CLINICAL STUDY PROTOCOL

Protocol Number: XYZ-301

A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of XYZ-2000 in Patients with Moderate to Severe Heart Failure with Reduced Ejection Fraction

Sponsor: Cardio Therapeutics, Inc.

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CONFIDENTIAL

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1. PROTOCOL SYNOPSIS

Protocol Title	A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safet
Protocol Number	XYZ-301
Sponsor	Cardio Therapeutics, Inc.
Phase	3
Indication	Heart Failure with Reduced Ejection Fraction (HFrEF)
Study Design	Randomized, double-blind, placebo-controlled, parallel-group, multicenter
Treatment Arms	XYZ-2000 100mg twice daily XYZ-2000 200mg twice daily Placebo twice daily
Randomization	1:1:1
Planned Enrollment	1,200 subjects
Number of Sites	Approximately 120 sites globally
Study Duration	52 weeks (4 weeks screening, 48 weeks treatment)
Primary Endpoint	Change from baseline in NT-proBNP at Week 24

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

To evaluate the efficacy of XYZ-2000 compared to placebo in reducing NT-proBNP levels in patients with moderate to severe heart failure with reduced ejection fraction (HFrEF).

2.2 Secondary Objectives

• To assess the effect of XYZ-2000 on cardiovascular mortality and heart failure hospitalization
• To evaluate the effect of XYZ-2000 on functional capacity as measured by 6-minute walk test
• To assess improvement in quality of life using the Kansas City Cardiomyopathy Questionnaire (KCCQ)
• To evaluate the safety and tolerability of XYZ-2000

2.3 Primary Endpoint

Change from baseline in NT-proBNP (pg/mL) at Week 24

2.4 Secondary Endpoints

• Time to first occurrence of cardiovascular death or hospitalization for heart failure through Week 48
• Change from baseline in 6-minute walk distance (meters) at Week 24 and Week 48
• Change from baseline in KCCQ Overall Summary Score at Week 24 and Week 48
• Proportion of subjects with ≥30% reduction in NT-proBNP at Week 24
• Change from baseline in left ventricular ejection fraction (LVEF) at Week 48

2.5 Safety Endpoints

• Incidence and severity of adverse events and serious adverse events
• Changes in vital signs, ECG parameters, and laboratory values
• Incidence of treatment discontinuation due to adverse events

3. STUDY DESIGN

3.1 Overall Design

This is a Phase 3, randomized, double-blind, placebo-controlled, parallel-group, multicenter study to evaluate the efficacy and safety of XYZ-2000 in patients with moderate to severe heart failure with reduced ejection fraction (HFrEF). The study will be conducted at approximately 120 sites globally.

3.2 Study Periods

Screening Period (Days -28 to -1): Subjects will undergo screening assessments to determine eligibility. All inclusion and exclusion criteria must be met before randomization.

Treatment Period (Weeks 1-48): Eligible subjects will be randomized 1:1:1 to receive XYZ-2000 100mg twice daily, XYZ-2000 200mg twice daily, or matching placebo. Subjects will be seen at clinic visits at Weeks 4, 12, 24, 36, and 48.

Follow-up Period: A safety follow-up visit will occur 30 days after the last dose of study drug.

3.3 Randomization and Blinding

Subjects will be randomized using an interactive web response system (IWRS) in a 1:1:1 ratio to one of three treatment groups. Randomization will be stratified by baseline LVEF (≤30% vs >30%) and region (North America, Europe, Asia-Pacific).

This is a double-blind study. Subjects, investigators, and the sponsor study team will remain blinded to treatment assignment throughout the study. The placebo will be identical in appearance to the active drug.

3.4 Sample Size

Approximately 1,200 subjects (400 per treatment arm) will be randomized. This sample size provides 90% power to detect a 15% difference in NT-proBNP reduction between XYZ-2000 200mg and placebo at Week 24, assuming a two-sided alpha of 0.05 and a 15% dropout rate.

4. STUDY POPULATION

4.1 Inclusion Criteria

1. Male or female subjects aged 18 to 85 years at screening 2. Diagnosis of heart failure with reduced ejection fraction (HFrEF) for at least 3 months 3. Left ventricular ejection fraction (LVEF) ≤40% documented by echocardiography within 6 months 4. NYHA Functional Class II-IV at screening 5. NT-proBNP ≥400 pg/mL (or BNP ≥100 pg/mL) at screening 6. Stable optimal medical therapy for heart failure for at least 4 weeks prior to screening, including ACE inhibitor or ARB (or ARNI), beta-blocker, and mineralocorticoid receptor antagonist unless contraindicated or not tolerated 7. Able to provide written informed consent

4.2 Exclusion Criteria

1. Acute decompensated heart failure requiring IV therapy within 4 weeks of screening 2. Myocardial infarction, coronary revascularization, or stroke within 3 months of screening 3. Heart failure due to uncorrected primary valvular disease 4. Planned cardiac surgery or percutaneous intervention during the study period 5. Estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m² at screening 6. Serum potassium >5.5 mEq/L at screening 7. Systolic blood pressure <90 mmHg or >180 mmHg at screening 8. Pregnant or breastfeeding women 9. Known hypersensitivity to XYZ-2000 or any excipient 10. Participation in another clinical trial within 30 days

5. STUDY PROCEDURES AND SCHEDULE

5.1 Schedule of Assessments

Assessment	Screen	Day 1	Wk 4	Wk 12	Wk 24	Wk 36	Wk 48	FU
Informed Consent	X							
Inclusion/Exclusion	X	X						
Demographics	X							
Medical History	X							
Physical Examination	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X
12-Lead ECG	X	X		X	X		X	
Echocardiogram	X						X	
Hematology (10 mL)	X	X		X	X		X	
Chemistry (10 mL)	X	X	X	X	X	X	X	X
NT-proBNP (5 mL)	X	X	X	X	X	X	X	
Urinalysis	X			X	X		X	
Pregnancy Test	X	X			X		X	
6-Minute Walk Test		X			X		X	
KCCQ Questionnaire		X			X		X	
Randomization		X						
Study Drug Dispensing		X	X	X	X	X		
Concomitant Medications	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X

Blood Volume: Total blood volume per visit is approximately 25 mL (approximately 1.5 tablespoons). Total blood volume over the study is approximately 225 mL.

6. INVESTIGATIONAL PRODUCT

6.1 Description of XYZ-2000

XYZ-2000 is a novel, orally administered, selective cardiac myosin activator that increases cardiac contractility by a mechanism distinct from traditional inotropes. XYZ-2000 is formulated as film-coated tablets for oral administration.

6.2 Dosage and Administration

Treatment Arm	Dose	Route	Frequency	Duration
XYZ-2000 Low Dose	100 mg	Oral	Twice daily with food	48 weeks
XYZ-2000 High Dose	200 mg	Oral	Twice daily with food	48 weeks
Placebo	Matching tablets	Oral	Twice daily with food	48 weeks

6.3 Storage and Handling

Study drug should be stored at controlled room temperature (15-30°C / 59-86°F) in the original container. Protect from moisture. Study drug must be stored in a secure area with limited access.

6.4 Dose Modifications

If a subject experiences symptomatic hypotension (SBP <90 mmHg with symptoms), the dose should be reduced by 50%. If symptoms persist after dose reduction, study drug should be temporarily discontinued. Subjects who discontinue study drug should continue to be followed for all study assessments.

7. SAFETY INFORMATION

7.1 Known and Potential Risks

Based on Phase 1 and Phase 2 clinical studies with XYZ-2000 involving approximately 800 subjects, the following adverse events have been reported:

Adverse Event	Frequency	Severity	Description
Headache	Very Common (>10%)	Mild	Usually resolves within first 2 weeks of treatment
Dizziness	Very Common (>10%)	Mild-Moderate	Often related to blood pressure changes
Hypotension	Common (1-10%)	Mild-Moderate	Symptomatic low blood pressure
Nausea	Common (1-10%)	Mild	Usually transient, improves with food
Palpitations	Common (1-10%)	Mild	Awareness of heartbeat, typically benign
Fatigue	Common (1-10%)	Mild	May improve over time
Diarrhea	Uncommon (<1%)	Mild	Usually self-limiting
Elevated Troponin	Uncommon (<1%)	Moderate	Requires monitoring

7.2 Serious Adverse Events

Any serious adverse event must be reported to the sponsor within 24 hours of the investigator becoming aware of the event. A serious adverse event is any event that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent disability, or is a congenital anomaly.

7.3 Pregnancy

XYZ-2000 has not been studied in pregnant women. Women of childbearing potential must use highly effective contraception throughout the study and for 30 days after the last dose. Any pregnancy occurring during the study must be reported immediately.

8. STATISTICAL CONSIDERATIONS

8.1 Analysis Populations

Population	Definition
Intent-to-Treat (ITT)	All randomized subjects
Modified ITT (mITT)	All randomized subjects who receive at least one dose of study drug
Per-Protocol (PP)	All mITT subjects who complete the study without major protocol deviations
Safety	All subjects who receive at least one dose of study drug

8.2 Primary Efficacy Analysis

The primary endpoint (change from baseline in NT-proBNP at Week 24) will be analyzed using a mixed-effects model for repeated measures (MMRM) with treatment, visit, treatment-by-visit interaction, stratification factors, and baseline NT-proBNP as covariates. The comparison of XYZ-2000 200mg vs placebo will be tested at a two-sided alpha level of 0.05.

8.3 Secondary Efficacy Analyses

Time to first cardiovascular death or heart failure hospitalization will be analyzed using a Cox proportional hazards model stratified by baseline LVEF and region. Kaplan-Meier curves will be generated for each treatment group. Changes in 6-minute walk distance and KCCQ scores will be analyzed using MMRM similar to the primary endpoint.

8.4 Safety Analyses

Safety analyses will be performed on the Safety population. Adverse events will be coded using MedDRA version 26.0 and summarized by system organ class and preferred term. Laboratory parameters and vital signs will be summarized descriptively with shift tables for clinically notable abnormalities.

8.5 Interim Analysis

An independent Data Safety Monitoring Board (DSMB) will conduct one interim analysis for efficacy and futility when approximately 50% of subjects have completed the Week 24 visit. The O'Brien-Fleming spending function will be used to control the overall Type I error rate.

— END OF PROTOCOL —