Clinical Study of Lipoic Acid on Ischemic Heart Failure

Statistical Analysis Plan

Research initiating unit: Zhongshan Hospital, Fudan University

Research units:

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一、Introduction

Heart failure is the common endpoint of various structural or functional heart diseases and represents the leading cause of death from cardiovascular diseases, with a 5-year mortality rate exceeding 50% [1-3]. Ischemic heart disease most frequently manifests as the clinical syndrome of heart failure, which is associated with high rates of morbidity and mortality, thereby garnering significant clinical attention. Current clinical treatments for ischemic heart failure primarily include pharmacological therapy, revascularization, therapeutic angiogenesis, and heart transplantation. Despite advancements in interventional techniques and the establishment of treatment strategies based on the "neuroendocrine," "hemodynamic disorders," and "immune-inflammatory" theories, which have partially improved clinical outcomes in patients with ischemic heart failure, these improvements fall far short of clinical needs. There is an urgent necessity to explore new therapeutic targets for heart failure from innovative perspectives.

Abnormalities in myocardial energy metabolism constitute a crucial pathological basis for heart failure, with mitochondria playing a pivotal role as the primary site for ATP production. Elucidating the role of mitochondrial energy metabolic remodeling in the pathogenesis of different types of heart failure and its potential for therapeutic intervention could facilitate the early identification of risk factors for heart failure and lead to more effective improvements in prognosis. Our previous research has focused on mitochondria in failing myocardium, and through proteomic analysis, we have identified nearly a hundred differentially expressed mitochondrial proteins, including aldehyde dehydrogenase 2 (ALDH2). ALDH2 is a key enzyme involved in mitochondrial glycolipid metabolism[4], possessing a redox-sensitive

thiol group at its active site. An increase in endogenous oxygen free radicals promotes the formation of disulfide bonds at the ALDH2 active site, leading to its inactivation [5]. Given the abundant distribution of this enzyme in the myocardium, we have conducted a series of studies on the role of ALDH2 in myocardial injury and protection. Our findings indicate that ALDH2 exerts cardioprotective effects by maintaining mitochondrial metabolic homeostasis under cardiovascular pathological stimuli such as ischemia, hypoxia, and alcohol exposure [6-12]. Furthermore, we have demonstrated that ALDH2 is a prerequisite for enhancing the therapeutic efficacy of stem cell transplantation in the treatment of heart failure [13-14]. The significance of ALDH2 in numerous human diseases is gradually being elucidated, with substantial evidence from domestic and international peers indicating its irreplaceable role in preventing other cardiovascular diseases, cerebrovascular diseases, hematological disorders, and malignant tumors, among others [15-18]. Autologous bone marrow-derived aldehyde dehydrogenase-bright (ALDH^br) cells, which express highly active ALDH2, have been proven safe and effective in patients with chronic myocardial ischemia [19]. Given its pivotal role in cellular protection and its significance in various human diseases, ALDH2 may represent a novel therapeutic target for the treatment of multiple diseases [20-21].

Lipoic acid (LA), a vitamin B-class compound, features a closed cyclic structure composed of sulfur and carbon atoms, conferring relatively high electron density and thus strong antioxidant properties. LA has demonstrated favorable preventive and therapeutic effects on diabetes and its complications, various brain and neurodegenerative diseases, and aging [22-24]. The role of LA in cardiovascular diseases has increasingly attracted attention, with studies confirming that LA can restore ALDH2 activity by reducing disulfide bonds at its active site in mitochondria,

thereby enhancing nitrate tolerance [25]. Subsequently, scholars have discovered that lipoic acid (LA) also exerts protective effects on diabetic cardiomyopathy and acute ischemia-reperfusion injury by restoring the activity of ALDH2 [26-28]. Given the protective role of ALDH2 in ischemic heart failure and the ability of LA to reduce ALDH2 activity, we propose the following scientific hypothesis: during the recovery phase of post-ischemic heart failure, lipoic acid can improve myocardial mitochondrial energy metabolism and inhibit oxidative stress by reducing the activity of ALDH2 in myocardial mitochondria, thereby protecting against post-ischemic heart failure.

This study employs a prospective, multicenter, randomized, and controlled research design to assess the clinical outcomes of patients with heart failure following myocardial infarction (MI) who are treated with a combination of lipoic acid capsules and standard pharmacological therapy. The evaluation will be conducted using a range of indicators, including primary endpoint events, secondary endpoint events, control of risk factors, and surveys on medication adherence. The primary objective is to elucidate the significant role of lipoic acid capsules in managing post-MI heart failure, thereby paving the way for innovative approaches in heart failure treatment.

二、Objectives

The clinical outcomes of lipoic acid capsules combined with standard pharmacological therapy in patients with heart failure following myocardial infarction are evaluated through indicators such as primary endpoint events, secondary endpoint events, control of risk factors, and surveys on medication adherence.

三、Study Design

This is a multicenter, double-blind, randomized, placebo-controlled, parallel-group, exploratory trial.

The aim of this study is to observe the efficacy and safety of ALA as a complementary therapy in patients with ischemic heart failure (IHF). Participants with IHF with myocardial infarction (MI) history and left ventricular ejection fraction (LVEF)≤50% were randomized 1:1 to receive ALA (600mg daily) or placebo on top of standard care for 24 months. The primary endpoint was the composite endpoint of hospitalization for HF or all-cause mortality events. The second endpoints included non-fatal MI, non-fatal stroke, changes of LVEF, 6 minute walking distance (6MWD) from baseline to 24 months.

四、General Statistical Considerations

4.1 General Principles

All statistical analyses were performed with R version 4.0.2. Normally distributed continuous variables were reported as the mean±SD, and non-normally distributed continuous variables were expressed as the median and interquartile range. Categorical data were expressed as absolute values and percentages and analyzed using the chi-square or Fisher's exact test. The Student's t-test or Wilcoxon rank-sum test was performed to determine differences between groups. Survival analyses were based on the time to the first event. The cumulative incidence of the time-to-event end points is expressed as Kaplan-Meier curves over 24 months after randomization. Cox proportional hazard models were used to estimate HR and 2-sided 95% CI. A linear mixed-effects model was used to analyze the differences in changes of LVEF and 6MWD between the ALA group and the placebo group, with study centers included as

random effects. The analysis of safety and efficacy was performed in full analysis set (FAS) adhering to the intention-to-treat principle. The type I error rate (2-sided α) was set at 0.05.

4.2 Handling of Noncompliance to Study

Individuals who provide their consent by signing an informed consent form but subsequently withdraw from the study prior to randomization, for whatever reason (such as not fulfilling the inclusion and exclusion criteria), are categorized as "screening failures".

Irrespective of the cause, a participant who was randomized but permanently ceased the study treatment prior to their scheduled End of Treatment Visit was deemed to have permanently discontinued the study medication. This category also encompasses those who were randomized but did not commence taking any study medication at all. The decision to permanently halt the administration of the study medication will be made in accordance with the reason for discontinuation.

When participants permanently discontinue the study medication, various follow-up approaches are deliberated upon to gather data on outcome events and vital status. These methods may encompass scheduled study visits, routine telephone communications with the patient or their primary care physician, or a contact at the study's conclusion. In cases where patients decline to participate in regular study visits, the investigator will strive, to the fullest extent possible, to persuade them to attend at least one concluding evaluation visit, as stipulated for the End of Treatment Visit.

All participants who have been randomized into the study will be actively encouraged to persist with the study treatment and remain under observation throughout the entire duration of the research. It's important to note that withdrawing informed consent cannot be achieved simply by discontinuing the study treatment. In situations where participants express

a desire to "no longer continue," investigators are tasked with clarifying whether this pertains to halting the study treatment, a reluctance to attend follow-up appointments, an unwillingness to engage in telephone communication, a general disinterest in maintaining any form of contact with study staff, or a refusal to permit contact with a third party, such as a family member or their doctor. To ascertain the survival status of all participants at the study's conclusion, every feasible effort will be undertaken to follow up with them. It is anticipated that only a negligible number of individuals will have incomplete follow-up (in any capacity) during the course of this trial.

A participant will be considered to have incomplete follow-up or be classified as lost to follow-up (meaning they have entirely failed to comply with follow-up procedures) if, despite exhaustive attempts, all investigators and dedicated site staff are unable to establish contact with the participant or a designated third party (such as a family member or doctor). In accordance with local legal provisions, every endeavor will be undertaken to reach the participant or a third party, with the aim of ascertaining the endpoint, survival status, and the rationale behind treatment discontinuation. However, the participant will not be deemed lost to follow-up if the database confirms that they are alive at the conclusion of the study.

4.3 Handling of Missing Data

In the subject data listing, all missing or partially recorded data will be presented exactly as they appear on the Case Report Form (CRF).

This includes the best estimate date provided by site investigators (as detailed below), which is gathered during clinical data collection. When an exact event date is unknown, site investigators are requested to furnish their most informed approximation of the event date. Even though the precise occurrence date remains uncertain, investigators often possess

information that can suggest an approximate date, such as the first week of a month, the autumn season of a year, the midpoint of a specific year, or at least the last date the subject was observed or contacted. By incorporating this contextual information into the computer program, the estimated date it generates is likely to be more accurate than that produced by a program lacking such knowledge. The estimated date should represent the midpoint of the period during which the event is known to have occurred. For instance, if the event took place in the first week of a month, the middle date of that month should be used as the estimate. Similarly, if the event occurred in the autumn, the middle date of that season should serve as the approximation. When no prior information about the subject is available before the event (start date of the plausible time period) and only the date of contact when information about the event became known (end date of the plausible time period) is available, the midpoint of this plausible time period should be recorded as the estimated date. This approach has been widely adopted in numerous studies and is endorsed by Dubois for date estimation purposes.As a precautionary measure, if the date/time information is insufficient to ascertain whether an event occurred before or after randomization, the event will be considered an outcome. In such cases, the event start date will be imputed no earlier than the randomization date.

4.4 Determination of Sample Size

This pilot and feasibility study was designed to evaluate the efficacy and safety of ALA in IHF patients and get the reference data on outcomes related to clinical events for the future RCT with large sample size. As a feasibility trial, 300 patients were expected to be enrolled during the study period. Assumptions for sample size calculation included the following: the event rate for primary outcomes was 35% at 24 months in the control group[29]; the HR for the ALA group was estimated to be

0.60 and a withdrawal rate of 5% at 24 months in both groups. A total of 300 (150 per group) subjects followed up for a fixed period of 24 months provides 80% power to reject the null hypothesis at the two-sided 0.1 significance level.

五、Analysis Set

5.1 Assignment of Analysis Sets

All subjects who were randomized in the study were eligible for assignment tothe analysis sets.

5.2 Intention-to-treat Analysis Set (ITT)

All participants who were randomly assigned will be included in the intention-to-treat analysisset, also known as the full analysis set in the International Conference on Harmonization(ICH) E9 standard.

5.3 Full Analysis Set (FAS)

The FAS set is defined as the set that adheres to the ITT principle with the additional condition that the investigational drug has been used for at least one month, allowing for reasonable exclusions and enabling the reflection of the investigational drug's efficacy and safety to the greatest extent possible. The analysis of the primary endpoint and other endpoint was based on the FAS dataset.

5.4 Safety Analysis Set

All randomized subjects who received at least one dose of study medication will be included in the safety analysis set.

5.5 Per-protocol Analysis Set

Subjects who do not have serious protocol violations in terms of inclusion and exclusion criteria, treatment, and measurement of main indicators.

六、Statistical Methodology

6.1 Population Characteristics

In the FAS set, baseline characteristics and demographic data will be summarized by treatment group. Continuous variables will be presented using summary statistics to provide a concise overview. For categorical variables, frequency tables will be utilized to display the distribution of responses across different categories.

6.1.1 Demographics and Medical History

An individual's demographic information includes their age, sex, body weight, systolic blood pressure, diastolic blood pressure and heart rate, which will be given as continuous variables.

We will employ frequency tables to assess the medical history data. These tables will illustrate the number of subjects who have medical history entries (such as previously diagnosed conditions, diseases, or surgeries documented on the CRF) that commenced prior to signing the informed consent and are deemed pertinent to the study.

6.1.2 Protocol Deviations

The frequency tables show the number of subjects with major protocol deviations overall grouped by type of deviation. The analysis will be based on FAS data. Types of major protocol deviations include significant inclusion criteria not fulfilled, significant exclusion criteria fulfilled, failure to obtain informed consent, prohibited medication use, failure to report SAE, failure to report outcome event.

6.1.3 Prior and Concomitant Medications

Frequency tables will be generated, categorized by the type of medication. These tables will present data on prior medications taken before randomization. Additionally, separate frequency tables will be compiled for concomitant medications that were continued after randomization. These tables will detail medication usage at Visit 1, Visit 3 the 12-month follow-up, and at the End of Treatment (EOT). The analyses will be conducted both by treatment group and in the FAS set.

6.2 Endpoints

6.2.1 Primary Endpoints

The primary endpoint is the time from randomization to the first occurrence of any of the components of the composite outcome, including the hospitalization for HF and all-cause mortality events during 24-month follow-up period.

6.2.2 Secondary Endpoints

The secondary endpoint included the time from randomization to the first occurrence of the hospitalization for HF and all-cause mortality events, non-fatal MI or non-fatal stroke. The changes of LVEF and 6MWD from baseline to 24 months were also assessed.

6.2.3 Analysis of the Primary endpoints

The primary analysis will include events adjudicated by the ICAC and will be based on the FAS analysis set. To evaluate whether ALA could prolong the time to a primary endpoints in patients with IHF. The following null hypothesis (H0) will be tested at the significance level of 0.05 using a Cox proportional hazards model:

H_o: HR≤1

H_1: HR>1

HR is the hazard ratio of primary outcome for ALA group in comparison to the placebo group.

6.2.4 Subgroup Analyses

Subgroup analysis was performed for age (whether≤65 years old), sex, smoking status, past medical history (hypertension, diabetes mellitus, atrial fibrillation), NYHA functional class, NT-proBNP levels, LVEF(whether≤30%), and whether the patient received triple therapy.

6.2.5 Analysis of the Secondary endpoints

To evaluate whether ALA could prolong the time to a secondary endpoints (SE) defined as the time from randomization to the first occurrence of any of the components of the hospitalization for HF, all-cause mortality events, non-fatal MI or non-fatal stroke in patients with IHF.

The following null hypothesis (H0) will be tested at the significance level of 0.05 using a Cox proportional hazards model:

H_o: HR≤1

H_1: HR>1

HR is the hazard ratio of secondary outcome for ALA group in comparison to the placebo group.

A linear mixed-effects model was used to analyze the differences in changes of LVEF and 6MWD between the ALA group and the placebo group, with study centers included as random effects.

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