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1 List of abbreviations

AV: Atrio-Ventricular
BP: Blood Pressure
CDMS: Clinical Data Management System
CI: Confidence Interval
CIP: Clinical Investigation Plan
CIR: Clinical Investigation Report
CLS: Closed Loop Stimulation
CRO: Contract Research Organization
CRT: Cardiac Resynchronization Therapy
CTL: Control group
ECG: Electrocardiogram
eCRF: electronic Case Report Form
ESC: European Society of Cardiology
HR: Hazard Ratio
ICD: Implantable Cardioverter Defibrillator
IPG: Implantable Pulse Generator (pacemaker)
IRB/EC: Institutional Review Board/Ethics Committee
ITT: Intention to treat
LVEF: left ventricular ejection fraction
RCT: Randomized Clinical Trial
SBP: Systolic Blood Pressure
TT: tilt table test

2 Introduction

The BIOSync CLS study is designed to test the hypothesis that DDD pacing with CLS stimulation is able to (completely or partially) prevent syncopal recurrences in patients with vaso-vagal syncope and documentated asysitolic pause at a pre-implant Tilt-Table test (TT).

3.1 Objectives

The study has the primary objective of comparing time to first syncopal recurrence between

- active group (CLS): CLS in addition to DDD pacing mode; and
- control group (CTL): sensing only, ODO mode.

Definition. According to the 2018 ESC guidelines[1], a syncopal recurrence is defined as a transient complete loss of consciousness characterized by rapid onset, short duration, and spontaneous complete recovery.

The Secondary endpoint will be the time to the first recurrence of pre-syncope or syncope, whichever comes first, compared between the study groups during follow-up

Definition. Pre-syncope is defined as any of the various signs and symptoms which are recognized by the patients as premonitory of imminent syncope but not followed by syncope.

3.2 Primary hypotheses

Null: the 2-year survival rate of syncopal recurrence in the treatment arm (S_{CLS}) is equal to the 2-year survival rate of the control arm (S_{CTL}), assuming an exponential distribution with proportional hazard rates.

H_0: $S_{CLS}(t=2 \text{ years}) = S_{CTL}(t=2 \text{ years})$

Alternative: the 2-year survival rate of syncopal recurrence in the treatment arm is different from the 2-year survival rate of the control arm.

H_1: $S_{CLS}(t=2 \text{ years}) \neq S_{CTL}(t=2 \text{ years})$

3.3 Secondary hypotheses

Null: the 2-year survival rate to the combined event of pre-syncope or syncope, $\Sigma(t)$, is equal in both study groups

H_0: $\Sigma_{CLS}(t=2 \text{ years}) = \Sigma_{CTL}(t=2 \text{ years})$

Alternative: the 2-year survival rate to the combined event of pre-syncope or syncope is different in the two study groups.

H_1: $\Sigma_{CLS}(t=2 \text{ years}) \neq \Sigma_{CTL}(t=2 \text{ years})$

3 Investigation plan

The study is a prospective, multi-center, double-blinded, randomized, intention-to-treat, placebo-controlled study.

Patients will be randomized to the active group or to placebo immediately after their enrolment and before any subsequent study-related procedure.

- Active group: before post-implant hospital discharge, IPG will programmed in a dual-chamber DDD pacing mode with the CLS function ON.
- Control group: before post-implant hospital discharge, pacing will be programmed in the ODO mode.

Randomization ratio will be 1:1, therefore the randomization procedure will ensure that each individual patient will have (approximately) 50% chance to be assigned to the active group or to the control.

Patient selection adheres to the class IIb indication for cardiac pacing of the guidelines of the European Society of Cardiology[1].

4.2 Inclusion criteria

Patients affected by clinical diagnosis of reflex (neurally-mediated) syncope who meet all the following criteria:

- age ≥40 years
- significant limitation of social and working life due to unpredictable frequent syncope recurrences, ≥2 of which within the last year.
- type 2B cardio-inhibitory response (VASIS classification) during TT performed according to the 'Italian protocol'.(7, 17)
- alternative therapies have failed or were not feasible.
- exclusion of other possible competitive causes of syncope.

4.3 Exclusion criteria

- Any classified indication to pacemaker different from reflex syncope with positive Tilt Test response
- Any classified indication implantable defibrillator (ICD), cardiac resynchronization therapy (CRT), according to current guidelines
- Any cardiac dysfunctions likely leading to loss of consciousness:
  - overt heart failure;
  - ejection fraction (LVEF) <40% (Echo-assessed within 3-month prior to study participation);
  - myocardial infarction;
  - diagnosis of hypertrophic or dilated cardiomyopathy;
  - clinically significant valvular disease;
  - sinus bradycardia <50 bpm or sinoatrial block;
  - Mobitz I second degree atrioventricular block;
  - Mobitz II second or third-degree atrioventricular block;
  - bundle-branch block;

- rapid paroxysmal supraventricular tachycardia or ventricular tachycardia;
- pre-excited QRS complexes;
- prolonged QT interval;
- Brugada syndrome;
- arrhythmogenic right ventricular cardiomyopathy;

- Symptomatic orthostatic hypotension diagnosed by standing BP measurement;
- Nonsyncopal loss of consciousness (eg, epilepsy, psychiatric, metabolic, drop-attack, cerebral transient ischemic attack, intoxication, cataplexy).
- Symptomatic cardio-inhibitory carotid sinus hypersensitivity.

4.4 Study procedures

At enrolment, patients will receive anonymous syncope and pre-syncope assessment questionnaires to be self-administered upon each primary and secondary endpoint ever experienced and mailed (by ordinary mail) to an external CRO every 3 months after discharge, using anonymous pre-paid and pre-addressed envelops.

Patients will be also visited in hospital at 12-, and 24-months. More frequent scheduled visits are optional and can be performed according to the site clinical practice. However, any scheduled or unscheduled in-hospital visit must be documented in the appropriate eCRF. Optionally, a 1-month (±14 days) in-hospital visit may be performed to repeat the TT.

4 Determination of sample size

The study sample size calculation is based on the minimum relative difference in the 2-year incidence of syncopal recurrences as compared with placebo (pacemaker OFF).

The 2-year incidence of the primary endpoint in the control group will be assumed equal to the incidence observed in the control arm of the ISSUE 3 trial: [2] this was reported as high as 57%. The ISSUE 3 trial was considered as it selected a large population, had very similar inclusion/exclusion criteria, was recently published.

The BIOSync CLS study is designed to detect a 40% relative reduction of the 2-years incidence of syncopal recurrences (from 57% to 34%) with a statistical Type I and II errors of 0.05 (bilateral) and 0.20, respectively.

Further assumptions:

- Exponential distribution of times to first recurrence
- Accrual time: 2 years
- Total study time: 4 years
- Randomization ratio: 1:1
- Loss incidence: 10% (both arms)

With these assumptions a sample size of 62 patients per study arm (124 in total) is required. This estimate must be further increased by 2%, due to the slight power loss induced by the interim analyses.

In summary, 128 subjects (64 per study arm) are necessary to reach the study primary objective with the required power.

The required number of primary endpoint events is 62, as calculated with the formula:

$$events = \frac{\left(z_{\alpha/2} + z_{\beta}\right)^2}{0.25 \log(HR)}$$

where $z_{\alpha/2}$ and z_{β} are the standard normal percentiles (α=0.05, β=0.20), HR is the expected hazard ratio. A HR of about 0.49 was calculated assuming an exponential model of the survival functions with proportional hazard rates.

5 Database cleaning

Data entered into the Clinical Data Management System (CDMS) is checked with programmed quality checks: input errors, discrepancies, missing data, and out-of-range values are resolved automatically (CDMS) or manually (clinical monitor, data manager) by means of data queries.
The CDMS supports detailed tracking of the query process. Corrections to eCRF can only be done by the designated site personnel and need to be signed by the investigator. All changes are automatically recorded in the system's audit-trail.

6 Statistical methods

7.1 General methodology

Continuous variables will be summarized using tables of descriptive statistics: number of patients with recorded observations, mean, standard deviation, median, interquartile boundaries, minimum and maximum. Categorical variables will be summarized using counts and percentages.
Descriptive statistics will be presented by randomization group.
Comparison of continuous parameters between two groups will be performed using T-test or Wilcoxon Man-Whitney U test, after testing for normality with the Shapiro-Wilk test. Categorical variables will be compared using the Chi-Square or Fisher exact test, as appropriate.
For the analysis of the primary and the secondary endpoint the Kaplan-Meier plots will be generated and the estimated survival functions of the study groups tested with the two- sided log-rank test. Dependence of survival on major baseline predictors will be evaluated with proportional hazard Cox models. Proportionality of hazards will be tested with the Schoenfeld Residuals test. Hazard ratios and relative 95% confidence intervals for each predictor will be calculated, respectively. Data will be censored at the date of last patient contact. The ITT principle will be applied.
All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. All confidence intervals presented will be 95% and two-sided. Statistical calculations will be carried out by using STATA 11.1 ®, SAS 9.3 © or R.

7.2 Handling of missing data

Missing or spurious data is not substituted; all data – as far as correctly measured – is analyzed. No method of imputation will be used for missing data.

In some cases, particular clinical data is excluded from analysis. The following reasons are possible:

- Data is not measured as described in the latest applicable version of the clinical investigation plan.
- Data is evaluated beyond the required follow-up schedule.
- Patient has to be excluded because she/he does not fulfill the exclusion/inclusion criteria.
- Patient requires that all recorded data has to be deleted.
- There is evidence of incomplete or incorrect patient consent process.

7.3 Baseline list variables

The following table reports the description of relevant baseline variables.

Variable name	List of values	Unit of measure	Description
rd_display	0 (ODO); 1 (DDD-CLS)	-	Randomization group
gi_age	Numeric	Years	Age at enrolment
gi_gender	0 (Male); 1 (Female)	-	Gender
hs_syncop_life	Numeric	Unit	Total number of syncope during lifetime
hs_syncop_lastyear	Numeric	Unit	Total number of syncope during last year
hs_age_syncop	Numeric	Years	Age of first syncope
hs_histor_presync	0 (No); 1 (Yes)	-	History of pre-syncope
prevepi_hosp_sync	0 (No); 1 (Yes)	-	Was the patient hospitaliz ed because of syncope?
com_hypertension	0 (No); 1 (Yes)	-	Hypertension
com_diabetes	0 (No); 1 (Yes)	-	Diabetes
com_neuro_disease	0 (No); 1 (Yes)	-	Neurological disease
hdis_hypertensive_disease	0 (No); 1 (Yes)	-	Hypertensive disease
hdis_ca_disease	0 (No); 1 (Yes)	-	Coronary artery disease
hdis_valvular_disease	0 (No); 1 (Yes)	-	Valvular disease
hdis_hyp_cardiomyopath	0 (No); 1 (Yes)	-	Hypertrophic cardiomyopathy
hdis_dilated_cardiomyopath	0 (No); 1 (Yes)	-	Dilated cardiomyopathy
arr_fst_av_block	0 (No); 1 (Yes)	-	First degree AV block
arr_atr_tachycardia	0 (No); 1 (Yes)	-	Atrial tachycardia
arr_paroxy_afib_af	0 (No); 1 (Yes)	-	Paroxysmal atrial fibrillation/flutter
arr_perm_afib_af	0 (No); 1 (Yes)	-	Permanent atrial fibrillation/flutter
ecg_normal_sr	0 (No); 1 (Yes)	-	Normal sinus rhythm at enrolment
ecg_afib_af	0 (No); 1 (Yes)	-	Atrial fibrillation/Flutter at enrolment
ecg_mean_hr	Numeric	Beats per minute	Mean heart rate
ecg_qrs	Numeric	msec	QRS duration
ecg_pr_interval	Numeric	msec	PR interval
echo_ejection_fraction	Numeric	%	Ejection fraction
ortho_supl_syst_bp	Numeric	mmHg	Supine systolic blood pressure
ortho_low_syst_bp	Numeric	mmHg	Lowest upright systolic blood pressure
ortho_max_syst_bp	Numeric	mmHg	Maximal drop in systolic blood pressure
carotid_rr_pause	Numeric	sec	Longest RR pause at carotid sinus massagge
carotid_syncope_presyncope	0 (No); 1 (Yes)	-	Syncope or pre-syncope at carotid

			sinus massagge
cm_ace	0 (No); 1 (Yes)	-	ACE Inhibitors
cm_anticoagulant	0 (No); 1 (Yes)	-	Anticoagulants
cm_antiplatelets	0 (No); 1 (Yes)	-	Antiplatelets
cm_b_blocker	0 (No); 1 (Yes)	-	Beta-blockers
tt_response	0 (Pre-syncope); 1 (Syncope)	-	Response to tilt test
tt_pause_max	Numeric	sec	Maximum asystolic pause at tilt test
tt_pause_type	0 (Sinus arrest); 1 (AV block)	-	Type of pause at tilt test
tt_time_pre_sync	Numeric	min	Time to symptom onset (from tilt up if positive during passive phase or from TNT administration if positive during the TNT phase)
tt_bp_pre_sync	Numeric	mmHg	Systolic BP at symptom onset
tt_low_bp	Numeric	mmHg	Lowest systolic blood pressure during tilt test

7.4 Baseline data analysis

*Baseline Analysis (STATA script)

*Age at enrolment
tabstat gi_age, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk gi_age
ranksum gi_age, by(rd_display)

*Gender
tabulate gi_gender rd_display, chi2 column exact

*Total number of syncope during lifetime
tabstat hs_syncop_life, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk hs_syncop_life
ranksum hs_syncop_life, by(rd_display)

*Total number of syncope during last year
tabstat hs_syncop_lastyear, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk hs_syncop_lastyear
ranksum hs_syncop_lastyear, by(rd_display)

*Age of first syncope
tabstat hs_age_syncop, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk hs_age_syncop
ranksum hs_age_syncop, by(rd_display)

*History of pre-syncope
tabulate hs_histor_presync rd_display, chi2 column exact

*Was the patient hospitaliz ed because of syncope?
tabulate prevepi_hosp_sync rd_display, chi2 column exact

*Hypertension
tabulate com_hypertension rd_display, chi2 column exact

*Diabetes
tabulate com_diabetes rd_display, chi2 column exact

*Neurological disease
tabulate com_neuro_disease rd_display, chi2 column exact

*Hypertensive disease
tabulate hdis_hypertensive_disease rd_display, chi2 column exact

*Coronary artery disease
tabulate hdis_ca_disease rd_display, chi2 column exact

*Valvular disease
tabulate hdis_valvular_disease rd_display, chi2 column exact

*Hypertrophic cardiomyopathy
tabulate hdis_hyp_cardiomyopath rd_display, chi2 column exact

*Dilated cardiomyopathy
tabulate hdis_dilated_cardiomyopath rd_display, chi2 column exact

*First degree AV block
tabulate arr_fst_av_block rd_display, chi2 column exact

*Atrial tachycardia
tabulate arr_atr_tachycardia rd_display, chi2 column exact

*Paroxysmal atrial fibrillation/flutter
tabulate arr_paroxy_afib_af rd_display, chi2 column exact

*Permanent atrial fibrillation/flutter
tabulate arr_perm_afib_af rd_display, chi2 column exact

*Normal sinus rhythm at enrolment
tabulate ecg_normal_sr rd_display, chi2 column exact

*Atrial fibrillation/Flutter at enrolment
tabulate ecg_afib_af rd_display, chi2 column exact

*Mean heart rate
tabstat ecg_mean_hr, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk ecg_mean_hr
ranksum ecg_mean_hr, by(rd_display)

*QRS duration
tabstat ecg_qrs, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk ecg_qrs
ranksum ecg_qrs, by(rd_display)

*PR interval
tabstat ecg_pr_interval, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk ecg_pr_interval
ranksum ecg_pr_interval, by(rd_display)

*Ejection fraction
tabstat echo_ejection_fraction, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk echo_ejection_fraction
ranksum echo_ejection_fraction, by(rd_display)

*Supine systolic blood pressure
tabstat ortho_supl_syst_bp, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk ortho_supl_syst_bp
ranksum ortho_supl_syst_bp, by(rd_display)

*Lowest upright systolic blood pressure
tabstat ortho_low_syst_bp, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)

swilk ortho_low_syst_bp
ranksum ortho_low_syst_bp, by(rd_display)

*Maximal drop in systolic blood pressure
tabstat ortho_max_syst_bp, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk ortho_max_syst_bp
ranksum ortho_max_syst_bp, by(rd_display)

*Longest RR pause at carotid sinus massagge
tabstat carotid_rr_pause, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk carotid_rr_pause
ranksum carotid_rr_pause, by(rd_display)

*Syncope or pre-syncope at carotid sinus massagge
tabulate carotid_syncope_presyncope rd_display, chi2 column exact

*ACE Inhibitors
tabulate cm_ace rd_display, chi2 column exact

*Anticoagulants
tabulate cm_anticoagulant rd_display, chi2 column exact

*Antiplatelets
tabulate cm_antiplatelets rd_display, chi2 column exact

*Beta-blockers
tabulate cm_b_blocker rd_display, chi2 column exact

*Response to tilt test
tabulate tt_response rd_display, chi2 column exact

*Maximum asystolic pause at tilt test
tabstat tt_pause_max, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk tt_pause_max
ranksum tt_pause_max, by(rd_display)

*Type of pause at tilt test
tabulate tt_pause_type rd_display, chi2 column exact

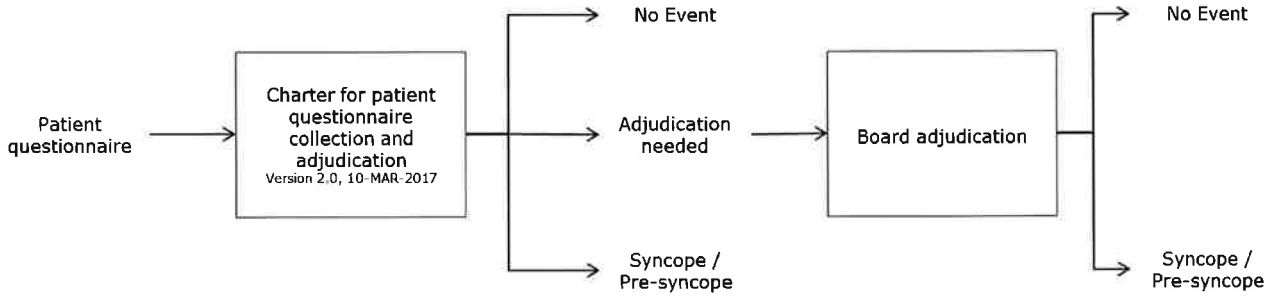
*Time to symptom onset(from tilt up if positive during passive
*phase or from TNT administration if positive during the TNT phase)
tabstat tt_time_pre_sync, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk tt_time_pre_sync
ranksum tt_time_pre_sync, by(rd_display)

*Systolic BP at symptom onset
tabstat tt_bp_pre_sync, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk tt_bp_pre_sync
ranksum tt_bp_pre_sync, by(rd_display)

*Lowest systolic blood pressure during tilt test
tabstat tt_low_bp, statistics(mean sd p50 p75 p25) by(rd_display) columns (statistics)
swilk tt_low_bp
ranksum tt_low_bp, by(rd_display)

7.5 Evaluation of study objectives

The BIOSync CLS study (BA103, CIP ver. 7.0) gathers primary and secondary endpoints' data through self-administered patient questionnaires. In order to ensure double-blinding in endpoint assessment, the questionnaires will be collected by external personnel of an appointed CRO.
This process is descripted in detail in the Charter for patient questionnaire collection and adjudication, version 2.0, 06-Mar-2017, and summarized in the below image.



At the end of the adjudication the following derived variables will be created.

Variable name	List of values	Unit of measure	Description
pe	0 (No); 1 (Yes)	-	Occurrence of syncope episode
time_pe	Numeric	days	If pe=1: time to the first post randomization recurrence of a syncopal episode; If pe=0: total follow-up time
se	0 (No); 1 (Yes)	-	Occurrence of pre-syncope or syncope episode
time_pe	Numeric	days	If pe=1: time to the first post randomization recurrence of a pre-syncope or syncope episode whichever comes first.; If pe=0: total follow-up time

The analysis of the primary and secondary study objectives will be perfomed with the following code (STATA language).

```
*Study objectives evaluation

*Primary endpoint

stset time_pe, failure(pe==1)
*Kaplan_meier plots generation
sts graph by(rd_display), ci risktable
*Comparison of survivors functions
sts test rd_display, logrank
*Proportional hazard Cox models for major baseline predictors
stcox rd_display
estat phtest
stcox gi_age
stcox gi_gender
stcox hs_syncop_life
stcox hs_syncop_lastyear
stcox ecg_mean_hr
stcox tt_response
stcox tt_pause_max
stcox tt_pause_type
stcox tt_time_pre_sync
```

```
stcox tt_low_bp

*Secondary endpoint

stset time_se, failure(se==1)
*Kaplan_meier plots generation
sts graph, by(rd_display) ci
*Comparison of survivors functions
sts test rd_display, logrank
*Proportional hazard Cox models for major baseline predictors
stcox rd_display
estat phtest
stcox gi_age
stcox gi_gender
stcox hs_syncop_life
stcox hs_syncop_lastyear
stcox ecg_mean_hr
stcox tt_response
stcox tt_pause_max
stcox tt_pause_type
stcox tt_time_pre_sync
stcox tt_low_bp
```

7.6 Interim analysis

Interim analyses will be performed at t = 0.4 and 0.7 of the required primary endpoint events or, equivalently, after 25 and 43 endpoint events will be collected. The final analysis will be performed after the study database has been closed out. In order to keep the overall type I error at the level of 0.05, two-sided, symmetric O'Brien–Fleming boundaries generated with the Lan–DeMets spending function approach to group-sequential testing will be assumed as early stopping rules for efficacy.

t	0.40	0.70	1.00
No. endpoint events	25	43	62
Z_{up}	3.3569	2.4445	2.0005
Z_{low}	-3.3569	-2.4445	-2.0005
p	0.0008	0.015	0.05

7.7 Clinical Investigation Plan (CIP) deviations

All CIP deviations will be classified with details of the type of deviation and the full-list will be provided in the Clinical Investigational Report (CIR).
The number (and percentage) of patients with major and minor protocol deviations will be summarized by randomization group.
A post-hoc per-protocol analysis according to the existing data gathered could be run after the evaluation of the provided deviations.

7 References

(1) Brignole M, Moya A, de Lange FJ, Deharo JC, Elliott PM, Fanciulli A, Fedorowski A, Furlan R, Kenny RA, Martín A, Probst V, Reed MJ, Rice CP, Sutton R, Ungar A, van Dijk JG; ESC Scientific Document Group. 2018 ESC Guidelines for the diagnosis and management of syncope. Eur Heart J. 2018; 39(21):1883-1948.

(2) Brignole M, Menozzi C, Moya A, Andresen D, Blanc JJ, Krahn AD, Wieling W, Beiras X, Deharo JC, Russo V, Tomaino M, Sutton R; International Study on Syncope of Uncertain Etiology 3 (ISSUE-3) Investigators. Pacemaker therapy in patients with neurally mediated syncope and documented asystole: Third International Study on Syncope of Uncertain Etiology (ISSUE-3): a randomized trial. Circulation. 2012; 125(21): 2566-71.