STUDY PROTOCOL

DYDA 2 TRIAL

Effects of the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin on left ventricular myocardial dysfunction in patients with type 2 Diabetes mellitus and concentric left ventricular geometry

Protocol Number G113

A national, multicentre, randomized, double blind, placebo-controlled, parallel group, Phase III study

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A study conducted in collaboration

AMD (Associazione Medici Diabetologi)

ANMCO (Associazione Nazionale Medici Cardiologi Ospedalieri)

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Protocol:
Effects of the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin on left ventricular myocardial DYsfunction in patients with type 2 DiAbetes mellitus and concentric left ventricular geometry (DYDA TRIAL)
A national, multicentre, randomized, placebo controlled, double blind, parallel group, Phase III study

Approved by:

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Signature page for the Principal Investigator

Protocol:
Effects of the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin on left ventricular myocardial DYsfunction in patients with type 2 DiAbetes mellitus and concentric left ventricular geometry (DYDA TRIAL)

A national, multicentre, randomized, placebo controlled, double blind, parallel group, Phase III study

I have read this protocol and I agree to carry out this trial in compliance with all protocol conditions and with the Declaration of Helsinki

Principal Investigator  Signature  Date
________________________________________  ______________________________  __________
## List of abbreviations

<table>
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<th>Definition</th>
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<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>DSMC</td>
<td>Data and Safety Monitoring Committee</td>
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<tr>
<td>DPP-4</td>
<td>Dipeptidyl-peptidase-4</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic polypeptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricular</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>sc-MS</td>
<td>Stress-corrected midwall shortening</td>
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<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
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SUMMARY

Glucagon-like peptide-1 (GLP-1) receptors have been found in myocardium and endothelium and the hypothesis that they exert a protective anti-apoptotic effects has been proposed. Experimental data suggest GLP-1 to have direct effects on the cardiovascular (CV) system, in addition to their classic glucoregulatory actions. These direct effects may be cardioprotective, contractility improving and vasorelaxant. A few preliminary clinical trials in humans appear to support a mechanical function improvement after GLP-1 administration to patients with a weakened left ventricle. GLP-1 receptors agonists such as exenatide have been shown to attenuate lethal injury to the post-ischemic reperfused myocardium. To date, little information is available on the effect of dipeptidyl-peptidase-4 (DPP-4) inhibitors and the recent, unexpected and unexplained increase of hospitalizations for HF of patients treated with the DDP-4 inhibitor saxagliptin in the SAVOR TIMI trial, warrants in-depth investigations in this field. In animal experiments (rats), a cardioprotective function of linagliptin in the setting of acute myocardial infarction and of uraemic cardiomyopathy has been demonstrated. On these grounds, the effect on left ventricular (LV) function of the addition of linagliptin in patients with type 2 diabetes mellitus (T2DM), fairly controlled with usual care, is addressed with a randomized, placebo-controlled, trial in patients with concentric LV geometry. The aim is to collect information on the efficacy and safety of linagliptin administered as add-on therapy in patients with T2DM with early-stage LV systolic dysfunction.
1. INTRODUCTION

A remarkable proportion of patients with T2DM without overt cardiac disease have asymptomatic LV systolic dysfunction (1-6) even though conventional echocardiographic indexes of chamber function such as LV ejection fraction are normal (7,8). This condition of subclinical systolic dysfunction is revealed by the assessment of stress-corrected midwall shortening (sc-MS), which identifies early systolic impairment of circumferential LV myocardial fibers (8). It has been shown that impaired sc-MS is an early and reliable indicator of the transition phase between normal cardiac function and clinically manifest congestive heart failure (9) as well as a potent predictor of adverse CV outcomes both in patients with hypertension (10) and in those with chronic heart failure but preserved LV ejection fraction (11). Such early impairment in systolic LV function is strongly associated with the presence of baseline LV hypertrophy and concentric LV remodeling (1-4, 7), so explaining the high prevalence of this pathologic condition among patients with T2DM, whose left ventricles typically tend to remodel toward concentric geometry (3,4). A significant, positive association between impaired sc-MS and risk of CV mortality was observed several years ago in non-diabetic patients with hypertension (10), in patients with chronic heart failure and preserved LV ejection fraction (11), and, more recently, in people with T2DM (12). Neither in hypertensives, in patients with chronic heart failure or in those with T2DM a randomized intervention trial aimed to test the effect of any drug on sc-MS and on clinical outcomes has never been performed.

DPP-4 inhibitors (gliptins) are oral incretin-based glucose-lowering agents with proven efficacy and safety in the management of T2DM. In addition, preclinical data and mechanistic studies suggest a possible additional non-glycemic beneficial action on blood vessels and the heart, via both GLP-1-dependent and GLP-1-independent effects. In patients with T2DM, DPP-4 inhibitors improve several CV risk factors such as glucose and blood pressure control, postprandial (and even fasting) lipemia, they reduce inflammatory markers and oxidative stress, improve endothelial function and reduce platelet aggregation (13). In addition, positive effects on the myocardium have been described in patients with ischemic heart disease. Results of a meta-analysis of phase 2/3 controlled trials suggest a cardioprotective effect with a significant lower incidence of major CV events with sitagliptin, vildagliptin, saxagliptin, linagliptin, or alogliptin compared with placebo or other active glucose-lowering agents (13). To date, little information is available on the effect of dipeptidyl-peptidase-4 (DPP-4) inhibitors on ventricular function. Recently, unexpected and unexplained increase of hospitalization for HF of patients treated with the DDP-4 inhibitor saxagliptin in the SAVOR TIMI 53 trial raised some questions on the safety of this compound (14). Likewise, a subsequent meta-analysis of randomized clinical trials of all DPP4 inhibitors, which
did not rule out the possible increased risk of HF in patients treated with this subclass (15), supports the need for an in-depth investigations in this field.

Linagliptin is an oral, once-daily DPP-4 inhibitor that prevents the inactivation of incretin hormones GLP-1 and glucose-dependent insulinotropic peptide, which stimulate glucose-dependent secretion of insulin. In large clinical trials undertaken in patients with T2DM, linagliptin as monotherapy or in combination with other oral antidiabetic drugs has shown clinically meaningful efficacy with a low risk of hypoglycemia and no weight gain (16-18). A meta-analysis of linagliptin phase III studies showed no increased CV risk with linagliptin (19). Furthermore, it has been demonstrated that linagliptin reduces blood pressure and improves intracellular Calcium mishandling and cardiomyocyte ultrastructure, which collectively result in improvements in diastolic LV function and reduces infarct size after myocardial ischemia/reperfusion in rats (20,21). The effects of linagliptin on systolic LV function in patients with T2DM is still unknown.

2. OBJECTIVES OF THE STUDY

2.1 Primary objective
The primary objective of the study is to evaluate the effect of linagliptin 5 mg daily versus the corresponding placebo on the LV systolic function (measured by midwall shortening analysis) in patients with T2DM and a documented baseline concentric LV geometry and LV systolic dysfunction.

2.2 Main secondary objectives
1. Assessment of changes in diastolic LV function. Transmitral peak E wave (pulse Doppler) and early diastolic Tissue Doppler velocity of mitral annulus (E’) will be used to classify LV diastolic function together with other parameters (E/A ratio of transmitral flow, deceleration time of E, left atrial volume, pulmonary artery systolic pressure) in 4 degrees: normal, mild dysfunction, moderate dysfunction and severe dysfunction. Changes from baseline and 48-weeks evaluation will be analyzed.

2. Assessment of changes in LV systolic longitudinal function (measured by tissue Doppler technique) (peak systolic velocity of S’ wave of mitral annulus). Changes from baseline and 48-weeks evaluation will be analyzed. Incidence of patients who have an improvement in S’>25% from baseline (comparison between treated and not treated group).
3. STUDY DESIGN AND SETTING

Multicentre, randomized, double blind, parallel group comparison of an DPP-4 inhibitor, linagliptin 5 mg od, versus placebo in patients with T2DM and a documented baseline concentric LV geometry and LV systolic dysfunction. Patients will be centrally randomized in a 1:1 ratio to receive either linagliptin or placebo.

During the double-blind study treatment period, the management of glycemia will be left to the Investigator’s judgment informed by clinical guidelines (see Appendix 1). The Investigator will therefore be allowed to undertake appropriate action, i.e.:

- Adjust the background antidiabetic treatment.
- Prescribe an additional antidiabetic medication according to its labeling (with the exclusion of other DPP-4 inhibitor or GLP-1 receptor agonist).

The enrollment period will last 12 months. The patients will be enrolled by Centers in which subjects with T2DM are usually managed by the clinical and diagnostic point of view, including standard echocardiographic examination performed in referred echo-lab. The patients will be followed up for 48 weeks from randomization.

At Visit 1 (Day -7 to -1) patients will be assessed for eligibility for study participation. The researchers will take into consideration the T2DM patients with a history of LV hypertrophy diagnosed and documented by an ECG and/or a history of concentric LV geometry diagnosed by standard echocardiography within 1 year before Visit 1. The diagnosis of LV hypertrophy by ECG evaluation can be made using one of the following criteria, all characterized for having a very high specificity: Gubner, Lewis, Perugia, Perugia 2, Framingham, Sokolow-Lyon, Romhilt-Estes, Cornell, RV6/RV5 >1.

Patients with LV hypertrophy diagnosed by an ECG and/or a history of concentric LV geometry diagnosed by standard echocardiography will be asked to give their consent to participate to the study and to perform a complete transthoracic ColorDoppler echocardiographic examination and an ECG in order to verify if they fulfill all the inclusion/exclusion criteria. Blood will be collected for local laboratory assessment of creatinine and HbA1c if not available within prior 3 months.

If eligible, the patient will be randomized at Visit 2 (Day 0) into one of the two treatment groups and blood samples collected for central analyses. The patient will then be treated with either 5 mg linagliptin or placebo once daily.

After the randomization the patients will have a control visit after 2 weeks (Visit 3) and at 3 months from randomization (Visit 4, week 12). At Visit 4 blood samples will be collected.
Afterwards the patients will have one control visit at 24 weeks from randomization (Visit 5) and a final visit at 48 weeks from randomization (Visit 6) with echocardiogram and ECG performed and blood samples collected.

Patients still on study treatment at the time of final visit (Visit 6) will have a post treatment safety follow up (clinical visit or phone contact) 30 days after the study treatment discontinuation.

4. STUDY POPULATION

4.1 Inclusion criteria

- Men and women aged equal to or more than 40 years at screening.
- Patients with history of T2DM lasting at least six month prior to the screening visit.
- HbA1c ≤ 8.0% (≤ 64 mmol/mol) at screening.
- Evidence of sinus rhythm at screening ECG evaluation
- No clinical signs/symptoms of a cardiac disease and no evidence of coronary artery disease on the basis of clinical, electrocardiographic and echocardiographic evaluation at screening.
- Evidence at baseline echocardiographic examination of concentric left ventricular geometry, defined as relative wall thickness ≥ 0.42. Relative wall thickness was calculated as the end-diastolic ratio 2* posterior wall thickness/LV diameter.
- Evidence at baseline echocardiographic examination of LV systolic dysfunction defined as Midwall shortening (MFS) ≤15%
- Obtained informed consent

4.2 Exclusion criteria

- Patients with a confirmed indication for an incretin treatment
- Uncontrolled diabetes: HbA1c > 8.0% (> 64 mmol/mol) or Fasting Plasma Glucose > 300 mg/dL measured at screening visit.
- Glitazones within the last three months
- Permanent atrial fibrillation
- Uncontrolled hypertension (defined as systolic blood pressure > 160 and/or diastolic blood pressure > 90)
• Unstable dosage and changes in type of antihypertensive, lipid lowering and antidiabetic drugs within 4 weeks before the screening visit.

• Severe chronic renal dysfunction (defined as estimated glomerular filtration rate < 30 ml/min/1.73 m²).

• Previous or current documented history of untreated (by using CPAP) obstructive sleep apnea syndrome

• Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis.

• Previous or current documented history of malignant disease

• Pregnancy and breast feeding

• Documented alcohol and drug abuse

• Anticipated poor compliance

• Current participation in a clinical trial with other investigational products

5. STUDY TREATMENTS

5.1 Description

Linagliptin is an inhibitor of DPP-4, an enzyme that degrades the incretin hormones GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). Thus, linagliptin increases the concentrations of active incretin hormones, stimulating the release of insulin in a glucose-dependent manner and decreasing the levels of glucagon in the circulation. Both incretin hormones are involved in the physiological regulation of glucose homeostasis. Incretin hormones are secreted at a low basal level throughout the day and levels rise immediately after meal intake. GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta-cells in the presence of normal and elevated blood glucose levels. Furthermore, GLP-1 also reduces glucagon secretion from pancreatic alpha-cells, resulting in a reduction in hepatic glucose output.

5.2 Treatment groups and randomization procedures

The following study drug will be provided: 5 mg linagliptin tablets and placebo tablets. All tablets are of identical appearance and are supplied in bottles. Linagliptin 5 mg and matching placebo will be taken orally once daily.

At Visit 1 patients will be screened for inclusion/exclusion criteria. To increase the chances for identifying patients with concentric LV geometry and impaired LV midwall shortening, the researchers will take into consideration for Visit 1 the T2DM patients who have a history of LV hypertrophy diagnosed and documented by an ECG and/or a history of concentric LV geometry.
diagnosed by standard echocardiography within 1 year before Visit 1. The diagnosis of LV hypertrophy by ECG evaluation can be made using 1 of the following criteria, all characterized for having a very high specificity: Gubner, Lewis, Perugia, Perugia 2, Framingham, Sokolow-Lyon, Romhilt-Estes, Cornell, RV6/RV5 >1.

Patients with LV hypertrophy diagnosed by an ECG and/or a history of concentric LV geometry diagnosed by standard echocardiography will be asked to give their consent to participate to the study and to perform a complete transthoracic ColorDoppler echocardiographic examination and an ECG in order to verify if they fulfill all the inclusion/exclusion criteria. Patients who don’t fulfill the echocardiographic inclusion criteria will be recorded in a log.

At Visit 2, all patients who fulfill all the inclusion/exclusion criteria and have given their consent to participate to the study will be centrally assigned the randomization number. Patients will be randomly assigned to receive either linagliptin or placebo in a ratio of 1:1.

To obtain the randomization number a telephone call-in system or a web-based randomization system will be used.

The following information will be required:

- Patient initials
- Date of birth
- Sex
- Left ventricular midwall shortening
- Left ventricular relative wall thickness

The system will also ask to confirm all inclusion and exclusion criteria. A patient will be considered randomized when the randomization system assigns the patient randomization number. This number will be entered in the CRF.

Randomization will be performed using the following procedure to insure that treatment assignment is unbiased and concealed from patients and investigator staff. A randomization list will be produced by or under the responsibility of the Coordinating Center using a validated system that automates the random assignment of treatment groups to randomization numbers in the specified ratio. The randomization scheme will be accessible only to authorized persons, until the time of the unblinding for the analysis of results.

The independent statistician of the Coordinating Center will receive a copy of the randomization scheme to enable him to prepare the necessary tables for the DSMC in a semi-blinded fashion.
At the conclusion of the trial, the occurrence of any emergency code break will be determined after return of all code break reports and unused drug supplies.

Within the randomization system, the randomization numbers will be used to link the patient identification number to the correct drug medication number on the treatment packs. Only when the study will been completed, the data file verified, and the protocol violations determined the drug codes will be broken and made available for data analysis.

5.3 Labeling
Each study site will be supplied with study drug in identically-appearing bottles. All bottles will be labelled according to Good Clinical Practices (Annexe 13). This label will have a detachable peel-off portion, which will be attached to the appropriate case report form (CRF) at the time of dispensing. Each part of this label contains the randomization number, corresponding to the treatment group according to the confidential randomization list and assigned centrally. The label will also contain a space for the patient’s initials, visit number and the date dispensed. This information will be completed at the site when medication is dispensed to the subject. Investigators staff will identify the study drug to dispense to the patient using the randomization number on the label.

5.4 Storage
All trial drugs will be stored in their original boxes in a lockable storage facility until dispensed to the subjects. Trial medication should not be frozen but should be stored protected from light.

5.5 Dosage and administration
Linagliptin and corresponding placebo will be supplied to the investigator in tablets formulation of 5 mg for oral use. Linagliptin 5 mg and matching placebo will be taken with or without a meal at any time of the day. When the trial drug is dispensed to a subject (see Table 1), the peel-off portion of the label will be attached to the appropriate CRF page. The investigator must verify the bottle reference number to ensure that each subject is given the correct trial medication as allocated by the randomization system.

If a dose of study linagliptin / linagliptin placebo is missed it should be taken as soon as the subject becomes aware that the dose has been missed. A double dose should not be taken on the same day.
Table 1: drug dispensation

<table>
<thead>
<tr>
<th>Visit</th>
<th>Number of bottles</th>
<th>Medication</th>
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</thead>
<tbody>
<tr>
<td>Visit 2 (Rand.)</td>
<td>3 bottles (30 tablets each)</td>
<td>Linagliptin 5 mg or placebo</td>
</tr>
<tr>
<td>Visit 4 (12 weeks)</td>
<td>3 bottles (30 tablets each)</td>
<td>Linagliptin 5 mg or placebo</td>
</tr>
<tr>
<td>Visit 5 (24 weeks)</td>
<td>6 bottles (30 tablets each)</td>
<td>Linagliptin 5 mg or placebo</td>
</tr>
</tbody>
</table>

5.6. Study interruption or study treatment discontinuation

Every effort must be made to ensure that patients remain in the study and on study medication for the duration of the study.

If either the study medication is discontinued or follow-up visits of a patient are missed, the reason(s) are to be collected and recorded in the Clinical Record Form (CRF).

All randomized patients must be followed until study completion whether or not the first dose of study medication is taken, or study medication is temporarily interrupted or permanently discontinued. For temporary interruptions, every attempt to reinitiate study medication should be made throughout the duration of the study.

5.6.1 Permanent discontinuation of study medication

A permanent discontinuation of study medication must be considered (and whenever possible discussed with the Coordinating Center) when one of the following conditions exist:

- A patient decides it is in his or her best interest;
- An investigator considers it advisable for a sound, explicit and documented clinical reason;
- A serious adverse event occurs that based on medical judgment require discontinuation;
- A serious adverse event (SAE) (including laboratory abnormalities) occurs that is suspected to be related to study medication (adverse drug reaction, ADR) – with a particular attention to severe hypoglycemia, abdominal pain, nausea or vomiting or acute pancreatitis suspicion - and/or that prevents patient’s continuation on study medication;

- Unblinding of study medication.

The list of these cases will be submitted periodically to the DSMC of the study for the safety evaluation of the study.

In all these cases, the scheduled follow-up visits must be followed as planned by the study protocol.

5.6.2 Discontinuation from the study

A patient will be considered discontinued from the study only if

- he or she withdraws the consent to be followed by the participating center;
he or she is lost to follow-up after exhausting all means of contact.

5.7 Blinding
Patients, investigator staff, persons performing the assessments, and data analysts will remain blind to the identity of the treatment from the time of randomization until database lock, using the following methods: (1) Randomization data are kept strictly confidential until the time of unblinding, and will not be accessible by anyone involved in the study conduct (2) the identity of the treatments will be concealed by the use of study drugs that are all identical in packaging, labeling, schedule of administration, and appearance.

Unblinding will only occur in the case of patient emergencies (see Section 5.8), and at the conclusion of the study.

5.8 Emergency procedures for unblinding
Emergency unblinding should only be done when absolutely necessary in order to treat the patient and if stopping the blinded treatment is judged to be not sufficient. Whenever possible, the Investigator should discuss any potential unblinding with the Coordinating Center by calling the Medical Help Line. In the event the treatment is unblinded, the Coordinating Center will maintain a record of the date and time of unblinding.

An unblinding scratch-off label to be used only in strict emergency will be provided to each site for each patient to be scratched open to ascertain the treatment given.

When the Investigator unblinds the treatment for a patient, he/she must note the date, time and reason for unblinding and retain this information with the CRF documentation. He/she must also immediately inform the Coordinating Center that the code has been broken. The integrity of unblinding labels at clinical centers will be checked during site monitoring. Patients for whom the treatment code has been unblinded must permanently discontinue study medication. Follow-up of these patients per protocol will be required for the duration of the study (see 5.6.1, above).
It is the investigators’ responsibility to ensure that there is a procedure in place to allow access to the code break cards in case of emergency.

5.9 Treatment compliance
Records of study medication dispensed and returned, will be kept by the site during the study. Drug accountability will be checked by the monitor during site visits and at the completion of the trial.
6. CONCOMITANT TREATMENTS

An effort to maintain the same anti-diabetes treatment throughout the study should be made. In case of decompensation and poor metabolic control glitazones, sulfonylureas, metaglinides, acarbose or basal insulin are allowed. DPP-4 inhibitor or GLP-1 receptor agonist are not permitted, unless a specific clinical indication for treatment according to the Investigator judgment emerges during the course of the study. Rifampin decreases linagliptin exposure suggesting that the efficacy of linagliptin may be reduced when administered in combination with a strong P-gp or CYP3A4 inducer. Therefore, use of alternative treatments will be strongly recommended during the period of the study.

7. VISITS SCHEDULE AND ASSESSMENTS

Figure 1 summarizes graphically the sequence and the characteristics of the procedures and of the information requested throughout the study.

**Figure 1 – Trial Plan**

<table>
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<th>Visit n.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td></td>
</tr>
</tbody>
</table>

* only for patients still on study treatment at final visit (visit 6)
7.1 Information to be collected at visits

A common core of data and controls is foreseen for all study visits, with a few differences for visit 1 (screening) and Visit 2 (randomization) and at study end. All information collected at Visit 1 will be reported on the specific CRF of the day of the randomization.
The details of the information required and the way they should be collected are set-out in the CRF and in the operational manual, as appropriate.
It must be recalled that the same scheme of visits and controls apply to all randomized patients, independently from their possible discontinuations (see above 5.6).

7.2 Treatments

All concomitant medication, including over-the-counter medication, taken during the course of the study must be recorded on the CRF.
Records of study medication dispensed and returned, and intervals between visits will be kept by the site during the study. Drug accountability will be checked by the monitor during site visits and at the completion of the trial.

7.3 Echocardiograms

Transthoracic ColorDoppler-echocardiography will be performed in all patients at screening and at final visit (Visit 6-48 weeks) following a standardized protocol. Images of randomized patients will be stored on CD or MO disks and forwarded for final interpretation at the DYDA Echocardiography Core Laboratory. For further details please refer to Appendix 2.

7.4 12 Lead Electrocardiography

A 12-Lead ECG will be recorded in all patients at screening and at final visit (Visit 6-48 weeks). ECG tracings of randomized patients will be sent to a central laboratory for analysis. For further details please refer to Appendix 3.

7.5 Blood collection

Blood will be collected at screening for local laboratory assessment of creatinine and HbA1c if not available within prior 3 months. Blood samples will be also collected in all patients at randomization (Visit 2), at 12 weeks (Visit 4) and at final visit (Visit 6-48 weeks) for safety local analyses. The samples will be then sent to a central laboratory for analysis.
8. EFFICACY AND SAFETY PARAMETERS AND ASSESSMENTS

8.1 Primary Efficacy Variable
Changes from baseline to 48-weeks of LV systolic function measured by midwall shortening analysis.

8.2 Secondary Efficacy Variables
- Changes from baseline to 48-weeks in diastolic LV function: Transmitral peak E wave (pulse Doppler) and early diastolic Tissue Doppler velocity of mitral annulus (E’) will be used to classify LV diastolic function together with other parameters (E/A ratio of transmitral flow, deceleration time of E, left atrial volume, pulmonary artery systolic pressure) in 4 degrees: normal, mild dysfunction, moderate dysfunction and severe dysfunction.
- Changes from baseline to 48-weeks in LV systolic longitudinal function measured by tissue Doppler technique (peak systolic velocity of S’ wave of mitral annulus); Incidence of patients who have an improvement in S’> 25% from baseline.

8.2.1 Exploratory assessment
Asymptomatic LV systolic function could also be evaluated by analysing myocardial contractility of circumferential and longitudinal fibers using the “Speckle-Tracking Echocardiography” (STE) technique, so called “Global Strain”, which allows to measure such parameters with a method less dependent of LV load and end-systolic stress. As previously reported for midwall shortening, changes between baseline and 48 weeks values of LV systolic function derived by STE will be detected in both study groups and differences will be compared.

8.3 Safety parameters assessment
Safety assessments will consist on monitoring and recording the pre-defined safety and tolerability end-points, all serious adverse events, and the regular measurements of vital signs.

8.3.1 Predefined safety and tolerability end-points and study medication discontinuation
Background knowledge suggests adopting a generalized policy of surveillance specifically on:

–symptomatic or severe hypoglycemia.

Severe hypoglycemia is defined as an event requiring assistance of another person to actively administer carbohydrate, glucagons, or other resuscitative actions. These episodes may be
associated with sufficient neuroglycopenia to induce seizure or coma. Plasma glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

- severe pain in upper stomach spreading to back, nausea and vomiting, loss of appetite, fast heart rate (symptoms possibly related to pancreatitis)

- fever, sore throat, "runny nose", and headache with a severe blistering, peeling, and red skin rash.

The study medication will be discontinued in case of:

- severe hypoglycemia which, according to the Investigator’s judgment, is attributable to study medication;

- abdominal pain, nausea or vomiting or acute pancreatitis suspicion which, according to the Investigator’s judgment, is attributable to study medication;

- any serious clinical event or condition, especially those potentially life-threatening which, according to the Investigator’s judgment, is attributable to study medication.

Specific attention will be paid to patients at high risk, such as those receiving insulin secretagogue and those with a history of pancreatitis (although it is unknown whether this condition is a risk factor for the development of new episodes of pancreatitis while using linagliptin).

Specific tables containing information on safety aspects of these subgroups of high risk patients will be periodically reviewed by the DSMC.

The following biomarkers will be evaluated: hs-CRP, NT-proBNP, UACR, HbA1c

The following clinical combined outcomes measure will be considered a safety end-point: cardiovascular death, non fatal-MI, non-fatal stroke, hospitalization for heart failure, hospitalization for coronary revascularization procedure, acute pancreatitis, any type of cancer. These events will be adjudicated by a Clinical Event Committee.

8.3.2. Adverse events, serious adverse events and reporting

Adverse events (AEs)

An AE is defined as any untoward medical occurrence in a patient or in a clinical trial subject administered an investigational product and which does not necessarily have a causal relationship with this treatment.

All AEs fall into either the category “non serious” OR “ serious”.

20
**Serious Adverse Events (SAEs)**

A serious adverse event is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening (“Life-threatening” means that the patient was at immediate risk of death at the time of the SAE; it does not refer to a SAE that hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or prolongation of existing hospitalization (This means that hospital inpatient admission or prolongation of hospital stay were required for the treatment of the AE, or that they occurred as a consequence of the event. Visits to a hospital by ambulance without admission will not be regarded as hospitalization unless the event fulfils any other of the serious criteria);
- Results in persistent or significant disability or incapacity (“Persistent or significant disability or incapacity” means a permanent or significant and substantial disruption of a person's ability to carry out normal life functions);
- Is a congenital anomaly or birth defect;
- Is an important medical event.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. A diagnosis of cancer/malignant tumor during the course of a treatment should always be considered as medically important. This category also includes medical events requiring intervention to prevent permanent impairment or damage.

**Reporting of AEs**

In this study all SAEs will be monitored through the data collected in the CRFs. Clinic staff will report any SAE indicating whether they are likely to be due to study treatment or not. Other adverse events not considered serious (as defined above) will only be recorded if they are suspected to be related to study treatment (ADRs) (see Table 2).

As recommended by regulatory authorities, every SAE suspected by the Investigator to be related to study medication (Suspected Serious Adverse Reaction – SSAR), both expected and unexpected (SUSAR) must be reported to the study Coordinating Center within 24 hours of learning of its occurrence which will transfer immediately the information to the drug’s manufacturer.

Documentation supporting the clinical combined outcomes measure will be forwarded to the Coordinating Center for adjudication by a Clinical Event Committee.
Table 2. Relationship of adverse events to study drug

| Not suspected | The temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event. |
| Suspected | The temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event. |

Other events to be treated as serious adverse events

Exposure to drug during pregnancy or lactation. In principle, pregnancy and the lactation period are exclusion criteria for clinical studies involving investigational drugs, which are not directly related to the respective conditions. In the event of a pregnancy occurring during the course of this particular study, the subject must stop study treatment and be closely followed-up during the entire course of the pregnancy and postpartum period.

The Sponsor must be notified without delay. Parental and neonatal outcomes must be recorded even if they are completely normal and without AEs. Off-spring should be followed up for at least 8 weeks after delivery.

9. DATA ANALYSIS

9.1 Sample Size

The study is designed to show that treatment of patients with type 2 diabetes mellitus and concentric LV geometry and LV systolic dysfunction with a dipeptidyl-peptidase-4 inhibitor determines an improvement on the LV systolic function measured by midwall shortening analysis.

A sample size of 93 patients in each treatment group will be estimated to provide a 95% power at the 0.01 level of statistical significance to detect an improvement in midwall shortening of 10%, assuming a mean midwall shortening of 13.6%, a standard deviation of 2.0% and a drop out rate of 15%. This assumption is based on the analysis of the DYDA Registry (1,2). According to the above point, 186 patients will have to be randomized in the trial. The sample size calculations have been done by using the PS Power and Sample Size Calculations software version 3.0.
9.2 Statistical analysis
The primary hypothesis to be investigated is whether linagliptin is superior to placebo in terms of improvement in LV systolic function using the midwall shortening as the primary efficacy variable. The treatment effect on the echocardiographic measurements will be tested by one-way ANOVA on the intra-subjects difference between basal and end of treatment response using treatment group as factor and basal response as covariate. Secondary continuous variables will be analyzed in the same way using the one-way ANOVA on the response and including the difference and the basal values as covariate. Data will be summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, and safety observations and measurements.

9.3 Populations
Primary analysis population:
The primary analysis population will consist of all randomized patients with ECHO baseline and 48 weeks available. On analyses based on this population, the echocardiographic measures detected at the entry visit will be compared with the echocardiographic measures detected at study end (48 weeks).

Per-protocol population:
The per-protocol population will consist of all patients who receive, at least 40 weeks of study medication and who are still on treatment at the final echocardiographic evaluation.
Safety population:
The safety population will consist of all patients that received at least one dose of study medication and had at least one post-baseline safety assessment. Patients will be analyzed according to treatment received.

9.4 Background and demographic characteristics
Appropriate summary statistics will be provided for the primary analysis population by treatment group for demographic and medical history characteristics, and for HbA1c, fasting plasma glucose, blood pressure, heart rate, body weight, urinary albumin/creatinine ratio, hs_CRP, and NT-proBNP measured at the entry visit. All descriptive data will be given as mean value ± SD. Differences between patients will be compared by unpaired t testing (1-way analysis of variance) and frequency of parameters by the chi-square test. P-values from comparisons of the treatment groups with respect to these variables will be provided.
9.5 **Study medication and concomitant therapy**

Summary statistics for duration of exposure to trial medication will be calculated by treatment group. Summary statistics of concomitant therapy will be provided as appropriate.

9.6 **Efficacy evaluation**

*Primary efficacy variables*

The primary efficacy variable is the modification of midwall shortening. This will be calculated for each surviving patient as the difference between the final echocardiographic measures and those done at randomization.

Statistical model: The treatment effect on the echocardiographic measurements will be tested by one-way ANOVA on the intra-subjects difference between basal and end of treatment response using treatment group as factor and basal response as covariate.

Criteria for efficacy: Linagliptin will be considered superior to placebo if the difference between these treatment arms, using the primary analysis population, is statistically significant in favor of linagliptin using a two-sided p level ≤0.05.

*Secondary efficacy variables*

Secondary efficacy variables are defined in Section 8.2. Secondary continuous variables will be analyzed in the same way using the one-way ANOVA on the response and including the difference and the basal values as covariate.

9.7 **Safety evaluation**

The assessment of safety will be based mainly on the frequency of the pre-defined safety and tolerability parameters and serious adverse events suspected by the investigator to be related to study medication. Other safety data (e.g., electrocardiogram, vital signs, and special tests) will be considered as appropriate.

AEs will be coded using the MedDRA dictionary and summarized for each treatment group, by presenting the number and percentage of patients having any adverse event, having an adverse event in each body system and having each individual adverse event.
10. DATA REVIEW AND DATABASE MANAGEMENT

10.1 Site monitoring and data recording

The Steering Committee of the trial has delegated the GCP monitoring aspects of the study to the Coordinating Center. The responsibilities and the operational procedures are set-out in the monitoring SOP.

Recording of data and retention of documents

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations. The trial staff will notify the Investigator(s)/Institution(s) when the study-related records are no longer required. The Investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

- IRB approvals for the study protocol and all amendments
- Source documents and laboratory records
- CRF copies
- Patients' informed consent forms
- Any other pertinent study document.

Auditing procedures

Inspections by Regulatory Authorities during the study, and/or after its completion, could be expected. Auditing visits on the behalf of the Sponsor of the study can be envisaged.

10.2 Data collection

Investigators must enter the information required by the protocol into the CRFs. The CRFs will be forwarded to the study data management center. One copy of the CRF will be retained at the investigational site. Once the CRFs are received by the data management center, their receipt will be recorded, and they will be forwarded to the responsible data management staff for processing.

Transthoracic Doppler-echocardiography supporting the primary and secondary endpoints will be forwarded to the data management center for final interpretation by the DYDA Echocardiography Core Laboratory.

Documentation supporting the clinical outcome measures will be forwarded to the data management center for adjudication by the Clinical Event Committee. The required documentation will be reported in a Clinical Event Committee Manual, pre-defined by the members of the Committee.
Blood samples supporting the safety endpoints will be forwarded to a central laboratory for analysis.

12-Lead ECG will be forwarded to the data management center for central reading.

10.3 Database management and quality control
Database management and quality control for this study are under the responsibility of the Coordinating Center.

At the Coordinating Center, a physician will review the CRFs for completeness and accuracy. Omissions or questions will be entered on data query forms, which will be returned to the investigational site for resolution. After the investigator response is received at the data management center, the resolutions will be entered into the database. A copy of the signed data query form will be kept with the CRFs. Quality control audits of all key safety and efficacy data in the database will be made at designated times during the study.

When the database has been declared to be complete and accurate, the database will be locked and unblinded.

11. STUDY COMMITTEES

Steering committee
The Steering Committee acts as the Sponsor of the study, and has the full responsibility for planning, conduct, analysis, and publication of study protocol and results. In addition the following Committees are established.

Executive committee
The Executive Committee is composed by 3 members of the Steering Committee with the responsibility to coordinate all the procedural activities of the study.

Clinical Event Committee (CEC)
The Clinical Event Committee members will be independent and will not have direct contact with patients randomized into this study.
The main roles and responsibilities of the Clinical Event Committee are:
- To agree on definitions for the clinical outcome measures and on standard procedures for assessing these outcomes. An Event Validation Committee manual will be pre-defined, by the members of the Committee.
- To validate blindly the events recorded and reported by the Investigators as clinical outcome measures of the study.

Data and Safety Monitoring Committee (DSMC)
The roles and responsibilities of the DSMC will be defined by the same DSMC with special focus on intensive monitoring of the safety aspects in the whole study population.
No specific interim analysis is foreseen for efficacy.
The study may be amended, or stopped early, should any of these be deemed necessary based upon DSMC recommendations. The Chairman of the DSMC will discuss such recommendations with the Chairman of the Steering Committee. Any significant amendment (including recommended discontinuation) to the study will be notified as appropriate to the designed representative(s) of the industrial supporter of the study.

12. ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

12.1 Good Clinical Practice
The procedures set out in this protocol are designed to ensure that the Principal Investigator abide by the principles of the Good Clinical Practices guidelines of the ICH, and of the Declaration of Helsinki (1996). This study also will be carried out in keeping with local legal requirements.

12.2 Informed Consent
The Coordinating Center will supply a proposed informed consent form, which is part of the protocol and complies to regulatory requirements which must be approved by the IRB/IEC together with the protocol. IRB/IEC can ask to modify the informed consent. The final agreed version must be forwarded (together with the documentation of protocol approval) to the Coordinating Center.
The Investigator or a person designed by the Investigator, and under the Investigator’s responsibility, should fully inform the Patient of all pertinent aspects of the Clinical Trial. Patients will be allowed adequate time for consideration and making an informed decision. All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand. In patient willing to participate in the trial, informed consent will be obtained from the patient (or his/her legally authorized representative) according to the regulatory and legal requirements of Italy. This consent form must, signed and dated by the patient, must be retained by the Investigator as part of the study records. The Investigator will not undertake any investigation specifically required for the clinical study until valid consent has been obtained. The date and time when consent was obtained will also be documented in the CRF.
A copy of the signed and dated written Informed Consent Form will be provided to the patient.
12.3 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC and Competent Authorities. This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IEC and Competent Authority approval prior to implementation (if appropriate). Administrative changes (not affecting the patient benefit/risk ratio) may be made without the need for a formal amendment.

12.4 Duration of the study

The enrollment period will last 12 months. The maximum duration of the study for a patient will be approximately 52 weeks. The study will close when all patients have completed their follow-up (i.e. post treatment safety follow-up (Visit 7) if on treatment at 48 weeks visit or 48 weeks follow-up (visit 6) if treatment stopped early).

12.5 Confidentiality

All study findings and documents will be regarded as confidential. The anonymity of participating patients must be maintained. Patients will be identified on CRFs by their patient number, not by name. Documents that identify the patient (e.g., the signed informed consent) must be maintained in confidence by the Investigator.

13 PUBLICATION POLICY

The publication of the primary efficacy results as defined by the protocol shall be published on the behalf of the study group. An appropriate Appendix will include the names of the members of the study committees, of the coordinating center and of representatives from each individual study site.

After the publication of the main paper, the database will be available for further analyses to all participating Investigators and Committee members. Requests for further analyses to support ancillary publications must be submitted to the Steering Committee for review and approval. The Steering Committee must receive a copy of any presentation, manuscript, or abstract prior to any outside submission. A period of 5 working days for presentational materials and abstracts and 15 working days for a journal submission will be required for the Steering Committee review.

Authorship of the ancillary analyses will be determined according to the current medical community rules. According to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, designation as an author must satisfy at least two conditions. The author must have (a) contributed substantially to the conception and design of the study, the acquisition of data, or their analysis and interpretation and (b) drafted or provided a critical revision of the article.
14 REFERENCES


6) Koh YS, Jung HO, Park MW, et al. Comparison of left ventricular hypertrophy, fibrosis and dysfunction according to various disease mechanisms such as hypertension, diabetes mellitus and chronic renal failure. J Cardiovasc Ultrasound. 2009;17:127-134.


27) Sohn DW, Chai HI, Lee DJ, Kim HC, Kim HS, Oh BH, Lee MM, Park JB, Choi YS, Seo JD, Lee YW. Assessment of mitral annulus velocity by tissue Doppler imaging in the evaluation of left ventricular diastolic function. *J Am Coll Cardiol* 1997; *30*:474-480.


APPENDIX 1

GLYCEMIA MANAGEMENT GUIDELINES

The reference guideline for the DYDA TRIAL is “Italian Standards of care for Diabetes Mellitus 2014” by AMD (Italian Association of Diabetologists) and SID (Italian Society for Diabetes) (see Reference).

BRIEF SUMMARY OF RECOMMENDATIONS

Assessment
HbA1c should periodically be measured to assess glycemic control over time. Patients with recurrent or severe hypoglycemia should be evaluated for precipitating factors that may be easily corrected (e.g., missed meals, incorrect administration of insulin [dosage or timing], and exercise). Patients with diabetes should be assessed for knowledge, performance skills, and barriers (e.g. psychosocial, personal, or financial) to full compliance.

Glycemic control target range
Each patient’s glycemic target range must be individualized, based on the provider’s appraisal of the risk-benefit ratio for that individual, and the patient’s medical, social, and psychological status.

The risk of hypoglycemia should be specifically considered in recommending the target goal.

For patients with very mild or no microvascular complications of diabetes, and those free of major concurrent illnesses and with a reasonable life expectancy, the HbA1c target should be <7% (<53 mmol/mol). HbA1c target should be kept < 9% (<75 mmol/mol) for all patients to avoid symptoms of hyperglycemia.

For patients with advanced microvascular complications and/or major comorbid illness, or who have a shortened life expectancy (5 to 10 years), aggressive glucose lowering may not be warranted because of limited benefit in reducing the absolute risk of complications.

Individual treatment goals must be established with the patient based on the extent of the disease, comorbid conditions, and patient preferences.

Treatment options
Diet and exercise and lifestyle modification should be encouraged.

Institution of diet and exercise is usually the appropriate initial management in patients with new onset T2DM, depending upon the severity of the symptoms, psychosocial evaluation, and overall health status.

On at least a temporary basis, the use of intermediate- or long-acting insulin* for controlling fasting plasma glucose, alone or in addition to oral agents, should be considered for patients with T2DM in whom oral agents have proven ineffective, intolerable, or are contraindicated or rapid restoration of
euglycemia is desirable (e.g., patients with persistent symptoms of diabetes or with hyperglycemia in perioperative and/or critical care settings).

If treatment goals are not achieved with diet and exercise alone, drug monotherapy should be initiated and initial monotherapy with metformin should be used as first line drug therapy. If the glycemic target level is not achieved with one oral agent alone, combination oral and/or insulin therapy is recommended. Metformin may be combined with a thiazolidinediones*, sulfonylurea, DPP-4 inhibitors, GLP-1 receptor agonists. Baseline and follow-up efficacy (at 3-6 months) are necessary for continuation of oral therapy.

Selected individuals may benefit from three-drug oral agent therapy. Alpha-glucosidase inhibitors may be used in conjunction with other oral agents in combination in patients whose blood glucose is inadequately controlled, especially if postprandial glucose is out of range.

Addition of insulin therapy, both basal or prandial to an existing combination oral agent regimen may be a treatment option when the glycemic control target is not achieved by an all-oral regimen. Metformin can be considered as an adjuvant therapy to insulin for the purpose of achieving glycemic target goals. Metformin is the preferred agent to add to an existing insulin regimen. Insulin therapy may also be used and given in multiple daily doses, if the glycemic control target has not been reached with oral therapy.

Dietary counseling and individualized education should accompany initiation or maintenance of any anti-diabetes therapy.

**Follow-up**

Patients should be scheduled for appropriate follow-up to evaluate response, tolerability to therapy, goal assessment and management of acute and chronic problems.

The frequency of care provider visits for patients with diabetes who are meeting treatment goals and who have no unstable chronic complications should be individualized.

When there is a sudden change in health status or when changes are made to the treatment regimen, follow-up within one month or sooner may be appropriate.

Treatment goals should be periodically reassessed based upon patient-specific factors, including changes in the patient’s health status, adverse drug reactions, adherence to therapy, and preferences.

* In this trial thiazolidinediones use in the last three months before enrollment is an exclusion criteria

APPENDIX 2

ECHOCARDIOGRAPHIC PROTOCOL

Transthoracic Doppler-echocardiography was performed following a standardized protocol. Images were stored on CD or MO disks and forwarded for final interpretation at the DYDA Echocardiography Core Laboratory.

LV chamber dimensions and wall thicknesses were measured by the American Society of Echocardiography guidelines and LV mass calculated using a necropsy validated formula [22]. LV mass was normalized for height to the 2.7 power and LVH was defined as LV mass ≥51 g/m².7 [23]. Relative wall thickness was calculated as two times the posterior wall thickness/LV diastolic diameter ratio independently of the presence of LVH and used as index of LV geometry. Index ≥0.42 was considered indicative of concentric geometry.

LV volumes and stroke volume were measured by the biplane method of disks and used to calculate ejection fraction (LVEF), as the primary measure of systolic function. Stroke volume was used to estimate cardiac output. Stroke index and cardiac index were generated by normalization for height at the respective allometric powers to account for the non-linear variations with body size. Total peripheral resistance was calculated as mean arterial pressure / cardiac index times 80.

LV systolic function was also assessed by measuring the systolic shortening of the LV minor axis at the midwall level. Midwall fractional shortening (MFS) was calculated as previously reported, taking into account the apparent epicardial migration of midwall during systole [24,25]. Systolic LV dysfunction was defined as MFS ≤15% [26].

Tissue Doppler study (pulsed wave spectral analysis) was used to measure peak mitral annular systolic velocity (peak S’, expressed as mean of 4 measurements obtained in septal, lateral, inferior and anterior mitral annular position), as an estimate of longitudinal LV function [27]. Peak S’ < 8.5 cm/sec (10th percentile of a reference caucasian healthy population) indicated systolic dysfunction of LV longitudinal fibers (28-30).

Diastolic function was evaluated using transmitral and pulmonary vein pulsed wave Doppler curves and early diastolic Tissue Doppler velocity of mitral annulus (E’): they were assessed according to the recommendations of the American Society of Echocardiography [31]. Pulsed wave
transmitral Doppler signal was obtained with the sample-volume placed between the tips of mitral leaflets and in the LV outflow tract in apical four and five-chamber views to measure peak early (E) and late (A) transmitral flow velocity (cm/sec) and deceleration time of E velocity (DTE) (msec), thus E/A ratio was calculated. Tissue Doppler spectral signal was obtained at the septal mitral annulus to measure peak E’ diastolic mitral annular velocity. The ratio E/E’ was used to estimate LV filling pressure (normal LV filling pressure was defined as E/E’ ratio < 8) (31). All these measurements were averaged from 5 consecutive cycles. E/E’ ratio was used to classify LV diastolic function together with other parameters (E/A ratio of transmitral flow, deceleration time of E and the difference in duration of atrial wave on pulmonary vein flow and atrial wave on transmitral flow and maximal left atrial volume) in 4 degrees as proposed by Redfield et al. (32): normal, mild dysfunction, moderate dysfunction and severe dysfunction. Maximal left atrial volume was computed from 2D apical 4-chamber view using the area - length method and was normalized for body surface area.
APPENDIX 3

12-lead ECG

The 12-lead ECG is recorded on special graph paper which is divided into 1 mm² grid-like boxes. The ECG paper speed is 25 mm/sec and therefore each 1 mm horizontal box corresponds to 0.04 second (40 ms). Vertically, standard calibration is used (10 mm equals 1 mV).

ECG test will be previously examined by the respective medical staff of enrolled centre and then it will be send to the reading centre.

ECG test is performed at screening and after 48 weeks from randomization.

The following items must be raised for a good technical execution:

1. After standard calibration mark, in every lead must be present at least 3 ventricular complexes.
2. The standard ECG test must be recorded during a brief tele-espiratory apnoea to avoid substantial modifications of complexes amplitude and of electrocardiographic segments.
3. The maximum deflection of ventricular complex must be recorded entirely in the ECG grid; if ECG grid can’t include complexes with high amplitude, it is recommended to use half calibration (marking the procedure on a free space of the grid). At the end of the exam, the electrocardiographic test (or a readable photo static copy) must be enclosed into the form (with title page entirely completed) supplied by the Coordinating Center and must be send to the Coordinating Center.