BOOK CHAPTER

DOI: 10.34256/br2017

Bulletin of Medical and Clinical Research

Cardiac disease: Current approaches to gene therapy

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Received: 12-11-2019
Accepted: 19-01-2020

Abstract: **Background:** Last decade over the world, the cardiac disease becomes a leading cause of death. Gene-based therapies become a promising treatment for patients affected by cardiovascular diseases, such as myocardial infarction (MI), arteriosclerosis, heart failure and so on, but also underline the require for reproducible results in preclinical and clinical studies for efficacy and safety. **Aim:** This book chapter describes the current research prospect of gene therapy for cardiac disease. We focus on the various models to deliver genes using viral, non-viral vector, delivery methods, targets gene, recent clinical trials, inherited cardiomyopathies target genes and Present advances of CRISPR/Cas 9 for cardiovascular gene therapy. We recapitulate some challenges that require being overcome, future directions of gene therapies for cardiac disease. **Materials and Methods:** All required information regards Lef-7 was generated by exploring the internet search engine like as (PubMed, Wiley, ScienceDirect, CNKI, ACS, Google Scholar, Web of Science, SciFinder, and Baidu Scholar) and libraries. **Results:** In this book chapter, we focus on the present prospect of gene targets, gene delivery methods, and efficient vector to deliver gene, targets gene, recent clinical trials, inherited cardiomyopathies target genes and present advances of CRISPR/Cas 9 technology for the treatment of cardiac disease using gene therapy. Recent clinical trials require modifying vectors and gene delivery approaches to achieve effective results for cardiac gene therapy. **Conclusion:** In this book chapter, we integrate a historical perspective with recent advances that will likely affect clinical development in this research area.

Keywords: Cardiovascular disease, gene therapy targets, Gene delivery methods, the Potential vector for gene therapy, AAV-associated gene therapy, CRISPR/Cas9 for cardiovascular gene therapy.

Introduction

Cardiovascular diseases are the major cause of morbidity and mortality worldwide. Gene therapy is a vital treatment target for acquired and inherited cardiovascular disease

including severe peripheral and cardiac ischemia, vein graft failure, heart failure, and some forms of dyslipidemias [1].

The cardiac disorder like hypertension causes around 20 million cardiomyocytes each year in the absence of a diagnosed heart disease and acute myocardial infarction (MI) approximate 25% of cardiomyocyte in a few hours from left ventricle [2]. Recent advances in gene therapy provide promising approaches to treat and repair damaged cardiovascular tissue from nonfunctional to functional state [3, 4].

At the same time, hyperlipidemias became a target for gene therapy, and conditions like in-stent restenosis, vein graft stenosis, heart failure, arrhythmias, refractory angina, and peripheral vascular disease were recognized as potential targets for gene therapy. In the clinics, the pioneering work of Isner et al. used plasmid gene transfer to treat severe peripheral vascular disease, and adenoviral vectors were, for the first time, used for local endovascular catheter-mediated gene therapy in humans[5].

In spite of these difficulties, during the last 2–3 years, significant clinical and conceptual progress has been made in cardiovascular gene therapy. The first gene-drug approved in the Western world, Glybera, is indicated for the treatment of severe lipoprotein lipase deficiency. Even though this condition is ultra-rare, it was an important milestone for the entire field of gene therapy [5]. Additionally, gene delivery techniques have been significantly improved, particularly concerning catheter-based approaches, and targeting of powerful new therapeutic genes to the myocardium has recently produced promising results[5]. Thus, a new generation of cardiovascular clinical trials is primed to evaluate the potential of gene therapy in carefully selected patient populations. This wave of trials must include carefully documented gene transfer effects and objectively measurable changes in parameters like blood flow, metabolic activity, and cardiac function [5] because the impact of these results cannot be underestimated. Potential targets for cardiovascular gene therapy are presented in Figure-1.

This book chapter describes the current research prospect of gene therapy for cardiac disease. We focus on the various models to deliver genes using viral, non-viral vector, delivery methods, targets gene, recent clinical trials, inherited cardiomyopathies target genes and Present advances of CRISPR/Cas 9 for cardiovascular gene therapy. We recapitulate some challenges that require being overcome, future directions of gene therapies for cardiac disease.

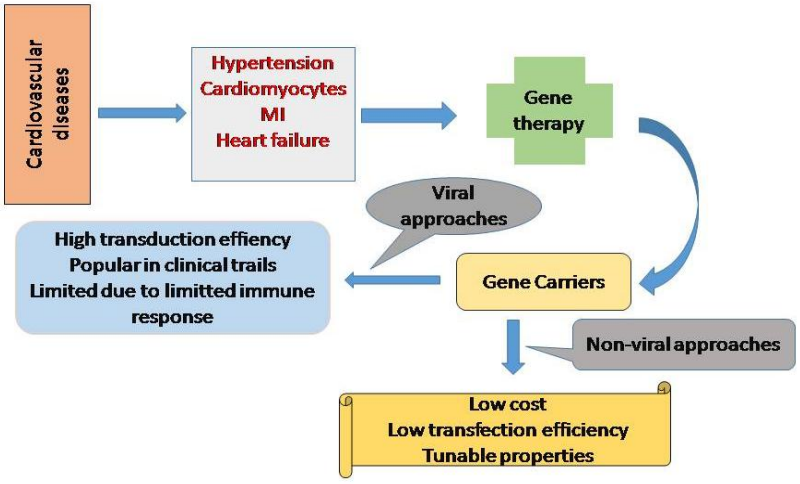

Figure 1 Schematic diagram for cardiac gene therapy.

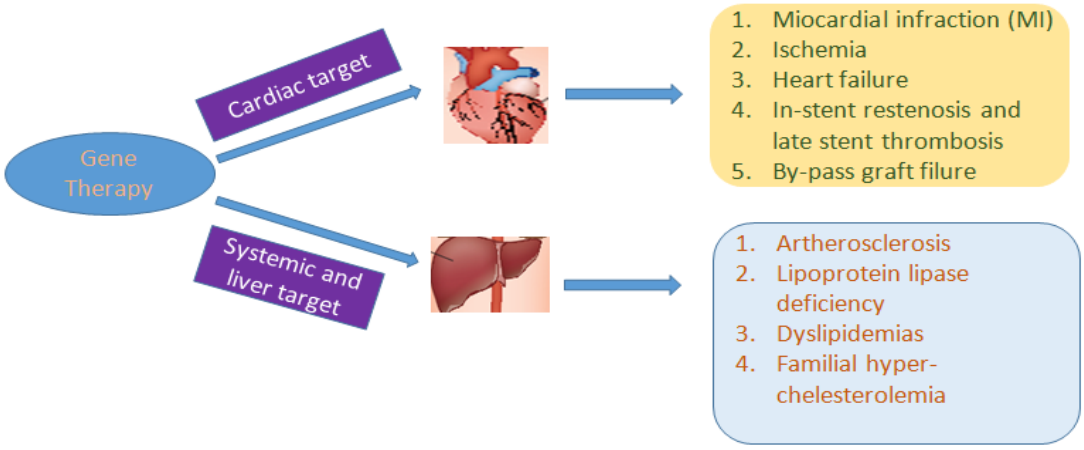

Figure 2. Potential target for cardiovascular gene therapy

Table 1 Recent clinical trials for cardiac diseases

Disease	Trial and Vector	Study Design	Therapeutic Agent	Delivery	n	Primary Endpoint	Main Result	Reference
CAD	ASPIRE; Ad	phaseIII, open-label	FGF-4	Percutaneous i.c. injections	100	Reversible perfus Defect (SPECT)	N/A	[52]
CAD	VEGF-A116A; Ad	PhaseI/II, open-label	VEGF-A_{116A}	Thoracotomy i.my. injections	41	timeto1mmST depression on ETT	N/A	ANCT01757223
CAD	ReGenHeart;Ad	phaseII, RCT	VEGF-D^{dNdC}	Percutaneous i.my. NOGA/PET guided injections	180	6-min walking test, perfusion reserve in ischemic area (PET/SPECT)	N/A	NCT03039751
CAD	AWARE; Ad	PhaseIII, RCT	FGF-4	Percutaneous i.c. injections	300	Is chemic ECG changes on ETT	N/A	NCT00438867
CAD	HGF-X7; Ad	phaseI, open label, no controls	HGF	Percutaneous i.my. injections	12	safety	N/A	[53]
HF	CUPID2; AAV1	phaseII, RCT	SERCA2a	Percutaneous i.c. injection	250	time to recurrent cardiovascular events	negative	[54]
HF	AGENT-HF;AAV1	phaseII, RCT	SERCA2a	Percutaneous i.c. injection	44	Changes in left Ventricular end-systolic volume	N/A	NCT01966887
chronic HF in patient swith LVAD	SERCA-LVAD; AAV1	phaseII,RCT	SERCA2a	Percutaneous i.c. injection	24	Safety and feasibility	N/A	NCT00534703
Heart failure	AC6;Ad	phaseI/II, RCT5	adenylylcyclasetype6	Percutaneous i.c. injection	56	Combined ETT and Cardiac function before And during doputamine Stress	N/A	NCT00787059

heartfailure	RETRO-HF; PI	phaseI/II, open-labelpartandRCTpart	SDF-1	Percutaneous retrograde Injection via coronary vein	52	6-min. walking distance	N/A	NCT01961726
heartfailure	STOP-HF;PI	phaseII,RCT	SDF-1	Percutaneous i.my. helicalinfusion catheter-mediated injections	93	6-min. walking distance	negative	[55]
PAD	Neovasculgen; PI	Phase II/III,RCT	VEGF-A_{165}	i.m. injections	100	pain-free walking distance	positive, pain-free walking distanceincreased167% at1year	[56]
PAD	HGF-X7 (NL003); PI	PhaseII,RCT	HGF	i.m. injections	200	ulcer area, visual analog scale	N/A	NCT01548378
PAD	MULTIGENE ANGIO;RV	phaseI, open-label, no controls	VEGF-A165, Ang1with Cell therapy	Percutaneous i.a. injections	23	safety, amputation-free survival	positive, amputation-free survival72%at1year	[57]
PAD	HGF-X7 (VM202)	phaseII;PI	HGF	i.m. injections	50	Visualan alog scale	N/A	NCT01064440
PAD	JVS-100	phaseII;PI	SDF-1	i.m.injection	48	safety, amputation rate	N/A	NCT01410331
PAD	KAT-PAD101	phaseI,RCT;Ad	VEGF-D^{dNdC}	i.m. injection	30	safety, perfusion intreated area(PET)	N/A	EudraCT 001019-22

Note: CAD, coronary heart disease; HF; heart failure; PAD, peripheral vascular disease; RCT, randomized controlled trial; i.a., intra-arterial; i.c., intracoronary; i.m., intramuscular; i.my., intramyocardial; Pl, plasmid; RV, retrovirus; Ad, adenovirus; AAV, adeno-associated virus; ETT,exercise to lerance test; N/A, not available.

Potential targets for cardiovascular gene therapy

Gene therapy focuses on a few targets to treat cardiovascular disease. Several target points mentioned in (figure-2) for the treatment of cardiovascular disease. Among these target hyperlipidemias recognized as a potential target for gene therapy conditions includes heart failure, vein grafts enosis, in-stent restenosis, arrhythmias, refractory angina, and peripheral vascular disease[1]. Recently gene delivery technique has been significantly improved and targeting of the myocardium by new therapeutic gene produce promising results [6]. A new generation of cardiovascular clinical trials is prime to evaluate the potential of gene therapy and requires carefully documented gene transfer effects with parameters like as metabolic activity, blood flow and cardiac function [1, 6]. Several clinical trials have been conducted with negative and positive results. Despite these results, we need to consider protocol development/ or modification, Gene delivery methods, and gene delivery vectors to earn

effective, safety and reproducible results. In this article, we presented some recent clinical trials for coronary heart disease and peripheral vascular disease presented in table-1.

Gene therapy Vector/ Potential vector for gene therapy:

Non-viral vectors delivery

In terms of safety concern, non-viral vectors have the potential advantage instead of viral vector vectors basis gene therapy. Non-viral vectors have the potential to deliver more significant genetic elements, lower immunogenicity, and easy to synthesize compared to viral vectors. However, the most important downside with the utilization of non-viral vectors has been their poor gene delivery efficiency[7].

The development of the field of non-viral vectors in recent years has been focused on the rapidly progressing nanotechnology to produce improved nano-sized materials for more efficient gene delivery. One group of non-viral vectors that is progressively more utilized for the delivery of various cargoes over the past 20 years is the cell-penetrating peptides(CPPs)[8]. There are two predominant classes of CPPs; one requiring chemical linkage with the cargo and the second involving the formation of stable non-covalent complexes. CPPs have been successfully employed both *in vitro* and *in vivo* to switch the expression of several different targets such as NF-kB, which offers exciting potential for the treatment of inflammatory diseases [9]. Besides, CPP's strategies have been used to detect and target atherosclerotic plaques [10, 11]. Several CPP-based drugs are currently under preclinical and clinical evaluation, and the majority of these uses a covalent approach to attach the cargo [12]. However, recent development has been focusing on the design of novel non-covalent CPP vectors transporting siRNAs and nanoparticles with higher efficiency, selective targeting, and fewer side effects [13, 14].

Different technologies have been used to non-viral vector approaches for cardiovascular disease like liposome-DNA complexes that enhance plasmid stability, though the plasmid is rapidly cleared from the systemic circulation [15]. Polymer-based DNA complexes based on polyethyleneimine (PEI) and poly-l-lysine (PLL) products facilitate cellular uptake and protect plasmids from nuclease digestion. However, *in vivo* applications, these products tend to accumulate and aggregate in different tissues [16, 17]. Recent reports, the ultrasound-targeted micro-bubbles (UTM) strategies have shown benefits in HF and MI animal models for targeted delivery of DNA or microRNA. UTM enhances the delivery of microRNAs to cardiomyocytes without discernable toxicity but in vitro study demonstrated that UTM-mediated delivery of miR-133 in cardiomyocytes provokes reversal hypertrophy[18]. One of the major challenges in the large animal model or clinical trials to improve efficiency and/or short-term gene expression [19].

Viral delivery of genome-editing systems

Viral vectors are the most prominent approaches to transfer the gene into tissue/or cells. Advanced vector system improves production methods, boost transduction efficiency and improved safety profile have been achieved during the past decade [20]. Virus vector system provides significant insights to deliver genome editing systems for both clinical and research applications including *in vitro*, *ex vivo* and *in-vitro*.

Table 2 Characteristics of three major types of viruses apply for genome editing

Virus vectors	Genome	Packing capacity and capsid diameter	Expression duration	Immuno genicity	The primary setting for genome editing	In vivo tropism	References
Adenoviruses	Double-stranded DNA(non-integration)	8kb or 36 kb; 100nm	Long term (week to months)	high	*In vitro*, *In vivo* and *ex vivo*	Primarily liver	[58, 59]
Adeno-associated viruses	Single-stranded DNA(non-integration)	5kb;25nm	Long term (years) in quiescent cells	Low	*In vitro*, *In vivo* and *ex vivo*	Broad serotypes dependent	[60-62]
Integration deficient lentiviruses	Single-stranded DNA (non-integration)	8kb; 100nm	Transient (depends on the rate of cell division)	Low	*ex vivo* and *in vivo*	N/A	[59, 60]

Note: Lower expression levels in Liver, Proliferating cells, Brain, Kidney, Lung, Skeletal, vascular tissues, retina as well as demonstrated in muscle and the central nervous system too.

Viral vectors (adenoviral vectors, AAV vectors, and Retroviral vectors) have been frequently used in preclinical models, and they have been tested in several clinical trials basis on key properties in *ex vivo* and *in vivo* genome editing applications presented in table-2 [21].

Adenoviruses

Adenoviruses vectors have broad targets of cell tropism and easy to produce, which promotes efficient transduced all major types of cardiac cells. Recently, adenovirus is widely used in laboratory studies for cardiovascular gene therapy. In clinical application adenovirus significantly reduce duration gene expression, moderate transduction efficiency compared to adeno-associated viruses (log scale), provoke inflammatory, immunity, and tendency to infect all organs, especially the liver cell [22].

CAR is highly express in cardiomyocyte which is the primary receptor for serotype 5 adenovirus (Ad5), whereas its expression is reduced in vascular smooth muscle and endothelial cells. It has been reported that Ad vectors cannot easily cross the endothelial barrier after systemic administration, but selectively transduce endothelial cells in local administration. The transduction efficiency varies depending on the Ad serotype. In particular, Ad serotype 49 (Ad49) showed increased transduction of endothelial cells and smooth muscle cells *in vitro* and in vascular graft *ex vivo* [23, 24].

AAV-associated gene therapy

Cardiac gene therapy with the AAV vector holds promising approaches for the treatment of inherited diseases and cardiovascular diseases by efficient delivery of

the therapeutic transgene to the site of action such as cardiomyocytes [25].

The recent progress of adeno-associated virus (AAV) vectors have galvanized the field of gene therapy. In the large animal model of cardiac disease using a cardiotropic AAV serotype 9 demonstrated effective cardioprotective activities by using gene HO-1 and VEGF-B [26]. An AAV vector consists of several serotypes with each serotype having the tropism to a specific organ. Thus many researchers reported AAV-mediated gene therapy to their desired gene target and delivery methods [27, 28]. Previous studies in large animal models demonstrated that Heme oxygenase (HO)-1 and vascular endothelial growth factor (VEGF)-B protecting the heart from cardiac ischemia, and protective even with permanent coronary occlusion, inhibit remodeling in chronic stages [29].

Recombinant AAV vectors (rAAVs) particularly attractive for cardiac gene therapy is the long-term persistence of the viral genome in an extrachromosomal form, which leads to the durable expression of the therapeutic protein (at least in nondividing cells such as cardiomyocytes) [25]. There are 5 types of cardiomyopathies; arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy and left ventricular(LV) noncompaction cardiomyopathy [30]. Inherited cardiomyopathies provide numerous targets for AAV-based cardiac gene therapy. Some potential targets mentioned in table-3. Recent advancement of cardiac gene therapy provides several strategies to evaluate more specific protein functions, binding capacities and delivery specific target to the specific site of action by using rAAV vector as gene delivery of the therapeutic transgene to the cardiomyocytes.

AAV vectors are safe for long-term expression. Numerous molecular heart failure [31] targets have been successfully identified like as S100A1, βARKct and SUMO-1 in large animal models and close to clinical evaluation shortly [32]. In addition to HF, preclinical studies suggest the potential utility of AAV gene therapy for arrhythmias and biological heart pacing.

Table 3. Inherited cardiomyopathies target genes

Genes	Target Protein	Function	Types of CM	cDNA size	OMIM
LMNA	Lamin-A/C	The structural protein of the nuclear lamina	DCM; ARVC	~1.7-2	150330
TTN	Titin	Scaffolding protein	DCM; HCM	~82	188840
SCN5a	Sodium channel protein type5, α-subunit	Sodium transport	DCM	~6	600163
MYH6	Myosin 6 heavy chain	Muscle contraction	DCM; HCM	~5.8	160710
MYH7	Myosin 7 heavy chain	Muscle contraction	DCM; HCM	~5.8	160760

MYBPC3	Cardiac-type myosin-binding protein C	Muscle contraction	DCM; HCM	~3.8	600958
RBM 20	RNA-binding protein 20	Regulation of splicing of Several cardiac genes	DCM	~3.7	613171
PKP2	Plakophilin 2	Cardiomyocyte cohesion	ARVC	~2.5	602861
TNNI3	Cardiac troponin I	Muscle contraction	DCM; HCM	~0.6	191044
MYL2	Regulatorylightchainofcardiacmyosin -β	Muscle contraction	HCM	~0.5	160781
MYL3	Myosin light chain, ventricular isoform	Muscle contraction	HCM	~0.6	160790
DSP	Desmoplakin	Cardiomyocyte cohesion	ARVC	~8.6	125647
JUP	Junction plakoglobin	Cardiomyocyte cohesion	ARVC	~2.2	173325
DSC2	Desmocolin 2	Cardiomyocyte cohesion	ARVC	~2.5	125645
DSG2	Desmoglein 2	Cardiomyocyte cohesion	DCM; ARVC	~3.4	125671

Note: arrhythmogenic right ventricular cardiomyopathy (ARVC); cardiomyopathy (CM); dilated cardiomyopathy (DCM); hypertrophic cardiomyopathy (HCM); OMIM, Online Mendelian Inheritance in Man (http://omim.org).

Lentiviral vectors

The quintessential lentiviral vectors (LV) are enveloped single-stranded (ss) RNA vectors derived from HIV-1 that can integrate their genome as cDNA of both dividing and non-dividing target cells into the chromosomes [24]. LV demonstrated the long-term therapeutic effect for the treatment of monogenic hematopoietic disorders. However, *in vivo* gene, deliveries for the treatment of CVD/HF are limited due to their poor transduction of myocardium. LV vectors are another potential for cardiac gene therapy for their moderate immune response and able to transducing nondividing cells like cardiomyocytes. Like adenoviral vectors, lentiviral vectors have no specific tropism to cells of the cardiovascular system and they likely require intramyocardial injection as a vector delivery method [33]. It would be important to now validate the therapeutic potential of such endothelial specific LV in an experimental model of CVD. Despite their promise, safety issues in significant concern of using LV. Since LV can integrate randomly into the target cell genome with a preference for genes, that provoke insertional oncogenesis. However, focus on target cell types and optimizing the vector design can lead to reducing this risk. To date, lentiviral vectors have so far not been used in clinical cardiovascular gene therapy trials [24, 33].

Delivery Methods of cardiac gene therapy

Delivery of vector to the target is an important aspect of gene therapy. In this review, we discuss the adeno-associated virus (AAV) delivery methods for gene therapy. Several delivery approaches are available for the AAV vector to myocardium broadly divided into direct intramyocardial injection and transvascular administration.

Intramyocardial injection

The direct intramyocardial injection has several advances owing to delivered high local concentration, bypass endothelial barrier, minimized off-target organ transduction, and shun immunogenic response. The cardiac disorder like genetically inherited cardiomyopathies, and heart failure, intra-myocardial injection provide suboptimal delivery methods for expression of the transgene is restricted to a very small area surrounding the injection site. The intramyocardial injection might be a desirable approach for the delivery of biological beta-blockers or pacemakers [25, 34]. Percutaneous, catheter-based injections are minimally invasive and more desirable from safety points of view [25].

Transvascular delivery

In most clinical applications, the rAAV could be injected, preferably for trigger the near homogeneous expression of the therapeutic proteins in rodents not with large animals and human models. The development of the AAV vector could efficiently transduce human cardiomyocytes upon intravenous injection would offer tremendous therapeutic benefits for gene therapy [25].

Antegrade intracoronary injection

Antegrade intracoronary infusion with AAV vector for gene therapy extensively used in large animal models and percutaneous access to the coronaries is routinely performed during angioplasty [22, 25, 35]. Antegrade intracoronary injection (Aii) is the only delivery method to deliver clinically potential AAV vector [25].

Retrograde injection delivery

In heart failure, stenotic coronary arteries pose a significant hurdle to the successful delivery of rAAVs via the antegrade route. This barrier to efficient transduction can potentially be overcome by the delivery of the vector into the coronary vein. This delivery approach might lead to longer dwell times of the vector in the coronary vasculature, increasing the chance of extravasation of the vector [36]. However, to increase gene transfer, temporary occlusion of the left anterior descending coronary artery to increase the intravascular pressure was required. Although this increased vasculature pressure enhances gene transfer, it carries significant clinical risks, especially in patients with advanced heart failure [25, 37].

Present Prospect of CRISPR/Cas 9 for cardiovascular gene therapy

CRISPR/Cas9 technology provided promising therapies for treating all aspects of genetic diseases in clouding cardiovascular disease by disrupting genes or correcting gene defects in both somatic cells and germline *in vivo* [38-40]. CRISPR/Cas9 play key roles in regulating smooth muscle cell differentiation and cardiac fibrosis in mice and art model that carrying Tet mutation, which provides a precise model to discover novel mechanisms in cardiac remodeling [41-43]. Monkey has been considered to be a vital model for studying human disease due to physiological

and genetic similarity [44, 45]. CRISPR/Cas9 has made p^{53} mutant monkey by genetic engineering tools which particularly useful to investigate how p^{53} induce inflammation contributes cardiac dysfunction during pressure overload and why p^{53} induce cardiovascular cell senescence promotes arteriosclerosis [46-49]. Proprotein convertase subtilisin/ Kexin type 9 (PCSK9) is responsible for decreasing blood cholesterol levels after a few days of administration of an adenovirus expressing guide RNA and Cas9 [15]. PCSK9 is a major therapeutic target to treat coronary heart disease (CHD), PCSK9 normally bind with Low-density lipoprotein (LDL) receptor thus help to reduce LDL cholesterol level. The previous study demonstrated that PCSK9 in somatic cells provides a single shot to prevent CHD by using the CRISPR/Cas9 system which is more convenient than taking a regular basis drug-like as statin [31, 50, 51].

Conclusions

Gene therapies have promising success for the modern treatment of cardiac disease. Recent advances in gene therapy could target genes to overcome their nonfunctional state to functional conditions. Researchers have been designed several methods to deliver the gene into the target site by using viral vectors, non-viral vectors for the treatment of cardiac diseases. Numerous clinical trials have been conducted and provide significant improvement in gene therapy for cardiac diseases.

As far as the safety profile of cardiovascular gene therapy is concerned, most trials have shown a very good safety profile even after a 10-year follow-up. This should encourage clinical testing of new therapeutic approaches with improved vectors and gene delivery methods. Current imaging methods can be used to guide therapeutic approaches so that very low doses could be applied to targets like ischemic myocardium in combination with standard therapies.

Because a regulatory pathway for the approval of gene drugs has now been established, it is expected that several novel treatments for cardiovascular diseases will enter clinical testing shortly. There is a clear need to develop validated surrogate endpoints for cardiovascular trials based on tissue perfusion, collateral flow, metabolic improvements, and reduced burden.

Abbreviation: AAV: adeno-associated virus; CHD: coronary heart disease; LDL: Low-density lipoprotein; PCSK9: Proprotein convertase subtilisin/ Kexin type 9; Aii: Antegrade intracoronary injection; LV: lentiviral vectors; HF: heart failure; LV: left ventricular; ARVC: arrhythmogenic right ventricular cardiomyopathy, DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; rAAVs: Recombinant AAV vectors; HO: Heme oxygenase; VEGF: vascular endothelial growth factor; Ad49: Ad serotype 49; PEI: polyethyleneimine, PLL: poly-l-lysine; CPPs: cell-penetrating peptides.

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Acknowledgments: I would like to express my greatest and deepest gratitude to the supreme of the universe, the Almighty Allah to whom all praises go for enabling us to complete my book chapter work. I would like to express my heartfelt gratitude and indebtedness to my parents (Md. Montazur Rahman and Mrs. Chamely Rahman), brother (Md. Raihan-ul-ferdous), sister (Monira Akhter) and others (Qian Zhou, she was motivate me lot to put contribution in science and find literature from database), (Han Jing, she was entirely supported me help to find literatures from website), to endevour support and motivation to write and contribute to science. I express my heartiest thanks and gratefulness to all honorable teachers of Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. Lastly, I am grateful to all my friends and specially my family members for their moral support during the study. I would like to thank CAS-TWAS President's Ph.D. Fellowship Program authority for providing sponsorship during my Ph.D. study.

Authors' Contribution: Md. Reyad-ul-Ferdous (MRF) carried out the study from conception and design to writing and finalizing the manuscript. MRF collected data from online sources, critically reviewing and revising the manuscript. Md. Shofiul Azam (MSA) and MRF rewriting, revising and formatting the manuscript according to publisher guideline. The final version was read and approved by all the authors.

Funding: NIL

Conflict of Interest: NIL

Ethics approval and consent to participate: Not applicable

Consent for Publication: Not Applicable

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