STATISTICAL ANALYSIS PLAN

TRIAL FULL TITLE	Intravenous Iron supplement for Iron deficiency in Patients with Severe Aortic Stenosis (IIISAS)
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SAP Signatures

I give my approval for the attached SAP entitled IIISAS dated 11th February 2022

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Abbreviations and Definitions

Abbreviation or special term	Explanation
AE	Adverse event
CANTAB	Cambridge Neuropsychological Test Automated Battery
CRF	Case report form (electronic/paper)
CRP	C-reactive protein
DMC	Data monitoring committee
EQ-5D-3L	EuroQoL five dimensions three levels [Questionnaire]
EQ VAS	EuroQoL visual analogue scale
GCP	Good clinical practice
ICH	International Conference on Harmonisation
KCCQ	Kansas City Cardiomyopathy Questionnaire
NT-proBNP	N-terminal pro-B-type natriuretic peptide
SAE	Serious adverse event
SF-36	Short form 36 [Questionnaire]
SUSAR	Suspected unexpected serious adverse reaction
TAVI	Transcatheter aortic valve implantation
TnT	Cardiac troponin T

1 Introduction

1.1 Preface

Iron deficiency is associated with poor exercise capacity, lethargy, and reduced quality of life. Iron deficiency is prevalent in patients with severe aortic stenosis. These patients have reduced exercise capacity and quality of life compared with the age and gender matched general population. Despite successful valve replacement, cardiac function may not return to normal, and symptoms may persist. We assume that some of these symptoms are due to iron deficiency and hypothesise that intravenous iron supplement will improve the functional capacity assessed by six-minute walk distance and New York Heart Association (NYHA) functional class, health-related quality of life, and muscle strength in patients with severe aortic stenosis and iron deficiency who undergo transcatheter aortic valve implantation (TAVI).

1.2 Purpose of the analyses

These analyses will assess the efficacy and safety of ferric derisomaltose (formerly known as iron isomaltoside) in comparison with placebo and will be included in the clinical study report.

2 Study Objectives and Endpoints

2.1 Study Objectives

The main goal of this study is to evaluate the effect of a single dose of intravenous ferric derisomaltose on exercise capacity after TAVI in patients with severe aortic stenosis and iron deficiency defined as serum ferritin < 100 µg/l or ferritin ≥100 and <300 µg/l in combination with a transferrin saturation < 20 %.

Secondary objectives are to assess the impact of treatment on: (i) quality of life (ii) NYHA functional class, and (iii) muscle strength, and (iv) iron stores.

Exploratory endpoints are to assess the impact of treatment on: (i) body composition, (ii) cognitive function, (iii) myocardial structure and function, (iv) markers of myocardial disease and inflammation, and (viii) safety and tolerability.

2.2 Endpoints

The primary endpoint will be the baseline-adjusted distance walked on a 6-minute walk test performed approximately 6 months after the trial intervention.

Secondary endpoints:

- Quality of life as assessed by the KCCQ, SF-36, EQ 5D 3L, and EQ-VAS questionnaires
- NYHA functional class
- Muscle strength as measured by the Kern MAP hand-held dynamometer

Exploratory endpoints:

- Body composition
- Myocardial structure and function
- Cognitive function as assessed by the CANTAB battery
- N-terminal pro-B-type natriuretic peptide (NT-proBNP)
- Cardiac troponin T (TnT)
- C-reactive protein (CRP)
- Inflammatory and vasoactive peptides

Safety endpoints:

- Complications during TAVI procedures
- Safety

Explanatory endpoints

- Iron stores

3 Study Methods

3.1 General Study Design and Plan

This is a single centre, balanced, randomised, placebo controlled, double blind, parallel group trial. Eligible patients are randomised 1:1 and allocated to intravenous iron supplement or matching placebo at baseline, after providing written informed consent and performing baseline exams. The patients receive the study intervention immediately after randomisation, and no more than 1 days after performing the baseline tests. The patients then return for efficacy tests the day before the TAVI, and 3 months later; approximately 6 months after the study intervention.

3.2 Study plan and assessments

	Baseline		At hospitalisation for TAVI	3 months after TAVI	12 months after TAVI (telephone interview)
Time	0	Study drug infusion	3 months	6 months	15 months
Informed consent	x				
Clinical examination	x		x	x	
Electrocardiogram	x		x	x	
NYHA classification	x		x	x	x
Echocardiography	x			x	
Safety samples	x		x	x	
Randomisation	x				
Biobank samples	x		x	x	
6-minute walk test	x		x	x	
Hand grip strength	x		x	x	
Body mass composition	x		x	x	
Cognitive function	x		x	x	
Quality of life	x		x	x	
Essential Frailty Toolset	x			x	
Hospitalisation	x		x	x	x
Adverse events				<-x->	

3.3 Inclusion-Exclusion Criteria

Patients will be screened for eligibility upon admittance for evaluation of severe aortic stenosis. We perform approximately 500 TAVI procedures for patients with aortic stenosis per year. Per routine, we measure ferritin and transferrin saturation in all these patients. We plan to recruit patients who are admitted for evaluation for surgery, and who have iron deficiency defined as serum ferritin < 100 µg/l or ferritin ≥100 and <300 µg/l in combination with a transferrin saturation < 20 %

3.3.1 Inclusion criteria

The following conditions must apply prior to administering the investigational medicinal product:

- Patients with aortic stenosis and peak flow velocity (>3.5 m/s) who are referred for aortic valve replacement with TAVI
- Iron deficiency defined as serum ferritin < 100 µg/l or ferritin ≥100 and <300 µg/l in combination with a transferrin saturation < 20 %.
- Age > 18 years.
- Signed informed consent and expected compliance with protocol.

3.3.2 Exclusion criteria

Patients will be excluded from the study if they meet any of the following criteria:

- Anaemia (Haemoglobin < 100 mg/l)
- Haemochromatosis
- Haemosiderosis
- Porphyria cutanea tarda
- Blood dyscrasias or any disorders causing haemolysis or unstable red blood cells
- Decompensated liver disease (Child-Pugh score 7 or higher)
- End-stage renal failure, i.e. eGFR < 15 ml/min or on renal replacement therapy
- Planned major surgery within 6 months
- Unresolved cancer predisposing to chronic bleeding or associated with life expectancy < 2 years
- On erythropoietin analogues
- Known sensitivity or intolerance to iron isomaltoside or other parenteral iron preparations
- Intravenous iron supplement within 6 months prior to inclusion
- A clear indication for intravenous iron supplement
- On oral iron substitution (unless the subject agrees to stop treatment prior to randomisation)
- Alcohol or drug abuse within 3 months of informed consent that would interfere with trial participation or any ongoing condition leading to decreased compliance with study procedures or study drug intake
- Intake of an investigational drug in another trial within 30 days prior to intake of study medication in this trial or participating in another trial involving an investigational drug and/or follow-up
- Failure to obtain written informed consent
- Inability to walk at least 100 meters over 6 minutes
- Women of child-bearing potential

3.4 Randomisation and Blinding

The Research Support Unit at Oslo University Hospital generated a balanced, permuted, variable block size randomisation list (in a 1:1: ratio for the two study arms). No stratification factors were included in the randomisation. A complete, sealed randomisation list containing details of all patient numbers and study group is stored as essential documentation within the Trial Master File in a locked office. A nurse who does not participate in the trial and who has no competing interest regarding study outcomes marks cards with "ferric derisomaltose 20mg/kg" or "placebo" and place them in sealed and numbered envelopes. Once the informed consent has been signed and the patient has received a trial number, a third-party nurse opens the corresponding envelope and prepares the study drug according to the information on the card. The administration of the study drug is performed by nurses who are not part of the study team. The study participants (patients) and all study personnel, including investigators, personnel assessing outcomes, study nurses, data analysts and treating physicians and nurses, are blinded to allocation to study drug.

3.5 Study Variables

Primary endpoint:

The primary endpoint, the six-minute walk distance, will be measured as the number of metres that the subject is able to walk unaided over six minutes. The patients walk back and forth on a 30-meter marked walking course on level ground in a hospital corridor with markings for each meter. Standardised encouragement is provided. Oxygen saturation, the level of exertion as measured by the Borg scale, and any reason for premature interruption are noted. The absolute number of metres walked will be used for the endpoint analysis regardless of whether the test was prematurely interrupted because the test subject stopped walking due to physical exertion or for medical reasons. However, if the test is interrupted due to logistical or external reasons, a new test will be performed later the same day, with a minimum of one hour's rest from the first test.

Secondary endpoints:

QoL: The first secondary endpoint is health-related quality of life. The overall summary score from the KCCQ, scaled from 0 to 100, will be the principal measure of quality of life. Important explanatory endpoints are whether the subject's summary score increases or decreases by more than 5 points (on the 0 to 100 scale) from baseline to follow-up, and the scores from the subscales of the KCCQ. For in-depth analyses of the impact of intravenous iron on quality of life in this population, we will also measure quality of life as assessed by the EuroQoL and SF-36 tools. The EuroQoL 5D 3L will be assessed as the sum of scores (1-3) for the five questions. Forms with more than one missing value will be discarded, whereas an average value will be imputed for forms with one missing variable. The scores for the SF-36v2 will be summed up and transformed to a based score of (mean ± standard deviation) 50 ± 10 as recommended for this version. If more than 20 % of values are missing, the forms will be regarded as invalid, and the patients with these forms will be excluded from the modified intention to treat analysis. If 20 % or fewer variables are missing, we will impute mean values derived from the existing values in the same subscore (mental or physical).

Iron deficiency: Iron deficiency will be reported as a dichotomous variable (whether the patient has iron deficiency defined as serum ferritin < 100 µg/l or ferritin ≥100 and <300 µg/l in combination

with a transferrin saturation < 20 %) at follow-up. As explanatory variables, we will also report ferritin and transferrin saturation values at follow-up, and the change in these parameters from baseline to follow-up.

Muscle strength: Muscle strength is measured with the KERN MAP Hand grip dynamometer. The participant's dominant hand is tested three times, and the best result is registered. Men and women are tested at a resistance of 40 kg and 20 kg respectively. The best result of the dynamometer test is reported in kg.

Myocardial function: Myocardial function is measured by echocardiography. Most patients with aortic stenosis have preserved left ventricular function as measured by ejection fraction. Strain imaging provides more sensitive measures of subtle improvements in left ventricular function. The principal measure of myocardial function in the IIISAS trial is peak left ventricular global longitudinal strain. Strain is a unitless measure of left ventricular deformation (stretching). When the left ventricle contracts, the longitudinal shortening (i.e., the shortening of the myocardium in the direction from the mitral valve insertion to the apex) is expressed as a percentage of the original length. The shortening of each left ventricular segment in a 16-segment left ventricular model will be averaged to generate global longitudinal strain. For in-depth analyses of cardiac function, we will also measure left ventricular ejection fraction, the E/E' ratio, left atrial volume, tricuspid annular peak systolic excursion, and the peak trans-tricuspid regurgitation pressure as estimated by the Bernoulli equation.

4 Sample Size

This trial is designed to assess the effect of intravenous iron on the distance walked during 6 min (6MWT) in patients with severe aortic stenosis with iron deficiency. We consider an increase in walk distance of 30 m a clinically meaningful improvement. With a mean difference between the groups of 30 m and an expected repeat-measurement standard deviation of 50 m, a power of 80% and an α of 5 %, we will need at least 44 patients in each group. To account for loss of information due to drop-out and to improve power for secondary endpoints, we aim to include 100 patients. After 50 patients have been through visit 3 (3 months after TAVI), the blinded results of the repeat-measurement standard deviation of the walk distance will be revisited, and the number of participants may be expanded to ensure sufficient power.

5 General Considerations

5.1 Timing of analyses

The final analysis will be performed after the last patient has completed the last follow-up visit 3 months after TAVI, and all data have been transferred to a separate file. The data must have met the cleaning and approval requirements of the primary investigator, and this Statistical Analysis Plan must have been finalised and approved by the primary investigator. Only when these requirements have been met, will database lock occur, and the randomisation code be opened.

5.2 Populations for analysis

5.2.1 Modified intention-to-treat Population (Primary analyses)

- All subjects who were randomised. For the primary endpoint, only patients who (irrespective of receipt of actual treatment) received TAVI and performed an adequate 6-minute walk test at baseline and at follow-up can be analysed. The adequacy of the walk-test must be determined before database lock and the randomisation code is opened. The primary endpoint will be assessed in a complete-case analysis for reasons explained in section 5.4, but in compliance with the intention-to-treat principle, the analysis will not exclude patients who have not received the study drug or have missing data beyond the 6-minute walk test.

5.2.2 Per Protocol Population

- All subjects who received the study drug and who received TAVI and performed baseline and follow-up tests.

5.2.3 Safety Population

- All subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving treatment.

Each subject's inclusion or exclusion status regarding each analysis population must be determined prior to breaking the blind. The status must be documented in the final database prior to breaking the blind.

5.3 Covariates and Subgroups

The primary analyses (intention-to-treat population) will not be adjusted for baseline demographics. However, the primary endpoint and key secondary endpoints are calculated as the baseline-adjusted values (i.e. the follow-up result is adjusted for the baseline result of the same assessment in ANCOVA analyses).

We will perform binary subgroup analyses of the primary endpoint stratified by gender; age above or below median; whether the baseline transferrin saturation is $< $ or ≥ 20 %; by whether ferritin is below 30 µg/L; and by whether the baseline haemoglobin is below, or above or equal to the WHO definition for anaemia: 12.0 g/dl for women and 13.0 g/dl for men. A forest plot will be used to communicate the results. The subgroup analyses are exploratory only, as the trial is not powered to show differences across subgroups.

5.4 Missing Data

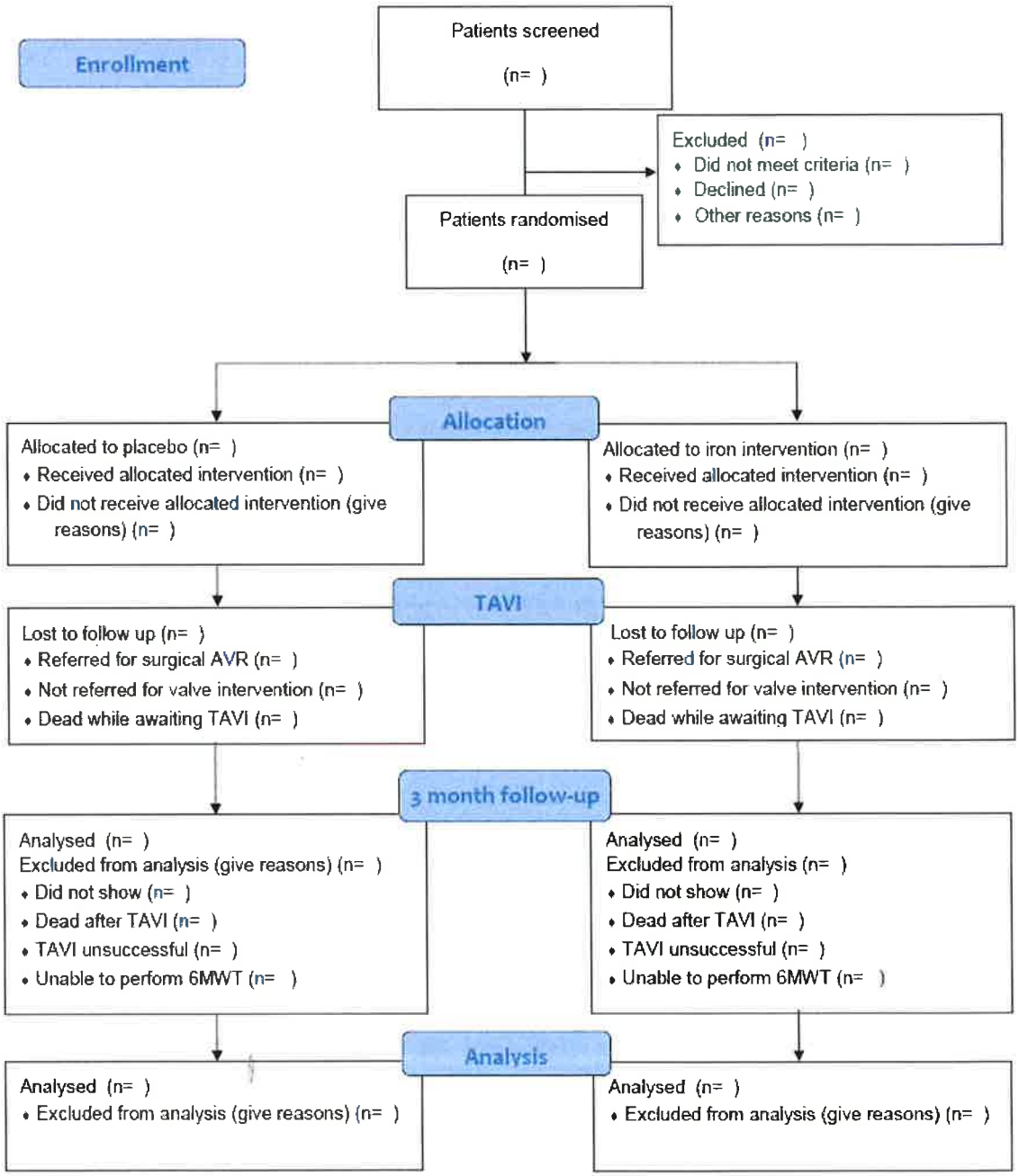
Because the efficacy data are collected twice only, and the endpoint analyses will be baseline adjusted ANCOVA or single point assessments only, there will be no imputation of missing data. The endpoint assessments will therefore be performed on the full analysis set in a modified intention to treat analysis. However, to elucidate possible biases this method entails, baseline data for patients for whom endpoint data exist will be compared with baseline data for patients who for some reason drop out or are unable to complete both sets of tests. We will also explore if the dropout rate differs between the treatment arms. The extent of missing data will be quantified for each endpoint variable.

We expect few missing baseline covariates and will not employ a strategy to impute missing baseline covariates.

6 Summary of Study Data

6.1 Subject Disposition

We will document how many subjects reached the various stages of the trial (the number screened, randomised, reached the 6-month follow-up visit and could be analysed for the primary endpoint), and how many dropped out and for what reasons (death, withdrew consent, failed to show up) according to the following CONSORT diagram:



6.2 Demographic and Baseline Variables

The baseline, intention-to-treat population demographics will be presented by columns for each treatment (Placebo, Intravenous ferric derisomaltose). All continuous variables will be summarised using the following descriptive statistics: mean ± standard deviation; median (interquartile range). The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. All summary tables will be structured with a column for each treatment in the order (Control, Experimental) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

The sample size of non-missing values for univariable summary statistics may be larger than the sample size of non-missing values in a complete-case analysis used in the primary regression (ANCOVA) analysis.

The following baseline variables will be presented:

Demography
Age, years
Male, sex
Smoker, current/previous smoker
Body mass index, kg/m^2
Systolic blood pressure, mmHg
Diastolic blood pressure, mmHg
Heart rate, rpm
Medical history
Previous cardiac arrest
Previous or current history of coronary artery disease
Hypertension
Diabetes mellitus
Hypercholesterolemia
Atrial fibrillation
Previous stroke or transient ischemic attack (TIA)
Peripheral vascular disease
Chronic obstructive pulmonary disease
Medication
Angiotensin-converting-enzyme (ACE) inhibitor/Angiotensin II Receptor Blockers (ARB)
Beta-blocker
Acetylsalicylic acid (ASA)
Non-ASA platelet inhibitors
Warfarin
Direct oral anticoagulation
Cholesterol lowering agent
Oral iron supplementation
Biochemistry
Hemoglobin, g/dL
N-terminal pro-B-type natriuretic peptide (NT-proBNP), ng/L
P-troponin T, ng/L
C-reactive protein (CRP), mg/L

Creatinine, µmol/L
Estimated glomerular filtration rate (eGFR), mL/min
Cholesterol mmol/L
LDL cholesterol, mmol/L
Ferritin, µg/L
Iron, µmol/L
Transferrin, g/L
Transferrin saturation (TSAT) (%)
Transferrin receptor, ng/mL
TIBC, µmol/L
Presence of ID, n (%)
Absolute (ferritin < 100 ug/L)
Functional (ferritin 100-300 µg/L and
TSAT (<20 %))
Echocardiography
Left ventricular ejection fraction, %
Aortic peak velocity, m/s
Aortic mean gradient, mm Hg
Aortic valve area, cm^2
NYHA functional class
Essential Frailty Toolset Score*
6 minute walk test (6MWT), meters
Hand grip strength, kilogram

6.3 Treatment Compliance

Because the study intervention consists of a single, intravenous infusion, there is no issue with treatment compliance. The intention-to-treat primary analyses will be performed regardless of whether or not the study drug infusion was completed, but the number of patients who did not receive the intervention will be reported for each group.

7 Efficacy Analyses

Data will be summarised by treatment group. N, Mean, Standard Deviation, Minimum and Maximum will summarise continuous efficacy variables, whereas number and percent will summarise categorical efficacy variables.

All analyses of the continuous efficacy variables (e.g. walk distance on the 6-minute walk test) will be performed as analysis of covariance with treatment group adjusting for study treatment (ANCOVA). Treatment groups will be tested at the 2-sided 5% significance level.

Secondary endpoints will be analysed in a non-hierarchical manner and without formal adjustment for multiple testing, but with due consideration to the fact that multiple testing increases the risk of chance findings, and that the results regarding secondary endpoints in a proof-of-concept trial must be regarded as hypothesis-generating only. A p-value < 0.05 will be considered statistically significant.

7.1 Primary Efficacy Analysis

The primary endpoint, the walk distance (in metres) on the 6-minute walk test three months after TAVI, will be analysed in the complete case set by ANCOVA. Treatment allocation will be the independent variable, and the baseline walk distance (in metres) will be the only covariate. A two-sided p-value of < 0.05 for the independent variable (i.e., that under the null hypothesis, such a large between-group difference will occur by chance alone in less than 5 % cases) will be taken as a reasonable cause to reject the null-hypothesis. In addition to the p-value, we will report the between-treatment group difference in baseline-adjusted walk distance with a 95% confidence interval.

7.2 Secondary Efficacy Analyses

All secondary continuous endpoints will be analyzed with ANOCVA, in the same manner as for the primary endpoint (see sec. 7.1).

All dichotomous endpoints will be analyzed with the Newcombe hybrid score interval and the Fisher mid-P test.

All categorical endpoints with more than two categories (i.e. NYHA) will be analyzed with the Wilcoxon-Mann-Whitney test.

Possible subgroup effects for continuous endpoints will be analyzed by adding an interaction term between the variable defining the subgroup and the treatment variable in the linear regression. The p-value for the interaction term will be reported, together with the within-subgroup treatment effects (in a forest plot).

7.3 Exploratory Efficacy Analyses

The effect of the treatment on cognitive function, myocardial structure and function, and markers of myocardial disease and inflammation, will be assessed in separate reports.

7.4 Explanatory endpoint analyses

The restoration of iron stores is not considered as a clinical endpoint, but is an important endpoint to explain any effect/lack of effect of the intervention on the clinically relevant endpoints listed above.

7.5 Sensitivity analyses

To determine the robustness of the analysis of the primary endpoint, we will perform sensitivity analyses with imputations for missing data. While we do not expect the trial intervention to affect dropouts, it is unreasonable to assume that dropouts will occur completely at random, and dropouts may therefore bias the final results. We therefore aim to do best-case, worst-case, no-change scenario imputations for missing data:

- Scenario 1 (best-case): missing data in intervention arm set to overall mean value + 1 standard deviation (SD); missing data in placebo arm set to overall mean value. The overall mean and SD are based on all observed values from both the intervention and placebo arms.
- Scenario 2 (worst-case): missing data in intervention arm set to overall mean value – 1 SD; missing data in placebo arm set to overall mean value

- Scenario 3 (no-change): all missing data in intervention and placebo arms set to mean value

8 Safety Analyses

Safety analyses will include tabulation of type and frequency of all adverse events. Any value of safety laboratory parameters outside normal ranges will be identified. The final report will include the absolute number of adverse and severe adverse events in each group. The results will be reported for the Safety Population only.

8.1 Adverse Events

Serious adverse events and adverse events of special interest; adverse reactions to the investigational medicinal products and paravenous leakage, will be reported with comprehensive narratives.