Clinical Trial Protocol

Epicardial Injection of Allogeneic Human Pluripotent Stem Cell-derived Cardiomyocytes to Treat Severe Chronic Heart Failure

Sponsor	Help Therapeutics Co., Ltd.
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Protocol version 6.1 was the sole version implemented throughout the trial, serving as the definitive operative version for all study activities, including screening, enrollment, and intervention.

Confidentiality

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PROTOCOL SYNOPSIS

Title	Epicardial Injection of Allogeneic Human Pluripotent Stem Cell-derived Cardiomyocytes to Treat Severe Chronic Heart Failure
Sponsor	HELP Therapeutics Co., Ltd. Nanjing, China
Objective	To evaluate the safety and efficacy of intramyocardial injection of Allogenic Human Induced Pluripotent Stem Cells-derived Cardiomyocytes in patients with severe chronic ischemic heart failure with coronary artery bypass grafting indications.
Principal Investigator	Dongjin Wang
Institution	Nanjing Drum Tower Hospital
Indication	Severe Chronic Ischemic Heart Failure
Study Phase	Exploratory
Study Design	single-center, open-label, randomized controlled trial
Study Population	Patients with severe chronic ischemic heart failure with definitive coronary artery bypass grafting (CABG) indications.
Sample Size	20
Treatment Groups	This exploratory trial will enroll 20 patients with severe chronic ischemic heart failure who meet the inclusion/exclusion criteria. After providing informed consent, subjects will be randomized into two groups: Control Group (n=10): Patients will undergo standardized coronary artery bypass grafting (CABG) alone. Cell Therapy Group (n=10): Patients will receive CABG followed by intramyocardial cell therapy (2×10^8 cells). Following the completion of graft anastomoses, human-derived regenerative cardiomyocytes will be administered via 10 targeted injections into the peri-infarct region.
Follow-Up Schedule	Patients will undergo three scheduled visits at postoperative intervals of 1, 6, and 12 months. During each visit, relevant clinical documentation and laboratory test results will be recorded. Following the completion of the study follow-up period, subjects will transition into post-study safety surveillance, which will continue until either mortality or loss to follow-up occurs.

Primary Endpoints	**Safety:** 1. Incidence of sustained ventricular tachycardia (1~6 Month Post-operation) o Defined as the proportion of patients experiencing ventricular tachycardia lasting more than 30 seconds 2. Incidence of newly formed tumors (12 Month Post-operation) o By comparing chest, abdominal and pelvic CT scan and PET-CT scan
Secondary Endpoints	**Safety:** 1. Incidence of perioperative serious adverse events (SAEs): o Such as Mortality, Non-fatal myocardial infarction, Stroke, Cardiac perforation, Cardiac tamponade, etc. 2. Allogeneic immune monitoring: o PRA, DSA (2 week, 1, 3, 6 and 12 Month Post-operation) 3. Perioperative cardiac monitoring: (1 Month Post-operation) **Efficacy:** 1. Left ventricular function assessment: o Echocardiography and cardiac MRI at 1, 6 and 12 months postoperatively to evaluate LVEF, LVEDV, and LVESV. o PET/ECT at 6 and 12 months postoperatively to assess myocardial contractility, diastolic function, and perfusion. 2. Six-minute walk test: o Distance walked measured at 6 and 12 months postoperatively. 3. NYHA functional classification: o Assessed at 6, and 12 months postoperatively. 4. Quality of life evaluation: o Minnesota Living with Heart Failure Questionnaire (MLHFQ) administered at 6, and 12 months postoperatively. 5. Major adverse cardiac events (MACE) incidence: o Mortality; Non-fatal myocardial infarction; Heart failure-related rehospitalization
Statistical Methods and Data Analyses	The trial results will be analyzed primarily using descriptive statistics.

	Continuous variables will be presented as mean ± standard deviation, along with median, minimum, and maximum values. 95% confidence intervals for means will be estimated using the t-distribution method. Between-group comparisons of means will be performed using Student's t-test. Categorical and ordinal data will be summarized as frequencies (percentages) and rates. 95% confidence intervals for rates will be calculated using the Miettinen method. Between-group comparisons of proportions will be conducted using Fisher's exact test. Longitudinal comparisons (e.g., PRA, DSA, 6-minute walk distance, NYHA functional class, MLHFQ scores) will be analyzed using repeated measures ANOVA to assess dynamic changes over time.
Duration	2 years

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LIST OF ABBREVIATIONS

iPSC	Induced pluripotent stem cells
HF	Heart failure
CABG	Coronary artery bypass graft
ICD	Implantable cardioverter defibrillator
LVEF	Left ventricular ejection fraction
LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume
MRI	Magnetic resonance imaging
CT	Computed tomography
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CKMB	Creatine phosphokinase-Mb
NT-proBNP	N-terminalpro-brain natriuretic peptide
PCT	Procalcitonin
TSH	Thyroid stimulating hormone
ECG	Electrocardiogram
NYHA	New York Heart Association
MACE	Major adverse cardiac events
MLHFQ	Minnesota Quality of Life Questionnaire for Heart Failure
6MWD	6min walking test distance
PCI	Percutaneous coronary intervention
HLA	Human leukocyte antigen
PRA	Population reactive antibody
DSA	Anti-donor specific antibodies
CEA	Carcinoembryonic antigen
CA125	Glycogen 125

AFP	Alpha-fetoprotein
ADR	Adverse reactions
AE	Adverse events
SAE	Serious adverse events
CRF	Case report form
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
GCP	Good Clinical Practice
CI	confidence interval
ICF	Informed Consent Form
HIV	Human immunodeficiency virus
HCV	Hepatitis C virus
HBV	Hepatitis B virus
TP	Treponema Pallidum

1 INTRODUCTION

1.1 Background

Heart failure (HF) is a serious condition with high rates of morbidity and mortality. HF is not an isolated condition, but rather the terminal phase of cardiovascular disease progression. Nearly all cardiac disorders—including myocardial infarction, cardiomyopathy, and hemodynamic overload—inevitably culminate in heart failure. Although therapeutic approaches such as pharmacotherapy, interventional procedures, and surgical interventions have seen continuous refinement in recent decades, they remain limited to symptom management rather than full functional restoration of cardiac performance. Consequently, the treatment of heart failure persists as one of medicine's most formidable global challenges.

Myocardial infarction, the most prevalent cardiac disorder, stands as a frequent precursor to heart failure. Mature cardiomyocytes are generally regarded as terminally differentiated cells with negligible regenerative capacity. Consequently, preserving and augmenting functionally competent myocardial cells emerges as the most rational therapeutic approach for various cardiac injuries. With advancing understanding of stem cell biology, harnessing their differentiation potential to regenerate cardiomyocytes, repair damaged myocardial tissue, and restore cardiac function has emerged as a promising novel strategy in contemporary heart failure treatment.

1.1.1 The mechanisms of cardiovascular regeneration

Traditionally, cardiac cells have been regarded as terminally differentiated and incapable of self-proliferation or repair[1-3]. Although some studies suggest the existence of cardiomyocyte turnover in adult human hearts, these findings remain unverified. Indeed, the regenerative capacity of the adult mammalian heart is profoundly limited[4]. When acute cardiac injury or chronic myocardial remodeling occurs, the substantial loss of cardiac cells far exceeds the heart's minimal intrinsic regenerative potential. This starkly contrasts with the robust cardiac regenerative abilities observed during embryonic development and in certain other species. For instance, in mammals, cardiomyocytes maintain vigorous mitotic activity throughout embryogenesis and the early postnatal period. However, this proliferative capacity rapidly diminishes thereafter, with mitotic activity becoming undetectable—lending credence to the conventional view that the adult heart consists of non-regenerative, terminally differentiated cells.

1.1.2 Cardiovascular Regeneration Therapeutics

Products in the field of cardiovascular regeneration can be categorized based on their regenerative strategies into: i) Exogenous regenerative products, which involve transplanted cells or tissues to replace damaged or dysfunctional cardiac structures; and ii) Stimulatory factors that activate endogenous regenerative mechanisms.

Stem cells, as the body's primordial cells, serve as the foundation for all tissues and organs. Stem cell therapy aims to repair diseased cells or reconstruct functional tissues, offering a fundamental treatment for numerous diseases. Consequently, leveraging their self-renewal and multipotent differentiation capabilities to treat heart failure has become a global research focus. Stem cells can be classified into skeletal myoblasts, bone marrow mononuclear cells, hematopoietic stem cells, mesenchymal stem cells, and pluripotent stem cells. Pluripotent stem cells possess unlimited proliferative and differentiation potential, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs, derived from the inner cell mass of blastocysts, hold significant promise in regenerative medicine. However, their application is constrained by ethical concerns, immune rejection, and limited source availability. In 2006, the Japanese scientist Yamanaka and his team[5] pioneered a breakthrough by introducing four pluripotency-associated transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) into mouse embryonic fibroblasts via retroviral vectors, generating cells resembling ESCs with self-renewal and multipotent differentiation capabilities. These reprogrammed somatic cells, termed iPSCs, circumvent the ethical and immunological challenges of ESCs, providing an invaluable tool for disease modeling and drug development. With advancements in culture conditions and reprogramming efficiency, iPSCs can now be derived from various somatic cells (e.g., skin fibroblasts, peripheral blood cells) across species. They can proliferate and differentiate into diverse cell types, including cardiomyocytes[6] — representing a key exogenous regenerative product. Within cardiac regenerative medicine, exogenous products encompass in vitro-differentiated cardiac cells, cardiovascular and endothelial progenitor cells, bioengineered cardiac tissues, and electromechanically functional vascular patches. Recent research has validated numerous approaches, some even demonstrating efficacy in primate models[7–9].

1.1.3 Preclinical and Clinical Research in Cardiac Regeneration

In pioneering studies on pluripotent stem cells, Laflamme et al.[10] successfully transplanted heat shock-treated human embryonic stem cell-derived cardiomyocytes (hESC-CMs) into the left ventricular wall of healthy athymic rats. The transplanted cells demonstrated excellent survival rates, with the majority exhibiting robust expression of myosin heavy chain (MHC). Notably, the hESC-CMs significantly induced angiogenic responses, accompanied by substantial neovascularization in the transplantation area. Echocardiographic and magnetic resonance imaging (MRI) assessments revealed ameliorated ventricular dilation four weeks post-transplantation. Subsequent research by Van Laake et al.[11] involved transplantation of hESC-CMs into myocardial infarction models using NOD SCID mice. The study documented progressive enrichment and maturation of cardiomyocytes, with MRI data indicating marked cardiac functional improvement at four weeks, though this beneficial effect diminished by three months post-transplantation. More recently, Chong et al.[8] made the groundbreaking discovery that hESC-CMs could regenerate cardiac tissue in large non-human primates. Their transplantation into infarcted monkey hearts 14 days post-myocardial infarction resulted in successful regeneration of the damaged myocardial regions. The latest findings by Zhu et al.[12] further demonstrated that transplantation of hESC-derived cardiovascular progenitor

cells in non-human primates led to significant enhancement of left ventricular ejection fraction within 28 days post-intervention.

Induced pluripotent stem cell (iPSC) technology offers a promising alternative by circumventing immunological rejection and ethical or religious controversies. The application of iPSCs in myocardial tissue repair and cardiac functional restoration has opened new therapeutic avenues for heart failure treatment. Miki et al.[13] engineered myocardial sheets from murine iPSCs using in vitro tissue engineering techniques and transplanted them into rats with chronic myocardial infarction. Histological analysis confirmed the survival of the transplanted myocardium at the epicardial site four weeks post-transplantation. Compared to the control group, the transplanted group exhibited significant improvements in cardiac function and attenuation of left ventricular remodeling. Higuchi et al.[14] introduced red fluorescent protein-labeled murine iPSC-derived cardiomyocytes into infarcted rat hearts. Functional assessments revealed progressive enhancement of left ventricular ejection fraction beginning three days post-transplantation. By two weeks, the engrafted cardiomyocytes expressed cardiac myosin and connexin 43 on the cardiac surface. Kawamura et al.[15] demonstrated that transplantation of human-derived regenerated cardiomyocytes into pigs with ischemic cardiomyopathy substantially improved cardiac function, mitigated left ventricular remodeling, and maintained detectable cell survival for eight weeks without teratoma formation. Recent advances in tissue engineering have further expanded therapeutic possibilities. Weinberger et al.[16] reported that iPSC-based artificial cardiac tissue transplantation restored cardiac function in guinea pigs with myocardial injury. Similarly, Gao et al.[17] found that human-derived engineered myocardial patches significantly enhanced left ventricular function, reduced infarct size, attenuated myocardial hypertrophy, and reversed infarction-associated alterations in striated muscle actin-regulated protein phosphorylation in a porcine myocardial infarction model. A landmark 2016 study published in Nature demonstrated that allogeneic iPSC-derived cardiomyocytes achieved robust electrical and mechanical coupling with host myocardium, successfully regenerating cardiac tissue in non-human primates.[18]

With the continuous advancement of preclinical research, clinical studies exploring stem cell-based regenerative therapies for heart failure have progressed rapidly. The first clinical trial in this field was conducted in 2002 to evaluate the potential of stem cells in preventing heart failure[19]. Assmus et al.[20] demonstrated significant improvement in left ventricular ejection fraction (LVEF) among 103 heart failure patients following intracoronary infusion of autologous bone marrow mononuclear cells. Similarly, a Phase I clinical trial involving percutaneous trans-coronary-venous delivery of autologous skeletal myoblasts showed LVEF improvements of 3-8% in 6 out of 9 treated patients[21]. The landmark SCIPIO trial revealed that transplantation of autologous c-kit+ cardiac stem cells substantially enhanced left ventricular function while reducing infarct size[22]. Further supporting these findings, the PROMETHEUS randomized controlled trial demonstrated that mesenchymal stem cell therapy in ischemic heart failure patients led to improved LVEF and reduced scar tissue formation in surgical patients[23]. These clinical trials collectively underscore the promising therapeutic potential of stem cell-based approaches as a novel treatment paradigm for heart

failure. A pivotal milestone was achieved in 2015 when Prof. Menaschᵉ's team published the first human clinical trial of pluripotent stem cell-derived cardiac cells in the European Heart Journal[24]. Subsequent reports confirmed the therapeutic potential of hESC-derived cardiovascular progenitor cells in treating severe ischemic left ventricular dysfunction. Six patients (mean age 66.5 years, baseline LVEF 26%) exhibited marked improvement in systolic function post-transplantation, with no observed arrhythmias or tumor formation during long-term follow-up[25].

Through systematic literature review, we have identified that stem cell therapy has been predominantly investigated for ischemic heart disease treatment. Current research efforts encompass over 100 clinical trials targeting acute myocardial infarction and more than 90 studies focusing on chronic ischemic heart failure (see Table 1 for comprehensive summary). Our analysis included 48 systematic reviews and meta-analyses[26], all demonstrating remarkable consistency in supporting the feasibility and safety of stem cell-based interventions. These studies collectively report significant improvements in both cardiac function and clinical parameters among patients with acute myocardial infarction and chronic ischemic left ventricular dysfunction. In conclusion, cardiac regenerative therapy has been conclusively established as an effective, feasible, and safe therapeutic strategy for patients with ischemic heart disease. The substantial body of clinical evidence underscores its potential as a transformative treatment modality in cardiovascular medicine[27].

Table 1. Summary of Randomized Clinical Trials in Cardiovascular Regenerative Medicine

Disease Classification	Regenerative Product	Safety Profile	Overall Efficacy
Acute Myocardial Infarction (n=2732)	BMMNC[28-38]	Safe	Neutral
	BM-MSC[39]	Safe	Neutral
	Specific BM Cells[40-42]	Safe	Neutral
	ADSC[43]	Safe	Neutral
	CDC[44]	Safe	Positive
Ischemic Heart Failure (n=2035)	SM[45-48]	Safe	Neutral
	BMMNC[49-52]	Safe	Neutral
	BM-MSC[53, 54]	Safe	Positive
	Specific BM Cells[20, 55-60]	Safe	Positive
	CSC[61]	Safe	Positive

	Gene therapy[62-64]	Safe	Neutral
Refractory Angina (n=353)	BMMNC[65-68]	Safe	Positive
	Specific BM Cells[69-71]	Safe	Positive
	ADSC[72]	Safe	Positive
Non-Ischemic Heart Failure (n=166)	BMMNC[73]	Safe	Neutral
	Specific BM Cells[74, 75]	Safe	Neutral
	BM-MSC[76]	Safe	Neutral
Peripheral Artery Disease (n=1217)	BMMNC[77]	Safe	Positive
	Specific BM Cells[78, 79]	Safe	Positive
	Gene therapy[80, 81]	Safe	Neutral

1.2 Study Rationale

Extensive large-animal studies on post-myocardial infarction heart failure have demonstrated that direct transplantation of exogenous cardiomyocyte products can reverse left ventricular remodeling and enhance systolic function, as evidenced in numerous acute and chronic models. Pluripotent stem cell-derived cardiomyocytes show promise in delaying the progression of post-infarction heart failure, offering new therapeutic prospects. Large-animal experiments reveal that allogeneic transplantation of terminally differentiated iPSC-derived cardiomyocytes with physiological cardiac function following infarction-induced heart failure enables effective electromechanical coupling with host cardiomyocytes. This intervention has been shown to improve cardiac function in treated subjects[18]. Our research team has also validated the therapeutic efficacy of human-derived regenerative cardiomyocytes in cynomolgus monkeys. Echocardiographic and magnetic resonance imaging analyses demonstrated significant functional improvement in treated groups compared to untreated controls. Histopathological staining revealed markedly reduced infarct size and fibrosis in the intervention group. Additionally, electrocardiographic monitoring detected no malignant arrhythmic events in either the cell therapy or cell-plus-medium treatment groups during the observation period. Biochemical analyses confirmed no significant hepatic or renal dysfunction in these cohorts. While preclinical primate studies using human-derived regenerative cardiomyocyte transplantation have yielded promising results for heart failure treatment, further investigation is required to translate these findings to human clinical applications. Our team has systematically reviewed, synthesized, and developed the current experimental protocol

based on eight years of closely related clinical research (Table 1: Summary of Randomized Clinical Trials in Cardiovascular Regenerative Medicine).

Notably, in March 2019, Japan initiated the world's first clinical trial using induced pluripotent stem cells (iPSCs) for heart failure treatment. Professor Yoshiki Sawa's team at Osaka University's Department of Cardiovascular Surgery received approval from the Ministry of Health, Labour and Welfare for this groundbreaking study - marking Japan's second application of iPSC-derived cell therapy since the pioneering 2014 retinal cell trial. Preliminary experimental treatment in three cardiomyopathy patients demonstrated favorable safety profiles. This clinical investigation will primarily evaluate therapeutic efficacy through a one-year validation period. These developments provide objective scientific rationale for our current trial.

2 OBJECTIVES AND ENDPOINTS

2.1 Study Objective

To evaluate the safety and efficacy of intramyocardial injection of Allogenic Human Induced Pluripotent Stem Cells-derived Cardiomyocytes in patients with severe chronic ischemic heart failure with coronary artery bypass grafting indications.

2.2 Study Endpoints

2.2.1 Primary endpoints

Safety:

1. Incidence of sustained ventricular tachycardia (1~6 Month Post-operation)
   - o Defined as the proportion of patients experiencing ventricular tachycardia lasting more than 30 seconds
2. Incidence of newly formed tumors (12 Month Post-operation)
   - o By comparing chest, abdominal and pelvic CT scan and PET-CT scan

2.2.2 Secondary endpoints

Efficacy:

1. Cardiac MRI Assessment of Left Ventricular Function:
   - o Comparative analysis of baseline versus postoperative measurements at 6, and 12 months, including: Infarcted size; End-diastolic lateral wall thickness; Focal septal thickness; Ejection fraction; End-diastolic volume; End-systolic volume; Stroke volume; Cardiac output; Myocardial density; End-diastolic myocardial mass
2. Echocardiographic Evaluation of Left Ventricular Function:
   - o Comparative analysis of baseline versus postoperative measurements at 6, and 12 months, including: End-diastolic septal thickness; Left ventricular end-diastolic

diameter; Left ventricular end-systolic diameter; Left ventricular posterior wall thickness at end-diastole; Left atrial diameter; Left ventricular ejection fraction; Mitral inflow velocity profile (E/A ratio)

3. Myocardial Perfusion Imaging (PET/ECT):

   o Comparative analysis of left ventricular perfusion at preoperative, 6-month, and 12-month postoperatively

4. Six-Minute Walk Test (baseline, 6 and 12 months)

5. NYHA Functional Classification (baseline, 6 and 12 months)

6. Minnesota Living with Heart Failure Questionnaire (MLHFQ) (baseline, 6 and 12 months)

7. Incidence of Major Adverse Cardiac Events (MACE) including: Mortality；Non-fatal myocardial infarction；Heart failure rehospitalization

Safety:

1. Incidence of Perioperative Serious Adverse Events (SAE) Incidence: Mortality；Non-fatal myocardial infarction; stroke; cardiac perforation; cardiac temponade, etc.

2. Alloimmune Response Monitoring (PRA, DSA): (baseline, 1, 6 and 12 months)

3. Perioperative Electrocardiographic Monitoring (Within 1 month postoperatively)

4. Laboratory Tests, including Complete blood count, Comprehensive metabolic panel; NT-proBNP;Troponin; Myocardial enzyme profile; Procalcitonin; Immunological panel (C3, C4, IgA, IgG, IgM); Coagulation profile, etc.

3 STUDY DESIGN

This is a single-center, open-label, randomized controlled trial involving 20 eligible subjects who meet the inclusion/exclusion criteria. Subjects will be randomly allocated in a 1:1 ratio into two groups:

- Control Group: Standard CABG treatment only
- Cell Therapy Group: CABG + cellular transplantation (dose: 2×10^8 cells) + perioperative immunosuppressive therapy

Postoperative Management:
All patients will undergo rigorous perioperative monitoring (within the first postoperative month) with comprehensive contingency protocols in place.

Follow-Up & Assessments:
Both groups will undergo identical follow-up evaluations at 1, 6, and 12 months, including:

Imaging studies (as per protocol), Laboratory investigations, Functional assessments. Documentation will be maintained via case report forms (CRFs) to ensure accurate and reliable data acquisition.

3.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

1. Aged 35-75 (including 35 and 75).
2. Have signed the Informed Consent Form.
3. Patients have chronic left ventricular dysfunction.
4. Patients have NYHA Class III-IV cardiac function in spite of optimal heart failure maximally tolerated guideline-directed medical therapy.
5. Patients have indications for Coronary Artery Bypass Grafting.
6. 20% $\leqslant$ LVEF $\leqslant$ 45% as determined by echocardiogram (data collected up to 6 months prior to inclusion evaluation are valid; data collected within 1 month since a myocardial infarction are invalid).
7. Weakening or absence of segmental regional wall motion as determined by standard imaging.

3.2 Exclusion Criteria

1. PRA $\geqslant$ 20% or DSA-positive.
2. Previous implantation of ICD, CRT or similar treatment.
3. Valvular heart disease or received heart valvular disease
4. Previous percutaneous transluminal coronary intervention (PCI)
5. Atrial fibrillation
6. History of sustained ventricular tachycardia or sudden cardiac death.
7. Baseline glomerular filtration rate <30ml/min/1.73m2.
8. Liver dysfunction, as evidenced by enzymes (AST and ALT) greater than three times the ULN.
9. Hematological abnormality: A hematocrit <25% as determined by HCT, white blood cell<2500/ul or platelet values <100000/ul without another explanation.
10. Known, serious radiographic contrast allergy, penicillin allergy, streptomycin allergy.

11. Coagulopathy (INR>1.3) not due to a reversible cause.

12. Contra-indication to performance of an MRI scan.

13. History of organ transplant.

14. Clinical history of malignancy within 5 years (patients with prior malignancy must be disease-free for 5 years).

15. Non-cardiac condition that limits lifespan <1 year.

16. Chronic therapy with immunosuppressant medication, such as glucocorticoids and TNF α antagonist.

17. Patients' allergy to or contra-indication to immunosuppressants.

18. Serum positivity for HIV, HBV, HCV, TP.

19. Currently enrolled in other investigational therapeutic or device study.

20. Female patient who is pregnant or breast-feeding.

21. Other conditions that researchers consider not suitable to participate in this study.

4 STUDY TREATMENT

4.1 Treatment and dosage

4.1.1 Treamtment

All enrolled subjects will undergo standardized coronary artery bypass grafting (CABG) in accordance with established clinical guidelines. For patients in the cell therapy group, the treatment protocol incorporates myocardial cell transplantation alongside the standard CABG procedure. Following completion of graft anastomoses, a total of 2×10^8 allogeneic human regenerative cardiomyocytes are administered via epicardial injection at 10 sites surrounding the infarcted myocardial region..

Patients in the Cell therapy group were administered the following immunosuppressive regimen: intravenous immunoglobulin (2.5 g, IV) on preoperative day 1, intraoperative day (IOD), and postoperative day (POD) 3, combined with methylprednisolone (500 mg, IV) on preoperative day 1; rituximab (20 mg, IV) on IOD and POD4 to target CD20+ B-cell depletion; oral tacrolimus started preoperative day 3 and dose-adjusted to maintain trough levels within the target range of 3-5 ng/mL, as monitored by blood drug concentration until POD28, concurrently with mycophenolate mofetil (1 g, orally on preoperative day 1, increasing to 1.5 g/day until POD28) and prednisone (20 mg/day started IOD until POD28). The Control group did not receive any immunosuppressive medications. All study subjects

undergo intensive postoperative monitoring in the ICU with comprehensive emergency protocols implemented as needed.

4.1.2 Dosage form and dosage

Cell Type: Allogeneic human regenerative cardiomyocytes

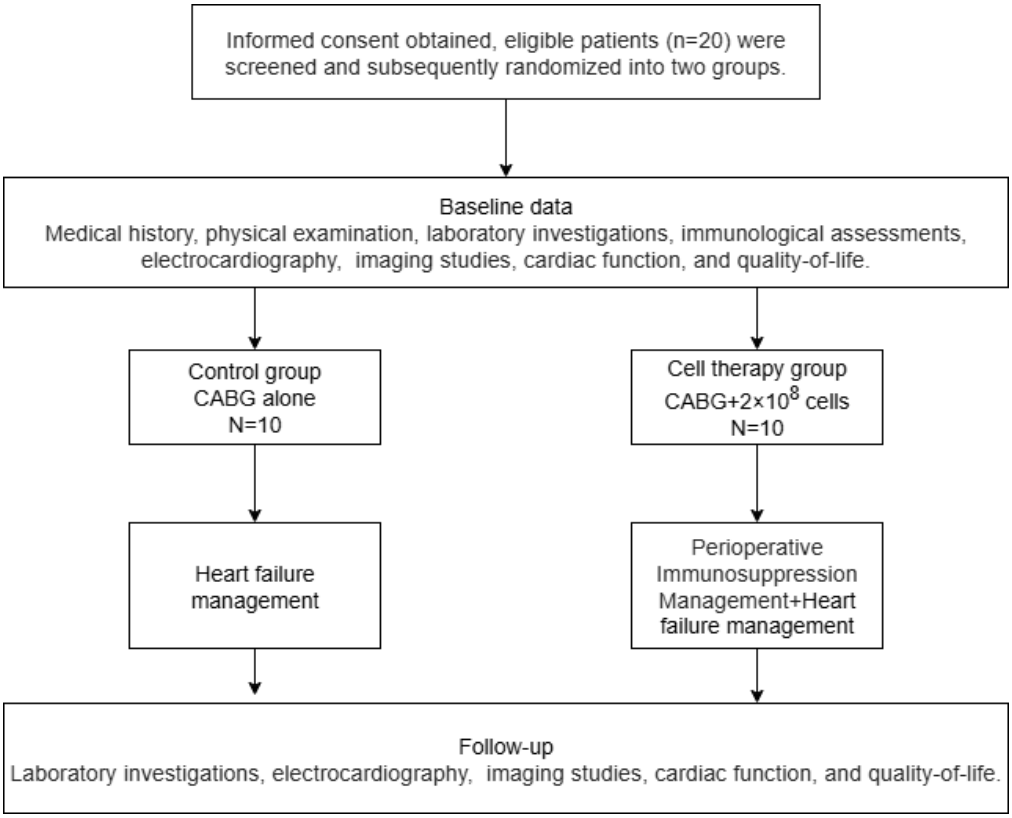
Dosage：A total of 2×10^8 cells are administered via intramyocardial injection, divided equally across 10 sites (250–500 μL per injection).

Administration: The procedure is performed intraoperatively following completion of graft anastomoses during CABG.

Control Group receive standardized CABG alone.

5 STUDY PROCESS

5.1 Study Flowchart



5.2 VISIT AND ASSESSMENT SCHEDULE

All data collection procedures for this study are detailed in Table 3

Table 3 Visit and Assessment Schedule

	Baseline	Treatment period				Follow-up		
Day (D) Month (M)	D-14 to D-1	D0	D1-D7	D14	D21	M1±7d	M6±14d	M12±14d
Informed Consent	X							
Medical History and Physical Examination	X					X	X	X
Vital Signs	X	X	X	X	X	X	X	X
12-Lead ECG	X		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
Coronary Angiogram (SYNTAX score)	X							
Cell transplantation documentation		X						
Echocardiography	X						X	X
Cardiac MRI	X						X	X
PET/ECT	X						X	X
CT Scan (Chest/abdomen/pelvis)	X						X	X
6 Minute Walk Test	X						X	X
NYHA classification	X						X	X
MLHFQ Score	X						X	X
Holter Monitoring (24-hour ECG)	X		X	X	X	X	X	X
Cardiac enzymes, troponin	X		X	X	X	X	X	X
NTproBNP	X		X	X	X	X	X	X
Complete Blood Count, Procalcitonin	X							
Blood type	X							
Biochemistry	X		X	X	X	X	X	X
Urinalysis, Fecal Analysis	X					X	X	X
Thyroid function tests (TSH, FT3, FT4)	X					X	X	X
Tumor markers	X						X	X
Immunogenicity	X			X		X	X	X
Viral Serology	X			X	X	X	X	X
Coagulation	X		X	X	X			
HLA Typing	X							
PRA	X					X	X	X
DSA	X					X	X	X
Cytokine Panel	X		X	X				

	Baseline	Treatment period				Follow-up		
Day (D) Month (M)	D-14 to D-1	D0	D1-D7	D14	D21	M1±7d	M6±14d	M12±14d
Adverse Events		X	X	X	X	X	X	X

6 STATISTICAL CONSIDERATIONS

6.1 Study Design

This is a single-center, open-label, randomized controlled trial. Study objective is To evaluate the safety and efficacy of intramyocardial injection of Allogenic Human Induced Pluripotent Stem Cells-derived Cardiomyocytes in patients with severe chronic ischemic heart failure with coronary artery bypass grafting indications. The sample size for this study is 20.

6.2 Study Duration

Patient enrollment is projected to require 6 months. All subjects will continue protocol-mandated follow-up through the 12-month (12M) visit as scheduled.

6.3 Sample Size Determination

This is an exploratory study. The total sample size is 20, with 10 allocated to the cell therapy group and 10 to the control group

6.4 Randomization and blinding

The trial employed an open-label design with single-blind outcome assessment. To mitigate evaluation bias, Assessors assessing treatment efficacy were blinded to group assignments.

Prior to trial initiation, a statistician generated 20 randomization codes using SAS PLAN procedure with 1:1 allocation. Sequentially numbered (1-20), opaque sealed envelopes containing group assignments were prepared, each bearing its corresponding identification number. Following written informed consent and successful screening, the designated envelope was opened immediately before surgery to reveal group allocation and initiate the assigned procedure. This ensured allocation concealment during screening and pre-randomization phases, preventing selection bias.

6.5 Statistical Analyses

6.5.1 Primary endpoint analysis

The primary endpoints, including incidence of sustained ventricular tachycardia (1~6 Month Post-operation) and newly formed tumors (12 Month Post-operation) will be analyzed using descriptive statistics (number of observations, mean, median, standard deviation, minimum, and maximum) for continuous variables and using frequencies and percentages for categorical and ordinal variables. A final analysis will be conducted when all subjects have completed the study participation.

6.5.2 Secondary endpoint analysis

Secondary endpoints were analyzed using descriptive statistics. The comparative assessment of treatment effects employed distinct analytical approaches based on variable characteristics. Continuous variables are presented with mean, standard deviation, median, maximum and minimum values, and range, compared using the t-test method. Categorical and ordinal data are reported as frequencies (percentages), compared using the Wilcoxon rank-sum test. A two-sided p-value of $\leqslant 0.05$ was considered statistically significant for all analyses.

6.5.3 Safety Analyses

Adverse events will be described using the preferred terms from the MedDRA terminology. All adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. For Major Adverse Cardiac Events (MACE), including arrhythmias, and for other adverse events and adverse drug reactions distinct from arrhythmias, the number of events and the number of subjects experiencing these events will be determined. Adverse events assessed as Serious Adverse Events (SAEs) will be identified, and the number of such SAEs and the number of subjects experiencing SAEs will be determined. For significant events related to Coronary Artery Bypass Graft (CABG), the number of events and the number of subjects experiencing these events will be determined. Adverse events occurring during or within a specified period following the first administration of the investigational medicinal product will be summarized by CTCAE grade.

Clinical laboratory parameters, vital signs, and physical examination findings will be summarized according to study visit. The analysis will describe the observed values at each visit during the trial as well as the changes relative to baseline values.

7 INFORMED CONSENT

Prior to any study-related procedures, all patients must provide written informed consent using the approved Informed Consent Form (ICF). The ICF has been reviewed and approved by the Ethics Committee. The informed consent process must be conducted in compliance with local laws and regulations. The content of the ICF must be understandable to the patient, and the patient has the right to have all questions regarding the study satisfactorily answered before signing. Consent must be voluntarily given by the patient without coercion. During the course of the study, if any amendments to the ICF are required, the revised form must be submitted by the Investigator to the Ethics Committee for review and approval. Newly enrolled patients must sign the most current, Ethics Committee-approved version of the ICF.

8 RISKS AND CONTROLS

The primary risks to subjects participating in this study include immune-mediated rejection (graft-versus-host disease) associated with the administration of allogeneic-sourced regenerative cardiomyocytes, adverse effects related to the administration of immunosuppressive medications, and the potential occurrence of ventricular arrhythmias following cardiomyocyte transplantation

8.1 Tumorigenicity Risk and Control Measures for Allogeneic Human-Derived Regenerative Cardiomyocytes

Stem cells share several characteristics with tumor cells, including sustained proliferative capacity and resistance to apoptosis, as well as similar growth regulatory mechanisms. Animal models have demonstrated that human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) can lead to both benign and malignant teratomas. However, the cellular product utilized in the present study consists not of stem cells, but of fully differentiated cardiomyocytes. Theoretically, this confers a substantially reduced tumorigenic risk, which is supported by the absence of tumor formation in prior small and large animal studies. Numerous international studies utilizing cardiomyocytes differentiated from mesenchymal stem cells (MSCs) report no definitive evidence of tumorigenicity associated with human MSCs, even beyond passage 50. Recent preclinical studies investigating allogeneic human-derived regenerative cardiomyocytes for heart failure treatment consistently report no teratoma formation following cell transplantation therapy [9,18,24-27]; relevant safety data are summarized in Table 1.

One of the safety assessments in this study involves monitoring for the incidence of newly developed tumors through scheduled CT scans and positron emission tomography (PET) examinations. The objective is to achieve timely detection and diagnosis to mitigate the severe consequences associated with tumor progression. Should any new tumor be identified, histopathological examination will be performed to definitively determine its origin and assess any potential association with the cell transplantation.

8.2 Graft-Versus-Host Disease Risk and Control

The presence of donor-derived T lymphocytes within the cellular graft carries a risk of eliciting a graft-versus-host response, which can manifest as a severe clinical condition known as Graft-Versus-Host Disease (GvHD). GvHD is classified based on the time of onset post-transplantation: occurrences within 100 days are designated as Acute GvHD, while manifestations arising after 100 days are classified as Chronic GvHD.

8.2.1 Acute GvHD

Acute GvHD, occurring early post-transplantation and representing a significant cause of early mortality, primarily affects the skin, gastrointestinal tract, and liver, with rare involvement of other organs. Cutaneous manifestations constitute the most frequent

presentation, characterized by erythema and maculopapular rash, often accompanied by pruritus or pain; lesions initially appear on the palms and soles, subsequently progressing to the face, ears, neck, trunk, and back, with severe cases demonstrating epidermal necrolysis and desquamation. Gastrointestinal involvement typically manifests as refractory diarrhea exceeding 1000 ml per day, associated with anorexia, nausea, and vomiting; advanced stages may feature abdominal pain with intestinal bleeding and obstruction. Hepatic involvement generally develops later, presenting as jaundice with elevated serum bilirubin and alkaline phosphatase levels. The majority of acute GvHD cases occur between 20 to 40 days following transplantation.

8.2.2 Chronic GvHD

Chronic GvHD typically manifests between 100 days and 1.5 years post-transplantation, with a minority of cases occurring up to 2 years. Incidence varies from 20% to 70% depending on graft source. Chronic GvHD frequently involves the oral mucosa, with xerostomia representing the earliest and most prevalent manifestation, often accompanied by oral pain or lichenoid mucosal changes. Cutaneous involvement initially presents as lichen planus-like lesions or polygonal papules, progressing in severe cases to generalized dermatopathy; late-stage skin changes include hyperpigmentation, atrophy, and fibrosis resembling scleroderma, potentially causing joint contractures and restricted mobility. Ocular involvement manifests as dry eye syndrome with significantly reduced tear production. Hepatic dysfunction is common, primarily evidenced by jaundice with histopathological findings of hepatic necrosis or cirrhosis. Gastrointestinal compromise frequently features esophageal pathology causing dysphagia and pain, potentially leading to weight loss; barium swallow studies demonstrate tapered esophageal strictures. Pulmonary involvement affecting bronchi and lung parenchyma may result in diminished pulmonary function and dyspnea. Additionally, Chronic GvHD is associated with immunodeficiency leading to recurrent infections, and persistent thrombocytopenia predisposing to hemorrhagic complications.

8.2.3 Treatment of GvHD

Standard first-line therapy for acute GvHD involves intravenous administration of methylprednisolone (a glucocorticoid), with potential dose escalation based on disease severity. Second-line therapy consists of one of the following agents: tacrolimus or mycophenolate mofetil; patients exhibiting suboptimal response to second-line therapy often receive combination therapy with methylprednisolone. Additionally, anti-T-cell monoclonal antibodies (e.g., anti-CD3 monoclonal antibody) may be utilized in patients refractory to methylprednisolone, while antibodies targeting the interleukin-2 receptor also demonstrate efficacy. For chronic GvHD, standard first-line therapy entails early initiation of prednisolone combined with azathioprine, followed by gradual tapering upon clinical improvement, with a total treatment duration of approximately one year. In cases accompanied by thrombocytopenia with reduced megakaryocytes in the bone marrow, prednisolone combined with cyclosporine A is preferred. Patients refractory to the

aforementioned therapies may receive second-line agents, including one of the following: tacrolimus or mycophenolate mofetil.

8.3 Immunosuppressive Medication Adverse Effects and Mitigation Strategies

The human immune system maintains highly sophisticated defense mechanisms against pathogenic agents, enabling the identification and targeted elimination of non-self entities including bacteria, viruses, foreign materials, allogeneic tissues, and synthetic implants. This complex immunological response represents a critical protective mechanism. Following allogeneic tissue or organ transplantation, the graft is recognized by the recipient's immune system as a non-self entity, triggering an immune-mediated attack that manifests as transplant rejection—a process involving graft destruction and clearance. Transplant rejection constitutes a primary determinant of graft survival. The administration of immunosuppressive medications represents an established therapeutic strategy for the effective prevention of transplant rejection.

Glucocorticoids : Adverse reactions associated with glucocorticoids are infrequent during short-term therapy but warrant vigilant monitoring. Potential adverse effects include: increased susceptibility to infections; drug hypersensitivity reactions; endocrine abnormalities; metabolic and nutritional disorders; gastrointestinal complications; skin and subcutaneous tissue disorders; musculoskeletal and connective tissue abnormalities; and menstrual irregularities.

Tacrolimus: This agent exerts its immunosuppressive effect primarily through inhibition of calcium-dependent signal transduction, thereby suppressing lymphokine production. A significant adverse effect is the inhibition of insulin secretion leading to hyperglycemia. Additional adverse reactions encompass hematological abnormalities; neurological manifestations; ophthalmic disturbances; tinnitus; respiratory disorders; gastrointestinal manifestations; renal impairment; dermatological effects; musculoskeletal complaints; and metabolic derangements.

Mycophenolate Sodium: This novel immunosuppressive agent employs a sodium salt substitution for the ester moiety present in mycophenolate mofetil, aiming to reduce gastrointestinal adverse effects associated with the ester group while maintaining efficacy in preventing transplant rejection. The most frequently reported adverse drug reactions include leukopenia and diarrhea. Additional potential adverse effects encompass infections; hematological abnormalities; headache; cough; gastrointestinal disturbances; abnormal liver function tests; elevated serum creatinine; and rash.

Basiliximab for Injection (e.g., Simulect): As an interleukin-2 (IL-2) receptor antagonist, basiliximab is associated with adverse reactions primarily including constipation, urinary tract infection, pain, nausea, hypertension, anemia, headache, hyperkalemia, hypercholesterolemia, surgical wound complications, weight gain, elevated serum creatinine, hypophosphatemia, diarrhea, and upper respiratory tract infection.

Safety Monitoring During Immunosuppressive Therapy: Subjects enrolled in this study will receive immunosuppressive therapy for a maximum duration of one month at reduced dosing levels. To mitigate the potential adverse effects associated with these agents, rigorous safety monitoring will be implemented throughout the dosing period. This includes scheduled assessments of hematological parameters, renal and hepatic function, quantitative immunoglobulin levels and lymphocyte subsets, as mandated by the protocol. Vigilant clinical monitoring for signs of adverse events or complications will be maintained. Prophylactic measures, including anti-infective prophylaxis and nutritional support, will be implemented per protocol-specified guidelines.

8.4 Post-Transplant Ventricular Arrhythmias Risks and Control

Fatal malignant ventricular arrhythmias constitute a potentially severe risk associated with myocardial cell therapy. Ventricular arrhythmias, particularly polymorphic ventricular tachycardia and ventricular fibrillation, represent significant in-hospital causes of early mortality in myocardial infarction (MI) patients. The resulting tissue architecture, comprising necrotic scar, border zones, and viable myocardium, creates regions of heterogeneous electrophysiology, particularly within the peri-infarct zone, fostering anisotropic conduction and reentrant circuits. Cell therapy targeting the infarcted myocardium inherently modifies this arrhythmogenic substrate. Theoretically, such interventions carry dual potential: therapeutic augmentation of the substrate may reduce arrhythmia susceptibility, while proarrhythmic exacerbation remains a concurrent possibility. Whether cell therapy exerts proarrhythmic effects depends critically upon multiple factors: (1) the specific cell type employed; (2) the degree of electrophysiological integration and coupling between transplanted cells and native myocardium; and (3) intrinsic electrophysiological properties of the transplanted cells themselves. Transplanted cells may introduce novel proarrhythmic substrates by establishing reentrant pathways within scar tissue or inducing arrhythmias via mechanisms of ectopic automaticity or triggered activity.

Animal experimental evidence regarding the proarrhythmic effects of cell-based therapies remains limited and conflicting. Some studies indicate that transplanted cells, which originally lack connexin 43 expression, can acquire this critical gap junction protein post-engraftment; connexin 43 is essential for facilitating coordinated electrical conduction within myocardial tissue. However, persistent concerns exist based on studies suggesting that cardiomyocytes derived from embryonic stem cells (ESCs) may harbor intrinsic proarrhythmic risks. Notably, research by Shiba et al. demonstrated that treatment of heart failure in nonhuman primates using induced pluripotent stem cell (iPSC)-derived cardiomyocytes did not result in fatal arrhythmias [18].

Clinical investigations initiated by Professor Menasche P. et al. (French Academy of Sciences) in 2015, involving multiple patients treated with human pluripotent stem cell (hPSC)-derived cardiac progenitor cells for heart failure [24, 25], have not reported any proarrhythmic effects to date. Nevertheless, further clinical data are required to definitively establish the arrhythmic safety profile of cell-based therapies. Collectively,

current clinical trial results across various cell therapy approaches have not reported significant arrhythmic risks; paradoxically, some evidence suggests stem cell therapy may represent a potential therapeutic strategy for post-infarction ventricular tachycardia. Specifically, transplantation of mesenchymal stem cells into myocardial infarct zones has been associated with myocardial repair, improved cardiac function, and no increased susceptibility to ventricular arrhythmias.

Given the theoretical potential for proarrhythmia associated with allogeneic human-derived regenerated cardiomyocyte therapy in this study, the following risk mitigation measures will be implemented: (1) Continuous electrocardiographic (ECG) monitoring will be employed for all subjects throughout the peri-procedural period. Following hospital discharge, subjects will be provided with remote cardiac monitoring devices at no cost to facilitate ongoing rhythm assessment. This remote monitoring infrastructure enables investigators to promptly evaluate subject cardiac rhythm status and detect potential arrhythmic events in a timely manner; (2) Arrhythmic risk stratification will utilize 24-hour Holter monitoring performed within 24 hours post-procedure; subjects identified as high-risk for ventricular arrhythmias (e.g., frequent premature ventricular contractions [PVCs] >6 per minute) will receive antiarrhythmic prophylaxis.

8.5 Donor Safety Risks and Control

The human-derived regenerative cardiomyocytes utilized in this study are cell suspension preparations obtained from the blood of healthy adult donors via in vitro preparation methods. To minimize potential risks associated with the donor source, a rigorous Donor Eligibility Determination was conducted for all donors, comprising detailed examinations and testing. This included comprehensive assessment of the donor’ s medical history and family history, alongside thorough laboratory investigations.Laboratory testing encompassed screening for relevant infectious diseases (HIV, HBV, HCV, HTLV, and syphilis); only healthy adults testing negative for all specified infectious agents were deemed eligible. Beyond routine laboratory screening, donors underwent karyotype analysis and whole-genome sequencing to exclude potential hereditary cancers and cardiac diseases.

All testing protocols were performed in strict accordance with the 3rd Edition of Research, Development and Quality Control of Biotechnology Pharmaceuticals and relevant FDA regulations (21 CFR Part 1271; http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073964.htm).

9 RESEARCH ORGANIZATION STRUCTURE

9.1 Sponsor and Responsibilities

Help Therapeutics Co., Ltd. serves as the Sponsor for this study and is responsible for study initiation and coordination. Specific responsibilities include:

1) screening of clinical investigators;

2) activating the research center upon receipt of all required documentation;

3) establishing the study database and assisting in data analysis;

4) reviewing the veracity, accuracy, and completeness of collected data;

5) ensuring all adverse events (AEs) and adverse drug reactions (ADRs) are reported and confirmed by the investigator. In the case of serious adverse events (SAEs) or death, the Sponsor is responsible for confirming reporting to the Ethics Committee.

9.2 Clinical Research Coordinator

The overall clinical coordinator for this study is Dr. Zhang He, a cardiologist.

Contact details are as follows: Nanjing Drum Tower Hospital, 321 Zhongshan Road, Nanjing 210008, Jiangsu Province, People's Republic of China.

Telephone: 15250969968.

Email: pumczhanghe@163.com

9.3 Investigator and Responsibilities

Professor Dongjin Wang serves as the Investigator and is the responsible person for the implementation of this study at the site. The Investigator is accountable for clinical decisions related to this study and for organizing/managing personnel involved in the study. The Investigator must conduct the study strictly in accordance with the protocol and signed agreements. By agreeing to conduct the study, the Investigator accepts monitoring, auditing, and supervision by the Ethics Committee. Responsibilities include:

1) Signing the Investigator Agreement;

2) Obtaining written approval from the Ethics Committee;

3) Implementing the study plan per protocol requirements;

4) Screening eligible patients;

5) Collecting data and accepting monitoring. Any omissions or errors in data must be promptly completed or corrected.

9.4 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs within 28 days from the previous ICF signature date.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The investigator or authorized designee will explain to each subject the objectives of the exploratory research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining specimens to be used for exploratory research. Subjects who decline to participate in this optional research will not provide this separate signature.

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