A Review on Gene Therapy for the Treatment of Heart Failure

By
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Bachelors of Pharmacy (Hons)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Ethics Statement

The thesis was done without involving any human or animal tests.

Abstract

Heart failure is a complex, fatal syndrome significantly affecting the quality of life, increasing cost and social burden. As a result, reducing its social and financial impact has been as a top global health priority. Researchers are considering gene therapy as a powerful emerging tool to significantly prolong heart failure patients' life. New vectors are being studied, novel delivery methods are being proposed and innovative targets are being identified for treating heart failure by gene therapy. Some modification and improvement are yet to be done in this field to make it an effective treatment option for patients. More than 64 million people suffer from heart failure worldwide. Hence, an urgent need to cure this disease has emerged. The goal of this review is to demonstrate the different vectors used, delivery methods and targets and to provide a comprehensive understanding of the current landscape of gene therapy in treating heart failure.

Keywords: Heart failure; gene therapy; gene delivery vector; delivery method; target.

Dedication

I dedicate this work to the lotus feet of Sri Sri Radhe Shyamsundar, my parent and my husband.

Acknowledgement

At first, I thank God for giving me courage, inspiration and energy throughout this journey. I would like to commence by expressing my gratitude to Him for the countless blessings and for making this journey much easier.

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List of Acronyms

AAV	Adeno Associated Viral Vector
AC	Adenyl-Cyclase
AIDS	Acquired Immune Deficiency Syndrome
ATP	Adenosine Triphosphate
AR	Adrenergic Receptor
AV	Adenoviral Vector
Bcl-2	B-cell lymphoma
BNP	Brain Natriuretic Peptide
cAMP	Cyclic Adenosine Monophosphate
CMV	Cytomegalovirus
CXCR4	Chemokine Receptor type 4
DNA	Deoxyribonucleic Acid
ESC	European Society of Cardiology
GRK	G-protein– coupled receptor kinase
HF	Heart Failure
JVS	Journal of Vascular Surgery
LV	Lentiviral Vectors
LVEF	Left Ventricular Ejection Fraction
MLWHFQ	Minnesota Living with Heart Failure Questionnaire
MRI	Magnetic resonance imaging

MWD	Mean Wheal Diameter
NYHA	New York Heart Association
PLN	Phospholamban
PKA	Protein Kinase A
PP1	Protein Phosphatase Inhibitor-1
RNA	Ribonucleic Acid
RYR	Ryanodine Receptor
SERCA	Sarcoendoplasmic Reticulum Calcium ATPase
SDF	Stromal Cell Derived Factor
SUMO1	Small Ubiquitin-like Modifier type 1
VEGF-B	Vascular Endothelial Growth Factor B

Chapter 1

Introduction

1.1 Background

There will be a 25% increase in prevalence and 215% increase in direct medical costs associated with heart failure (HF) from 2010 to 2030 (Heidenreich *et al.*, 2011). Heart failure is a condition where the heart is incapable of supplying sufficient blood flow in order to fulfill metabolic requirements or make room for systemic venous return (Kemp & Conte, 2012). According to ESC (European Society of Cardiology) guidelines, 2021, heart failure is characterized by a functional/ structural heart abnormality which results in reduced intracardiac pressures and insufficient cardiac output during exercise or at rest (McDonagh et al., 2021). Heart failure might be caused by diastolic/systolic dysfunction or, both. The presence of pathology of pericardium, valves, endocardium and heart rhythm abnormalities might also cause heart failure. Heart failure is associated with cardinal symptoms (e.g., breathlessness, ankle swelling and fatigue) with signs (e.g., pulmonary crackles, increased jugular venous pressure and peripheral oedema) (McDonagh et al., 2021). An emerging treatment in heart failure is gene therapy. Human gene therapy is a therapeutic approach which modifies or manipulates the expression of a gene or it alters the biological properties of the body's living cells for intended therapeutic use (Fda & Cber, 2020). The history of gene therapy in cardiac disease started in 1990 when Dr. Leiden's group published a seminal study in which rat cardiomyocytes were transfected in vivo. They injected plasmid DNA which contained the β-galactosidase gene in the left ventricular wall directly of rats. Activity of β-galactosidase was found in the myocardium after 4 weeks of treatment (Lin et al., n.d.). After phases of excitement and disappointment, and a decade of preclinical studies, clinical trials has officially launched in recent years. The initial one that reached phase II and the testing of delivery of gene of sarcoendoplasmic reticulum calcium ATPase had not given pleasing results. However, trials are still being continued to find a more effective viral vectors, and potential targets for gene therapy to treat heart failure. Current research focuses on gene repair, in order to treat hereditary forms of HF, in vivo, although experimental evidences show that specific microRNAs can produce regeneration when transported to the post-ischemic hearts, a result which was imagined to be possible only by the application of stem cell therapy (Gabisonia & Recchia, 2018).

1.2 Objective of the Review

The aim and objective of this review is to demonstrate the novel treatment strategies of heart failure by gene transfer, the possible vectors, delivery methods and targets as well as the current clinical results from the early clinical trials. The objective of this review is to gather data on the recent clinical trial, accumulate knowledge about new gene delivery vectors, delivery methods and the targets and identify knowledge gaps.

1.3 Rational of the Review

This review will give a better understanding and overview of the gene delivery vectors, gene delivery methods, targets for improving the signs and symptoms of heart failure and the major clinical trials performed recently which will be helpful for making modification and improvement in the field of gene therapy in the future.

Chapter 2

Methodology

The resources were found from research articles, peer-review studies found in well-known databases such as PubMed, Google Scholar, Embase and Scopus. The articles were searched based on important keywords like ‘gene therapy’, ‘heart failure’, ‘gene delivery vectors’, ‘target’, ‘clinical trial’ etc. The inclusion criteria include articles related to heart failure and gene therapy. The exclusion criteria include duplicate, unrelated article and articles which do not have full text or have abstract only. Relevant articles were collected.

Chapter 3

3.1 What is Gene Therapy?

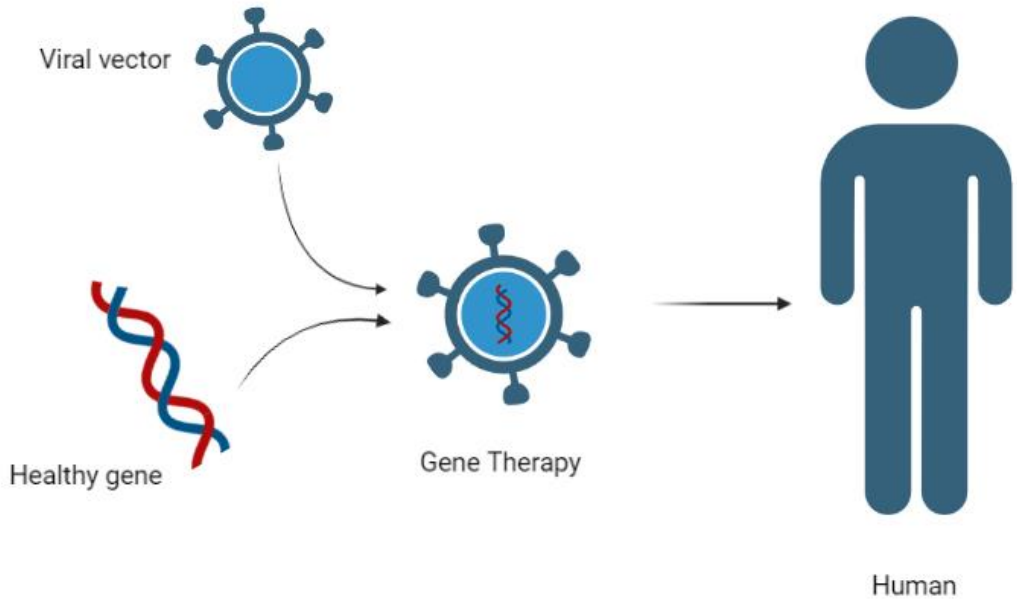

Figure 1: Schematic representation of gene therapy.

Gene therapy is a treatment in which a new healthy gene replaces a mutated diseased gene or a new gene is added inside a single cell or the cells of the body in order to cure or treat a particular disease (Gonçalves & Paiva, 2017). At first, a healthy gene is selected and then it is inserted into a viral or nonviral vector by a gene delivery method. Then the viral vector with healthy gene is inserted into the human body inside a specific target cell as shown in figure 1. Gene therapy is being used to treat certain types of disease such as cancer, AIDS, heart disease and so on. Gene therapy holds a promising future in the field of medicine (Gonçalves & Paiva, 2017).

3.2 What is Heart Failure?

A functional or structural heart irregularity that results in deficient cardiac output and decreased intracardiac pressures at rest or during exercise is commonly known as heart failure. Heart failure can occur due to systolic or diastolic dysfunction or due to both. Presence of pathology of pericardium, endocardium, valves and heart rhythm abnormalities can be also the reason of heart failure. The signs of heart failure are pulmonary crackles, peripheral oedema and increased jugular venous pressure. The cardinal symptoms of heart failure are breathlessness, ankle swelling and fatigue (McDonagh et al., 2021).

3.3 Gene Delivery Vectors

Gene delivery vectors are of two types. One is a viral-based vector and another is no-viral-based vector. Genetic material can be delivered to the heart successfully by these two types of vectors. Viral vectors can deliver nucleic acid much more efficiently to the heart. Viral vectors are able to provide gene expression for a long time and also, they have the ability to carry robust-sized genes. Only viral vectors are used in clinical trials till now (Kieserman et al., 2019). No-viral-based vectors are safe to use and have minimal immunogenicity. But they have insufficient transfection efficiency which is a big disadvantage (Tilemann et al., 2012). At first, exogenous genetic material is introduced into cells directly or into the vasculature. The viral vector has the responsibility to carry exogenous genetic material from that site where it has been inserted into cells directly or into the vasculature to the target cell's nucleus. A capsid is a protein shell adjacent to the viral genome. Sometimes capsids are surrounded by or an envelope or a lipid bilayer surround capsid. The envelope or lipid bilayer contains proteins which assist in coupling to targeted cells. The virus is transported over the cell membrane after binding with a specific receptor on the cell-surface (Kieserman et al., 2019). Then the genes are transported to the nucleus (Petrus et al., 2010). Viral vectors can cross the cell membrane more effectively than nonviral vectors. That's why they are widely used for inserting genes into the human heart. The parent virus's pathogenic genes are deleted in order to prevent the viral vectors from causing disease. However, a viral vector is challenging to use in vivo. Viral vector's manufacturing is complex and it has to be done carefully. In individual patients, immune responses toward the viral capsid can also be observed. In the United States, three viral vectors are currently under evaluation for targeting the cardiovascular system. They are adenoviral vector, lentiviral vector and adeno-associated viral vector (Kieserman et al., 2019).

3.3.1 Adeno Associated Viral Vectors

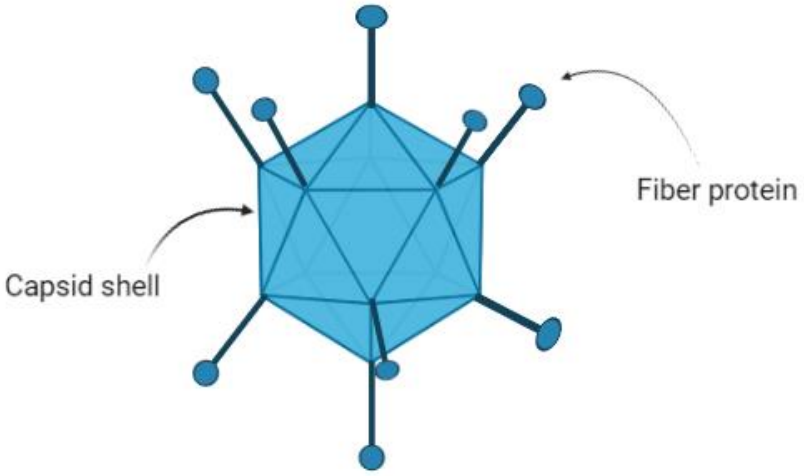

Figure 2: Schematic representation of adeno associated viral vector.

AAVs are DNA vectors which are single-stranded. They are small, nonenveloped and have icosahedral capsids as shown in figure 2. The genome is about 4.7-kb which is flanked by two viral inverted terminal repeats which comprise two genes: cap and rep. The rep protein aids AAV integration into the host chromosome's S1 site and that's why from therapeutic viral vectors, it is removed often (DiMattia et al., 2012). The recombinant AAV vector does not integrate without rep and yet in postmitotic tissues, it is capable of providing long-term episomal persistence. They exist commonly as big head-to-tail ring shaped multimeric concatemer structures. A replication mechanism of DNA which is a rolling circle-type and recombination is needed for the production of concatemers. AAVs might provide transgene expression stably (Athanasopoulos et al., 2000). Expressions which are tissue specific can be gained by using a promoter which is also tissue-specific. Furthermore, if a non-target organ is introduced in between 10^13 and 10^11 viral particles, the risk of exogenous DNA colocalizing with powerful promiscuous enhancer randomly is increased which may start gene transcription in an undesired location. The sporadic transduction takes place in a single cell, that's why it is not possible to find out whether the event has taken place or predict that it may take place. So, under way surveillance and long-term follow-up must be included in clinical protocols of all gene-therapy for possible malignancy (Kieserman et al., 2019). There are twelve strains of

AAVs (Kieserman et al., 2019). The different serotypes have the same size, structures, organization and distinct tropism based on the structure of their capsid protein structure (Tilemann et al., 2012). Serotypes which are the most cardiotropic are AAV1, AAV8, AAV6 and AAV9 (Zincarelli et al., 2010). But for gene delivery in the heart, AAV9 serotype is the most efficacious. In transduction efficiency, AAV9 contains a >200-fold higher level compared to AAV1 in the mouse, but, AAV8 transduction in myocardium is at ≈20-fold increased levels compared to AAV1(Kieserman et al., 2019). By coronary infusion, AAV9 is delivered the best (Fang et al., 2012). Delivery by direct myocardial injection is also possible for AAV9 (Prasad et al., 2011). There are no direct comparisons between viral vectors in nonhuman primates, as the cost of these experiments would be huge. However, AAV9 aids in efficacious transduction of a wide variety of cardiac genes into the myocardium (Kieserman et al., 2019). In AAV9, the capsid edges are smooth and the immunogenicity of AAV9 is much less (DiMattia et al., 2012). By including terminal repeats which are found from AAV2, AAV9 efficacy is increased. This variant's designation is AAV2/9. In spite of optimization of design, little immunogenicity remains in AAV vectors which may lead to myocarditis development, limited biologic effects and decreased transcription which limits gene therapy utilization. Preexisting anti-AAV antibodies which are neutralizing are present in 30% to 50% population and among different serotypes, cross-reactivity may happen. The efficiency of gene therapy approach might be altered by the adaptive immune system as AAV antibodies can remove the vector itself and blunt its transduction ability. An approach is proposed by investigators to limit the adaptive immune system's effectiveness: utilizing vacant vectors to overfill the system which needs further investigation (Kieserman et al., 2019). AAVs can infect nonhuman primates as humans but animal models might not estimate outcomes in treatment of humans (Rozas et al., 1997; Wahl et al., 2014). Because responses of preexisting T- cells in humans are distinct from those in nonhuman primates (Rozas et al., 1997). To inspect immune responses to adeno associated viral vectors, preclinical animal models were developed but they all have limitations. For instance, preexisting neutralizing antibodies seroconversion was not reduced effectively in a pig model by aggressive immunosuppressive therapy, but in humans, immune suppression has been efficacious for those who had inflammation. Hence, immune response to combination of a particular vector-gene is determined best in the early-phase of clinical trial. Two recent publications identified that AAV9's intrathecal administration might cause toxicity which was carrying survival motor neuron-1 gene. The reporters of the publication warn to use AAV9 carefully in children who have floppy baby syndrome (skeletal muscle atrophy) for clinical studies. Some studies proposed that the toxicity is dose dependent and species-strain dependent

(Hinderer et al., 2018; Hordeaux et al., 2018). Evidence of efficacy and safety was found from clinical outcomes. Survival motor neuron gene replacement in fifteen patients who had disease having either minimum doses [6.7×1013 vp (viral particles) per kilogram(kg) of the body weight; n=3] or maximum doses [2.0×1014 vp (viral particles) per kilogram(kg) of the body weight; n=12] showed the result that all fifteen patients survived at twenty months versus the survival rate of eight percent. Marked and significant progress in motor function is also seen in the patients. Aminotransferase levels in serum were decreased in four patients, but were retrieved with prednisolone (Mendell et al., 2017). Nevertheless, the most careful and provident approach would be to eliminate individuals with raised AAV antibody titers to particular AAV serotype, patient's follow-up throughout the late and early phases of therapy with caution, including echocardiography use and biomarkers use for inflammation, electrocardiography use and an algorithm prestudy development for inflammation treatment (Kieserman et al., 2019).

3.3.2 Adenoviral Vectors

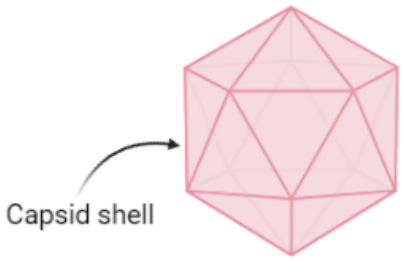

Figure 3: Schematic representation of adenoviral vector.

Adenoviral vectors do not have envelopes, contains icosahedral capsid as well as a non-integrating, linear and double-stranded DNA that can be seen in figure 3. By clathrin-mediated endocytosis, the vector penetrates the cell after attaching with the coxsackie-adenovirus receptor which are situated on the surface of cell. Seven species of AVs are present with a minimum 50 different serotypes (Gonçalves & de Vries, 2006). The presence of adenovirus contaminants which are replication-competent make it difficult to produce first generation AVs and it might result in viral-like symptoms (Kieserman et al., 2019). "Gutless" vectors or third generation AVs do not have any viral coding regions. During vector production, helper particles are needed which should be removed before human use (Alba et al., 2005). Adenoviral vectors have advantages: (i) AVs of third-generation may hold bigger transgenes (which is up

to 35 kb) (Merten & Gaillet, 2016), (ii) the DNA which is double-stranded is transferred to the nucleus and it provides effective transduction (Kieserman et al., 2019), (iii) within hours to days, transgene expression happens after transduction (Kieserman et al., 2019), (iv) cardiac transduction is achieved in high levels by them and (v) during cell division, AVs do not go to daughter cells as they are nonintegrating (Vassalli et al., 2003). Nevertheless, AVs have some limitations: (i) transgene expression is temporary which lasts for about two to four weeks, (ii) with noteworthy dose-related toxicity, the innate immune system can be activated by AVs and the adaptive immune system's preexisting antibodies might limit the efficacy of AVs (Kieserman et al., 2019) and (iii) intramyocardial injection might need to be given directly for AVs (Vassalli et al., 2003). However, a young man who had deficiency of ornithine transcarbamylase evolved a systemic inflammatory response to adenoviral vectors and died due to that. This report lowered the enthusiasm regarding adenoviral vectors (Raper et al., 2003).

3.3.3 Lentiviral Vectors

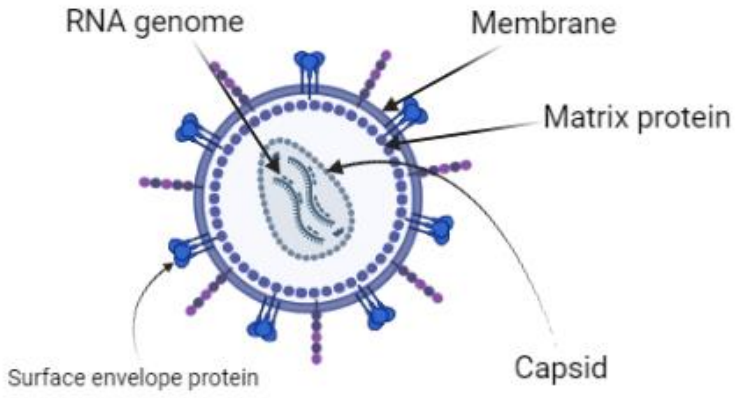

Figure 4: Schematic representation of lentiviral vector.

Lentiviruses are an enveloped virus as shown in figure 4. Lentiviruses are single-stranded RNA vectors which can transduce both nondividing and dividing cells. They exhibit relatively strong efficiency of transduction. In a study, mice exhibited decreased LV function after introduction of injection of doxorubicin, an anticancer drug. Then a lentiviral vector transporting SERCA2a transduction into the mice heart by direct injection showed improved myocardial function. Two weeks after a myocardial infarction in rats with LV dysfunctions was given SERCA2a-expressing lentivirus injection in the heart by hypothermic intracoronary delivery showed

enhanced SERCA2a expression, protected against heart failure and also affected remodeling and function (Mattila et al., 2016; Niwano et al., 2008). Furthermore, a mouse model having mucopolysaccharidosis type-7 was given LV-mediated gene therapy which showed the result of sustained expression of β-glucuronidase in the mouse model till twelve months (Derrick-Roberts et al., 2014). Lentivirus vectors can mediate insertional oncogenesis as they integrate into the genomes stably, their cargoes integrate into target cell's genome for targeting to the gene's coding regions (Papayannakos & Daniel, 2013). In terminally differentiated and postmitotic cells, for example, a cardiac myocyte, insertional oncogenesis's risk may be lower, but no promoter or gene delivery system can be 100% cardiac specific. That's why use of lentiviral vector is limited in the cardiovascular system and in clinical trials concerning heart failure (Kieserman et al., 2019).

Chapter 4

4.1 Gene Delivery Methods

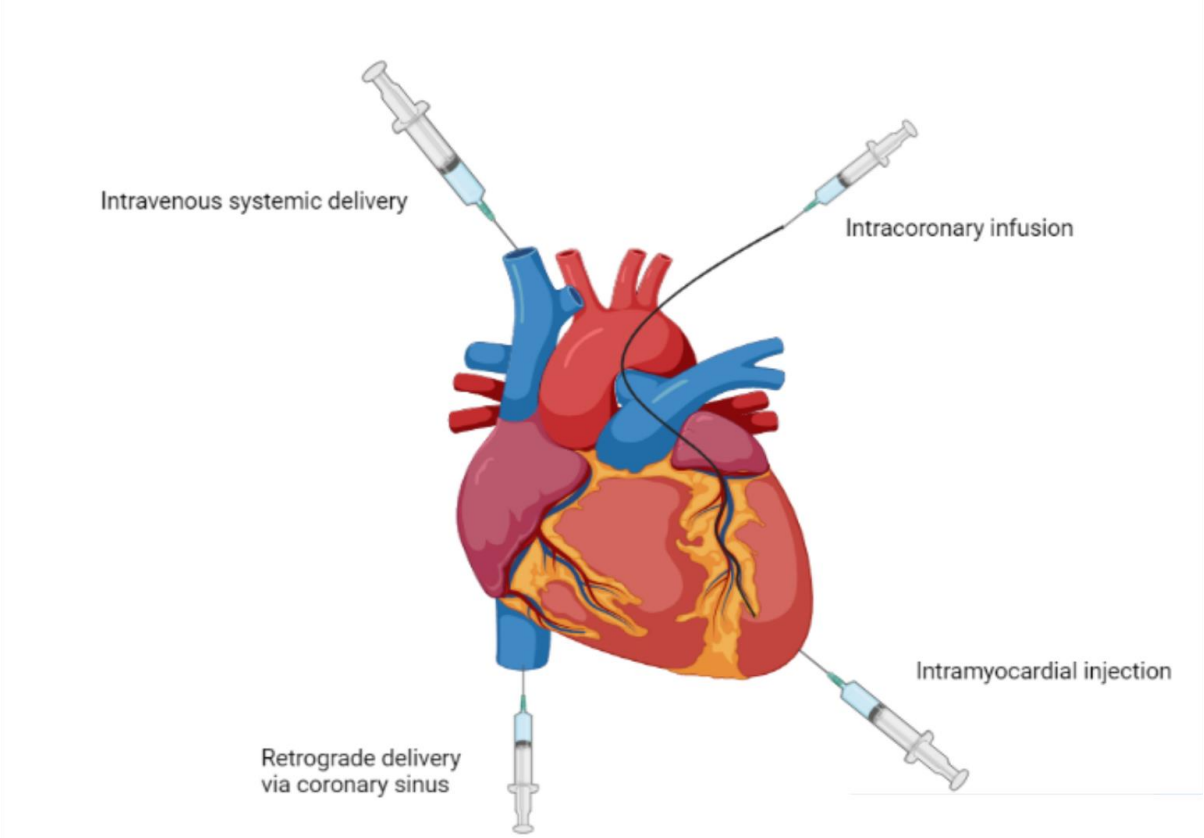

Figure 5: Schematic representation of different gene delivery methods.

4.1.1 Antegrade Arterial Infusion

Percutaneous coronary artery catheterization allows delivery of homogenous genes to each heart territory. It is a well-established, relatively safe and minimally invasive procedure. Thus, it attracts patients specially with end-stage HF. In a study of volume-overload–induced HF in a big animal model, gene transfer by antegrade coronary arteries restored cardiac function significantly. Antegrade coronary gene transfer efficacy depends on relatively fast vector transit through the vasculature (Tilemann et al., 2012a). Intracoronary infusion to the heart is shown in figure 5. Most proposed approaches for improving coronary artery infusion efficacy are enhancing the vector exposure time to the endothelium (Donahue et al., 1997). An approach for raising the residence time of vectors in the coronary circulation is the blockade of coronary venous. Myocardial gene expression was increased by a temporary occlusion of both coronary vein and coronary artery with antegrade coronary infusion (Beeri et al., 2010; Hayase et al.,

2005). This method inhibited ventricular remodeling and preserved function of left ventricle in a large HF animal model (Beeri et al., 2010). For maximizing vector exposure duration to endothelium and minimizing systemic distribution simultaneously, Kaye and colleagues invented an extracorporeal device which can drain blood, via an occlusion catheter, from the coronary sinus and then returns the oxygenated blood of coronary venous, using a peristaltic pump, to the main left coronary artery (V-Focus, St Paul, Osprey Medical Inc, MN) (Byrne et al., 2009; Kaye et al., 2007). In a large animal model study, infusion through an enlarged angioplasty catheter's lumen with short coronary artery occlusion might enhance expression of myocardial gene but this remains contentious (Boekstegers et al., 2000).

4.1.2 Direct Intramyocardial Injection

Intramyocardial injection is a commonly used method for gene transfer. Figure five shows this method. The vectors are injected into the area of target with a little gauge needle either epicardially or endocardially. Delivery of the vectors bypasses the barrier of endothelium and as a result, an enhanced local concentration is seen at the site of injection (Tilemann et al., 2012b). Also, the vector deactivation may be prevented by neutralizing antibodies or circulating DNAs, by preventing exposure to blood. There is also less vector exposure to nontarget organs (Bish et al., 2008; Grossman et al., 2002). Intramyocardial injection might be used for gene delivery locally as well. The endocardial approach needs imaging guidance modality for finding out the site of injection and a catheter having retractable injection needle. This involves electric mapping systems, echocardiography, fluoroscopy (Tilemann et al., 2012c) and MRI (Group, n.d.). Recently, the most widely used guiding system is NOGA electromechanical mapping system. Moreover, it is being used in clinical trials for angiogenesis (Tilemann et al., 2012c).

4.1.3 Retrograde Venous Infusion

Coronary venous system, for percutaneous delivery into the heart muscle, offers another possible route for therapeutic agents. In clinical settings, this approach can be used for patients having limited revascularization potential and damaged coronary artery circulation. Figure 5 shows retrograde venous infusion in the heart. This method can be used for cardioprotective drugs (Karagueuzian et al., 1986; Ryden, 1991). Percutaneous retrograde coronary infusion is a complex approach to the delivery of genes particularly to the myocardium. It is a less invasive and effective procedure, but requires some expertise to be performed safely.

4.1.4 Other Gene Delivery Methods

Other gene delivery methods are aortic cross-clamping (injection into aortic root where the pulmonary artery and the aorta are cross-clamped for some heart beats), intravenous infusion (injection into the vein), pericardial injection and a currently invented surgical gene ‘painting’ method (it is a transmural gene transfer method in both of the atria) (Tilemann et al., 2012c).

Chapter 5

5.1 Targets

Major systems which are targeted to repair the HF function are summarized below. When a target restore function in nonhuman models when heart failure has already occurred and arrhythmogenesis is dissociated with the rescue and establishment of gene-dose effect has occurred, the target is considered validated. For instance, increased gene expression of interest results in improvement in function. In HF, at multiple levels, excitation-contraction coupling might be dysregulated. That's why, various transporters, channels and critical protein had been pharmacologically targeted and to restore contractile function by genetic editing (Tilemann et al., 2012).

5.2 Targeting the -Adrenergic System

Multiple changes adversely affect the -adrenergic signaling that caused adrenergic receptor (AR) desensitization and downregulation. Critical G-protein– coupled receptor kinase (GRK)2 upregulation precipitate -AR signaling abnormalities. Myocardial -AR system's genetic manipulation might improve cardiac function (Tilemann et al., 2012).

5.2.1 Overexpression of -AR

Overexpression of human 1-ARs in transgenic mice resulted in severe cardiomyopathy (Engelhardt et al., 2004). In contrast, both intracoronary and direct myocardial delivery of the adenoviral vector which contained human 2-AR transgene showed the result of improved cardiac performance in mammalian and rodent models (Shah et al., 2000; Tilemann et al., 2012).

5.2.2 Inhibition of GRKs

In the heart, GRK2 is a highly expressed GRK. Development of a dysfunctional cardiac adrenergic receptor signaling is responsible for detrimental activity in HF (Hata et al., 2004). Study associated with HF caused by myocardial infarction in mice showed that ablation of selective GRK2 ten days after infarction showed results of halted ventricular remodeling, enhanced survival and increased cardiac contractile performance (Raake et al., 2008). A peptide ARKct can inhibit GRK2-mediated -AR desensitization in vivo in nonhuman. For

example, intracoronary delivery of adenovirus mediated ARKct transgene in rabbits resulted in a marked ventricular dysfunction reversal three weeks after the induction of myocardial infarction (Shah et al., 2001).

5.2.3 Activation of Cardiac Adenyl-Cyclase Expression

Overexpression of adenyl-cyclase (AC) type VI in the transgenic mice showed improvement in heart function following adrenergic stimulation with enhanced production of cAMP in the isolated cardiac myocytes (Gao et al., 1999). In a pig HF pacing model, adenovirus intracoronary delivery which encoded AC VI showed the result of improvement in function of left ventricle (LV) as well as remodeling, related to enhanced capacity of cAMP generation (Lai et al., 2004).

5.3 Targeting Ca2 Cycling Proteins

Faults in Ca2 handling proteins which are associated with excitation-contraction coupling are reversed in gene therapy techniques to improve a failing heart (Tilemann et al., 2012).

5.3.1 Overexpression of SERCA2a

Gwathmey et al about 20 years ago reported abnormal calcium cycling in human HF (Gwathmey et al., n.d.), and it was because of reduced SERCA2a activity despite of heart failure etiology (Tilemann et al., 2012). After SERCA2a gene transfer, cardiac contractility improved in experimental models of HF (del Monte, Harding, Schmidt, et al., n.d.; Miyamoto et al., n.d.). SERCA2a overexpression for long time by AAV intracoronary delivery which carried SERCA2a had been reported to improve ventricular remodeling and preserve systolic function in a swine volume-overload model of heart failure (Kawase et al., 2008). Besides improving contractility, gene transfer of SERCA2a also restored energetics state of the heart (Sakata et al., 2007, 2008), both in case of energy utilization and supply, reduce ventricular arrhythmias (Tilemann et al., 2012) and increase coronary flow by eNOS activation in the endothelial cells (Hadri et al., 2010).

5.3.2 Phospholamban Inhibition

Phospholamban (PLN) inhibition improves Ca2 handling. In human cardiac myocytes, reduced PLN improved relaxation and contraction velocities as seen with SERCA2a gene transfer (del Monte, Harding, Dec, et al., n.d.). For example, PLN expression silencing in a HF sheep model showed enhanced SERCA activity with enhanced diastolic and systolic LV function (Kaye et al., 2007).

5.3.3 Active Protein Phosphatase Inhibitor-1 and Inhibition of PP1

Reduced PP1 activity is seen in human HF patients which leads to PLN dephosphorylation. PP1 overexpression or protein phosphatase inhibitor-1 (I-1) ablation in murine hearts resulted in reduced adrenergic receptor mediated contractile responses, alleviated cardiac function as well as premature death in accordance with heart failure. An active I-1 expression in transgenic mice caused inhibition of PP1 with increased PLN phosphorylation and increased cardiac contractility (Tilemann et al., 2012).

5.3.4 S100A1

S100 is a part of Ca2-modulating proteins family includes S100 which promotes cardiac relaxation and contractile function through increasing RYRs and SERCA2a activity (Most et al., 2004). Currently AAV9 gene transfer of S100A1 improved contractile function dramatically in a preclinical ischemic cardiomyopathy model encouraging a future S100A1 gene therapy clinical trial for human HF (Tilemann et al., 2012).

5.3.5 Small Ubiquitin-Like Modifier Type 1

Currently, Kho et al described that the activity and levels of SERCA2a in the cardiomyocytes are regulated by cytoplasmic protein, SUMO1 (small ubiquitin-like modifier type 1). SUMO alters other proteins' functions in cells through sumoylation. Sumoylation increased the stability and activity of SERCA2a in the cell. For example, enhancing SUMO1 level by gene transfer of AAV9 restored SERCA2a levels, increased hemodynamic performance and decreased mortality in the animals having HF (Kho et al., 2011).

5.4 Homing of Stem Cells

SDF-1-CXCR4 system can assist homing of stem cells to the myocardium that is infarcted which made SDF1/CXCR4 complex an emerging therapeutic target in ischemic HF (Ghadge et al., 2011). A clinical trial is ongoing to investigate this (Kawase et al., 2011). A study found that SDF-1 reduced ex vivo myocardial contractility and on cardiac myocytes (Pyo et al., 2006). Overexpressing CXCR4 in rat hearts can increase ischemia-reperfusion injury (Chen et al., 2010), but potential complex interaction between the cardiovascular system and these chemokines are still being investigated. Pim-1 kinase can also increase proliferation, survival, lineage commitment, trafficking and cardiac progenitor cell's functional engraftment (Cottage et al., 2010; Fischer et al., 2009).

5.5 Targeting Cell Death

Apoptosis or programmed cell death play a role in myocardial damage in reperfusion/ischemia injury. In models of subacute and acute reperfusion/ischemia, antiapoptotic protein Bcl-2, PI3 kinase or Akt's overexpression decreased cardiomyocyte apoptosis rate and improved function of the heart (Matsui et al., 2001).

Chapter 6

6.1 Clinical Trials

The very first trial had been based upon the SERCA2a cDNA transfer. It was the most extensive trial. It had been based upon the finding that in a failing heart, sarcoplasmic Ca^{2+} ATPase had been downregulated and its restoration to normal level improved heart function in swine and mice both (Kranias & Hajjar, 2012). Following these experimental observations, a phase I/II trial showed safety of the AAV1 vector which expressed human SERCA2a cDNA by intracoronary infusion (Jaski et al., 2009). Based on this study's results, the CUPID trial (NCT00454818; Calcium Upregulation by Percutaneous Administration of Gene Therapy in Patients with Cardiac Disease) had been the very first clinical attempt using AAV gene therapy for treating heart failure. CUPID was a blinded, phase II, randomized, multicenter trial, placebo-controlled study which enrolled thirty-nine patients having severely decreased ejection fraction, symptomatic HF and fixable cardioverter defibrillator for primarily preventing sudden cardiac death. The patients had been randomized into four sections for receiving placebo or for receiving different AAV1.SERCA2a doses by intracoronary infusion (Jessup et al., 2011). The result of this experiment showed improvement in functional parameters and symptoms of HF and reduction in hospitalization for HF. This result encouraged a bigger randomized, phase IIb, multicenter trial, double-blinded, CUPID2 study (NCT01643330). 250 patients were enrolled in this trial who had stable symptomatic heart failure, of both nonischemic and ischemic etiology, along with harshly reduced ejection fraction, to undergo intracoronary infusion of $1\times10_{13}$ AAV1.SERCA2a viral particles or placebo, randomized in a 1:1 fashion (Greenberg et al., 2016). Sadly, the result of this treatment was devastating. This disappointing observation also stopped two other related clinical studies which used similar vectors. The two related clinical studies were the AGENT-HF study and the SERCA-LVAD study. In patients having LV assist devices, the AGENT-HF study was held to evaluate AAV1. SERCA2a efficacy (NCT01966887) (Hulot et al., 2017). The SERCA-LVAD study (Sarcoplasmic/Endoplasmic Reticulum Ca2+-ATPase/Left Ventricular Assist Device) took place to assess similar vector's potential to change left ventricular remodeling in patients having HF(NCT00534703). Both studies ended in 2016 prematurely. A secondary approach proposed for heart failure gene therapy is based on modulating reuptake of Ca^{2+} by overexpressing a I-1c's constitutively active form that binds with PP1. This intervention's ultimate effect is to raise SERCA2a levels and restore precise stimulation of β-adrenergic (Pathak et al., 2005). A large animal study was

held after the experimental success of transfer of I-1c gene in rodent model. The study was operated by intracoronary infusion of an AAV vector with a chimeric AAV2/AAV8 capsid or an AAV9 vector (Fish et al., 2013), which selectively transduced skeletal and cardiac muscle and traversed the blood vasculature while de-targeting the lungs and the liver (BNP116). Based on this study, a phase I clinical study was scheduled in 2020. It was a small study entailing BNP116.sc-CMV.I1c (under NAN-101 name) intracoronary infusion in 12 patients who had NYHA Class III HF (NCT04179643). A third proposed approach for improving cardiac function by gene transfer in HF is restoring the normal Ca2+ homeostasis which is done by blocking a response that is maladaptive because of changed stimulation of β-adrenergic receptors. A heterotrimeric G protein mediates signal transduction from those receptors. The heterotrimeric G protein activates adenylate cyclase (AC) which is situated in the receptor complex's cytosolic side, which catalyzes ATP conversion to cAMP and this activates PKA. It was found in preclinical experimentation that adenylate cyclase's (AC6) isoform-6's overexpression has an advantageous effect on heart failure (Cannatà et al., 2020). These encouraging results paved the way of a phase II, randomized, double-blinded, placebo-controlled clinical study which assessed five doses safety of an adenoviral vector that expressed AC6 (Ad5.hAC6, investigation name RT-100) by intracoronary infusion (Hammond et al., 2016). This trial resulted in partially improved systolic function in patient having nonischemic heart failure. A broad, phase III clinical trial had been arranged to start enrollment in 2018 (FLOURISH; NCT03360448). But it was withdrawn later because of re-evaluating the strategy and clinical recruitment plans. Recent study found that activity of AC6 (adenylyl cyclase type 6) might be mimicked by a protein's intracellular C2 and C1 segment's fusion protein, that may fit into AAV vector's backbone and this would improve safety and efficacy of gene delivery. Genes delivery coding for extracellular factors also improves function in failing hearts (Cannatà et al., 2020). A number of growth factors and cytokines has been proved to be effective over the years in big animal HF models, specially SDF (stromal cell–derived factor)-1α., (Penn et al., 2012), S100A1 (Pleger et al., n.d.), and VEGF-B (Cannatà et al., 2020). In patients having ischemic cardiomyopathy, SDF1α treatment reached phase I clinical experimentation utilizing naked DNA plasmid which was named JVS-100 (Penn et al., 2013). Nevertheless, a phase II, placebo-controlled, randomized, double-blind study (STOP-HF; NCT01643590) reported improvements in selected patients' groups, but it could not reach its primary endpoint (Chung et al., 2015). A parallel clinical study (STOP-PAD; NCT02544204) was conducted in crucial limb ischemia patients with the same SDF1α

plasmid, but this study also failed (Shishehbor et al., 2019). A third phase I/II trial (RETRO-HF; NCT01961726) in heart failure patients was initiated with similar plasmid which was delivered by the retrograde delivery method. This study was conducted in 2014 but had not been reported yet (Cannatà et al., 2020). Table 1 summarizes these main clinical trials.

6.2 Summary of Clinical Trials

Three proposed strategies for gene therapy have already reached clinical experimentation. Clinical studies for heart failure gene therapy are presented in the following table:

Table 1: Main gene therapy clinical studies for heart failure. Adapted from (Cannatà et al., 2020).

Trial Name	Phase	Gene	Delivery Method	No. of Patients	Study Design	Ischemic	NYHA III/IV	Mean LVEF	Follow-Up, (months)	Main Findings	Primary Endpoint
CUPID	I/II	SERCA2A	Intracoronary infusion	39	Open label	100%	100%	25%	12	Functional improvement	Composite: symptoms, BNP, exercise, echo, clinical outcomes
CUPID 2	IIb	SERCA2A	Intracoronary infusion	243	Double-blinded, randomiz	52%	82%	23%	12	Neutral	Time to chronic event (cardiovascular

					ed, placebo-controlled						events/worsening heart failure/hospitalization
AC6	II	AC6	Intracoronary injection	56	Double-blinded, randomized, placebo-controlled	48%	55%	31%	12	No changes in symptoms, slight increase in LVEF	Functional changes and LVEF changes
STOP-HF	II	SDF-1	Intramyocard-ial injection	93	Double-blinded, randomize-ed, placebo-	100%	68%	28%	12	Neutral	Functional: 6MWD and MLWHFQ at 4mo post-dosing

					controlled						
RETRO-HF	I/II	SDF-1	Coronary sinus infusion	72	Randomize-ed, open label	100%	n/a	n/a	4	Results were not reported	Functional: 6 MWD
NAN-101	I	I-1-c	Intracoronary infusion	12	Prospectiv-e, multicenter, open label	n/a	100 %	n/a	12	Recruiting	Number of major harmful cardiac events
n/a	I	SDF-1	Intramyocardial injection	17	Open-label, dose-escalation study	100%	100 %	33%	12	Safe	Number of major harmful cardiac events

Chapter 7

Advantages and Limitations of Gene Therapy

Table 2: Advantages and limitations of gene therapy. Adapted from (Korpela et al., 2021; Mendell et al., 2017; Porada & Almeida-Porada, n.d.).

Advantages	Limitations
AAVs can provide long-term transgene expression up to one year and they can also be tissue-specific.	The therapeutic effects found in clinical trials are limited. This can be because of the size difference between humans and experimental small animals. One single myocardial injection can reach a larger heart muscle area in experimental mice model than in human. The regenerative capacity of the human heart is less than healthy experimental animals that's why patients might suffer from other health complications.
Gene therapy can be used to cure disease when no other medication can.	The therapeutic effect might be limited by neutralizing antibodies and it can cause detrimental immune response.
Gene therapy has to be given only one time.	Gene therapy can be extremely expensive.
Positive effects of gene therapy can be passed down through generations.	Some people have ethical issues with gene therapy.

Chapter 8

Discussion

Gene therapy, a new treatment strategy, would ensure a promising future for heart failure patients. There are two types of gene delivery vectors; viral and nonviral vectors. Viral vectors are used more frequently in clinical trials due to their ability to deliver nucleic acid to the heart much more efficiently than nonviral vectors. Of all the gene delivery vectors in cardiac gene therapy, adenoviral vector, adeno-associated viral vector and lentiviral vector are the most studied vectors. Adeno-associated viral vectors are most effective for use in humans and it is being successfully used in clinical trials. Among the most cardiotropic serotypes AAV1, AAV8, AAV6 and AAV9 of adeno-associated viral vectors, AAV9 serotype is the most effective in transduction. Antegrade arterial infusion, direct intramyocardial injection and retrograde venous infusion are the most used gene delivery methods. Antegrade arterial infusion is a well-established, minimally invasive procedure and it is relatively safe. Increased vector exposure time to endothelium can improve efficacy of this method significantly. Direct intramyocardial injection results in enhanced local concentration at the site of injection and vector's less exposure to nontarget organs. One main target for heart failure gene therapy is the modulation of Ca2+ handling as it ensures normal function of the heart. A key factor for Ca2+ handling is SERCA2a gene transfer. Overexpression of SERCA2a improves Ca2+ handling, thus improving heart function. Phospholamban (PLN) inhibition improves Ca2 handling. An active I-1 expression leads to PP1inhibition and increased PLN phosphorylation. Enhancing SUMO1 levels and gene transfer of S100A1 improves SERCA2a level. The adrenergic system is also an important target. Overexpression of human 2-AR transgene and adenyl-cyclase (AC) type VI improved cardiac function. Among several clinical trials, a very few had been successful. Other clinical trials are taking place in the recent years and expected to give a successful result. This might be due to the wrong delivery vector, wrong delivery method, and wrong target which are yet under development. However, gene therapy is extremely expensive as mentioned in table 2. Nonetheless, this gives a hope for the future of cardiovascular gene therapy. More studies should be done in this regard.

Chapter 9

Future Direction

The safety and efficacy of cardiac gene therapy remain questionable. This might be due to inappropriate animal model, faulty vectors, inefficient delivery method, inappropriate target and so on. The vectors have to be safe and they should cause less side effects for the patient. They should be safe for people who are handling the vectors and environment friendly. Vectors have to be specific and efficient. It should be easy to produce vectors in large amount with a very high concentration. Healthier patients have to be included in clinical trials in the future. Selection of optimal patients by novel biomarkers are still under investigation. A lot of development is still required for viral vectors to minimize adverse effects and improve transduction efficacy. Current vectors modification is under study by minimizing empty vectors proportion, modifying promoters and changing the capsid properties. Researchers are developing the possibility of turning on and off the transgene expression by a drug. Due to the nature of gene therapy can cause a substantial placebo effect which should be taken into consideration in the future trials as a blinded controlled, randomized setup. Selection of a suitable objective endpoint should be emphasized in clinical trials. Study design must be carefully considered. In CAD studies, neovasculature's quantitative imaging must be considered of showing a potential causality in between other endpoints and therapeutic effects. Researchers should focus on the fundamental aspects and basic biology behind heart failure and angiogenesis as gene therapy might be proved as a promising treatment for heart failure patients (Korpela et al., 2021).

Chapter 10

Conclusion

Gene therapy has become a novel treatment strategy for heart failure. After trying for decades, cardiac gene therapy has achieved considerable improvement in many fields. New effective vectors and targets are yet to be found. Clinical trials are expected to be much more efficient in the near future. So, the day when gene therapy will be a trustworthy and economical treatment option for heart failure patients is not so far.

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