and documented by the Investigator.

7.3 Regulatory Authority Approvals/Authorizations

Regulatory Authority approvals/authorizations/ notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for Investigator sites responsible for conducting the study will be posted on a publicly accessible clinical registry(ies) as required by local law.

7.4 Subject Information and Consent

Subject information and ICF will be provided to Investigator sites. Prior to the beginning of the study, the Investigator must have the IRB/EC written approval/favorable opinion of the written ICF and any other written information to be provided to subjects. The written approval of the IRB/EC together with the approved subject information/ICF must be filed in the study files and a copy of the documents must also be provided to Sponsor by the Investigator site.

Written ICF must be obtained before any study specific procedure takes place. Subjects must provide written approval to specific ICFs prior to any Prescreening and Screening activities, respectfully. Participation in the study and date of ICF given by the subject should be documented appropriately in the subject's files. A signed copy of the subject ICF will be provided to the subject or subject's authorized representative.

7.5 Insurance

All subjects participating in the study will have insurance coverage by the sponsor, which is in line with applicable laws and/or regulations.

7.6 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names will not be supplied to the sponsor. Only the subject number and subject initials will be recorded in the eCRF, and if the subject's name appears on any other

document (*eg,* pathologist report), it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the sponsor, IRB/EC, or Regulatory Authorities may inspect their medical records to verify the information collected, and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The Investigator will maintain a list to enable subjects' records to be identified.

8 USE OF DATA AND PUBLICATION

Institution and the Investigator agree that the first publication shall be made in conjunction with the presentation of a joint, multi-center publication of the study results from all appropriates sites. If such a multi-center publication is not submitted within twelve (12) months after conclusion of the study at all sites or after Grifols confirms there will be no joint, multi-center publication, then institution and/or Investigator shall have the right, at their discretion, to publish, either in writing or orally, the results of the study performed under the protocol, subject to the conditions outlined below:

- The results of the study will be reported in the publicly accessible registry(ies).
- Institution and/or Investigator shall furnish Grifols with a copy of any proposed publication at least thirty (30) days in advance of the date of submission for publication.
- Within said thirty (30) day period, Grifols shall:
  - Review such proposed publication for Confidential Information (other than Study results) and for subject information subject to the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and other applicable privacy laws;
  - Review such proposed publication for the unauthorized use of the name, symbols and/or trademarks of Grifols;
  - By written notice to the Investigator, identify with specificity the text or graphics in such proposed publication that Grifols contends contains Confidential Information, protected subject information, or the unauthorized use of Grifols' name, symbols and/or trademarks so that the proposed publication may be edited appropriately to remove such text or graphics before publication; and
  - By written request, Grifols may delay proposed publications up to sixty (60) days to allow Grifols to protect its interests in Grifols Inventions described in such publications.
- Institution and/or Investigator shall give Grifols the option of receiving an acknowledgment for its sponsorship of the study in all such publications or presentation.

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APPENDICES

Appendix 1 Study Flow Chart and/or Schedule of Procedures

Visits Procedures/Assessments	Pre-screening Visit (-14 days)[a]	Screening Visit (-14 days)	Pre-Operative Visit (Day 0)	Operative Visit (Day 0)	ICU Admission (Day 0)	Post-Operative (Day 1)	Post-Operative (Day 2)	Post-Operative (Day 3)	Post-Operative (Day 5)	ICU Discharge	Follow-up Visit (Day 30±4 days)
Informed consent	X	X									
Inclusion/Exclusion criteria		X	X								
Medical history/demographics		X									
Randomization[b]			X[b]								
Concomitant medications		X	X	X	X	X	X	X	X	X	X
Vital signs		X	X		X					X	X
Physical examination		X	X		X					X	X
AT levels for isolated CABG/valve repair/replacements without preop heparin (local lab)[c]	X	X									
AT Levels (central lab)[c]		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X
Hematology and coagulation[d]		X	X		X	X	X	X	X	X	X
Hematocrit				X[e]							
Clinical chemistry[f]		X	X		X	X	X	X	X	X	
Pregnancy test (serum)		X									
Retention sample[g]		X	X		X						X
Heparin dose				X							
ACT				X							
Protamine dose				X							
Study drug infusion				X							
Operative assessments[h]				X							
Chest-drain blood loss[i]					X						
Clinical outcomes[j]				X	X	X	X	X	X	X	X

[a] Subjects undergoing complex/combined procedures will not be prescreened or screened for AT levels. Only applicable for subjects undergoing isolated CABG or single valve repair/replacements who will not be receiving preoperative heparin (see entry criteria) and whose eligibility will therefore be based on AT level < 80%. If it is logistically feasible, Prescreening visit must be performed prior to Screening visit, unless a subject's surgery is scheduled to occur in ≤ 72 hours. In the latter case, the Prescreening and Screening visit may be combined. All determinations for a separate Prescreening visit must be recorded on specific Prescreening sheets.

[b] Randomization may occur 24 hours before the day of surgery (Day 0).

[c] Baseline AT activity levels (hematology lab): Prescreening and Screening visits (-14 days).. Subjects undergoing isolated CABG or single valve repair/replacements will be prescreened for AT levels (AT <80%) if they are not receiving preoperative heparin (see entry criteria). These AT activity levels will be measured locally at the investigator site. The AT (local lab) determination at the Screening visits will not be required if the Prescreening visit is conducted. All further AT determinations will be assessed at a central lab. AT activity levels will be measured immediately before and after infusion of AT-III (Human) or Placebo at Operative visit (Day 0).

[d] Hemoglobin, hematocrit, red blood cells, white blood cells, platelets, aPTT, PT, INR, fibrinogen, TAT, and F1+2.

[e] Hematocrit only (local lab).

[f] Creatinine, total bilirubin, ALT, AST, BUN, LDH, glucose, sodium, potassium, chloride, and calcium.

[g] An appropriate volume of blood will be drawn in order to obtain a serum/plasma retention sample. The retention sample will be frozen and stored at -70ºC and stored in the event that additional testing is required in the future for purposes of this study only (i.e. repeat testing, or confirmation of virology results). Viral nucleic acid testing (NAT) and viral serology testing will be performed only if the subject exhibits clinical signs and symptoms of viral infection during the one month follow-up period.

[h] Type of surgical procedure, duration of CPB and aortic clamp, and transfusion requirements.

[i] Chest-drain blood loss in the first 12 and 24 hours postoperatively.

[j] Postoperative clinical outcomes (major morbidity composite, transfusion requirements, surgical reexploration, low cardiac syndrome, myocardial infarction, stroke, acute kidney injury, arterial or venous thromboembolic events, infections, and postoperative mortality) and chest-drain blood loss in the first 12 and 24 hours postoperatively.