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Cardiovascular gene therapy for myocardial infarction

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Abstract

Introduction—Cardiovascular gene therapy is the third most popular application for gene therapy, representing 8.4% of all gene therapy trials as reported in 2012 estimates. Gene therapy in cardiovascular disease is aiming to treat heart failure from ischemic and non-ischemic causes, peripheral artery disease, venous ulcer, pulmonary hypertension, atherosclerosis and monogenic diseases, such as Fabry disease.

Areas covered—In this review, we will focus on elucidating current molecular targets for the treatment of ventricular dysfunction following myocardial infarction (MI). In particular, we will focus on the treatment of i) the clinical consequences of it, such as heart failure and residual myocardial ischemia and ii) etiological causes of MI (coronary vessels atherosclerosis, bypass venous graft disease, in-stent restenosis).

Expert opinion—We summarise the scheme of the review and the molecular targets either already at the gene therapy clinical trial phase or in the pipeline. These targets will be discussed below. Following this, we will focus on what we believe are the 4 prerequisites of success of any gene target therapy: safety, expression, specificity and efficacy (SESE).

Keywords

efficacy; gene therapy; myocardial infarction; safety

1. Introduction

In the search for the green light

Gatsby believed in the green light, the orgastic future that year by year recedes before us. It eluded us then, but that's no matter. Tomorrow we will run faster, stretch out our arms farther and one fine morning...-

from *The Great Gatsby*, F. Scott Fitzgerald.

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Declaration of interest

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In the field of gene therapy there is a green light today too, perhaps not 'orgiastic,' but more real. Scientists are motivated to verify the presence of this light (green fluorescent protein, GFP) in the organs or in the cell types of interest as sign of presence (Expression) of our administered gene product. This review is about the current experimental gene therapies either in preclinical or in clinical development, and a discussion of the features that scientists and physicians aim toward when designing, testing and evaluating a novel gene therapy (Figure 1).

2. Adrenergic manipulation

2.1 Targeted inhibition of G protein-coupled receptor kinase 2

One of the features of heart failure (HF), regardless of its etiopathogenesis, is β -adrenergic receptor (β AR) system dysregulation [1]. Unfortunately, the hyperactivity of the sympathetic nervous system, initially a compensatory response to cardiac pump inefficiency, becomes detrimental over time, causing uncoupling and downregulation of β ARs in the heart and contributing to a vicious perpetuating cycle that has a negative effect on cardiac function with a loss of inotropic reserve [2]. Our lab's research over the last two decades has shown that a nodal regulator of β AR dysfunction and also cardiac function in the heart is the expression and activity of G protein-coupled receptor kinase-2 (GRK2), which is upregulated in the failing heart as a consequence of heightened catecholamines [3]. GRK2 appears to contribute to the pathogenesis of HF. When it is knocked down in cardiac myocytes post-MI (myocardial infarction) ventricular dysfunction and adverse remodeling is prevented [4]. Further, a peptide inhibitor, β ARKct [5], can robustly boost cardiac function and prevent HF development [3], especially when GRK2 is overexpressed in the heart. The β ARKct comprises the last 194 amino acids of GRK2 and competes with the membrane translocation and subsequent desensitization activity against receptor substrates through binding to the $\beta\gamma$ subunits of activated G proteins [3]. When β ARKct is expressed in transgenic mouse models or through adenovirus administration, it can block GRK2 activity *in vitro* and *in vivo* leading to improved cardiac function [6–9].

Importantly, a recent large animal preclinical model of HF in the pig showed that myocardial adeno-associated viral (AAV6) delivery of the β ARKct reversed ventricular dysfunction, remodeled the heart and lowered sympathetic outflow of catecholamines, indicating that β ARKct administration and GRK2 inhibition are the promising therapeutic targets in HF [10]. Inhibiting GRK2 is also important in acute cardiac dysfunction following MI [11]. The recently discovered mitochondrial localized GRK2 can act as a pro-death molecules in myocytes [12]. Table 1 summarizes the history to date of successes of β ARKct expression in small and large animal studies.

2.2 Adenylyl cyclase VI

Within the adrenergic signaling pathway, a primary downstream effector, adenylyl cyclase VI (AC VI), has also been shown to improve post-MI HF in animal models [13,14], and adenoviral-mediated gene transfer to HF patients is currently in an active Phase I/II clinical trial enrolling patients (<http://clinicaltrials.gov/show/NCT00787059>). AC comes in two isoforms in the heart (AC V and VI) [15], and their roles are related to the ability of

generating cAMP. Specifically, in preclinical animal models, adenovirus encoding AC VI injected into swine coronary arteries of isoproterenol [16,17] and pacing-induced HF models has resulted in amelioration of left ventricle contractility [16]. These results in large animal models, together with the evidence in a transgenic mouse model of increased cardiac function coupled with increased cAMP in response to adrenergic stimulation in cardiomyocytes, have rendered AC VI a strong candidate for human clinical trial where it is now under evaluation for safety and efficacy.

3. Ca cycling proteins

3.1 SERCA2a

One of the hallmarks of HF and a consequence of dysregulated β AR signaling is abnormal calcium (Ca^{2+}) handling [18,19]. Its major characteristic in the failing cardiac myocyte is decreased expression and activity of the myocardial sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase (SERCA2a) [20]. Reintroduction of SERCA2a to the failing heart has been a successful strategy in several animal models and has been shown to boost cardiac contractility [21]. As a result, restoring the SERCA2a level with gene therapy is a primary Ca^{2+} -handling target and has proven to be quite effective in small and large animal models of HF. Using a volume overload HF pig model, AAV-mediated gene delivery of SERCA2a ameliorated cardiac contractility and slowed down left ventricular (LV) remodeling. This beneficial effect was shown in additional large animal models such as sheep [22,23] and dogs [24]. AAV1-SERCA 2 has been tested in many disease models: ischemic HF, pacing-induced HF, and an MI with mitral valve regurgitation model [25]. Importantly, SERCA2a gene delivery represents the first-in-man HF gene therapy target as Phase I and II clinical trials in patients with advanced HF have now been concluded, showing, respectively, safety and better efficacy of higher doses of SERCA2 [26,27]. Clinical development of this gene product is, therefore, moving toward Phase III clinical trials.

3.2 Phospholamban

Phospholamban (PLN) is another Ca^{2+} -handling target that has been studied for its potential in HF therapy. PLN is an inhibitory accessory protein to SERCA2a, but when it is phosphorylated it becomes dis-inhibitory, which leads to increased SERCA2a activity [28,29]. At the molecular level it has been reported that expression and activity of SERCA2 are markedly increased typically in conjunction with a decrease in the expression and the activity of the regulatory protein PLN [30,31]. PLN knockout (KO) mice show improved cardiac performance and enhanced SR Ca^{2+} uptake [32], whereas mice overexpressing the constitutive active PLN showed deranged SR Ca^{2+} and developed cardiomyopathies. Gene transfer in hamsters [33] and rats [34] of dominant-negative PLN mutants, and PLN antisense RNAs transfer [35] have been used to increase SERCA2a activity and to rescue HF models. AAV shRNA vectors have been used successfully in a rapid-pacing adult sheep and rat model of transaortic constriction, respectively, to silence PLN and therefore rescuing HF [36,37]. Cardiac dilation was normalized, and cardiac hypertrophy, cardiomyocytes diameter and cardiac fibrosis were reduced significantly. The overall therapeutic translation of targeting PLN appears limited in humans since a null mutation has been found in humans, which leads to cardiomyopathy [38].

3.3 S100A1

Another protein found to be involved in Ca^{2+} regulation in the failing myocytes is S100A1, which is an EF-hand Ca^{2+} -binding protein abundantly expressed in the heart [39]. S100A1 appears to regulate myocyte Ca^{2+} handling at several nodal points including SERCA2a (activating it) and also the ryanodine receptor; thus, it can have multiple actions to improve failing myocyte function [39]. Importantly, S100A1 levels are decreased in human failing myocardium and studies have shown that restoring its downregulated levels in the failing heart through cardiac gene transfer can reverse cardiac dysfunction and provide novel inotropic support [40,41]. Recently, this has been shown using AAV technology, where intracoronary AAV6-S100A1 delivery to a rat model of HF significantly reversed LV dysfunction remodeling chronically [41]. Further, in a large animal preclinical model of HF (Pig), AAV9-S100A1 gene delivery using a retrograde perfusion strategy of delivery through the great coronary vein reversed LV remodeling and dysfunction, showing its potential clinical utility [42].

3.4 Parvalbumin

Diastolic dysfunction can be a feature of post-MI remodeling [43]. Parvalbumin is a small cytosolic protein, which belongs to the superfamily of EF hand $\text{Ca}^{2+}/\text{Mg}^{2+}$ -binding proteins. It is normally not present in the heart, and when expressed has shown to correct slowed myocyte relaxation in diastole [44,45], at the expense of shortening Ca^{2+} transients with final result of impaired contractility. To overcome this unwanted latter effect, a new parvalbumin, where glutamine has been substituted with glutamate in position 12 of the second cation-binding loop domain of parvalbumin (ParvE101Q), has been redesigned allowing the formation of a new ultra-delayed Ca^{2+} buffer [46]. Expression of ParvE101Q corrected abnormal myocardial relaxation in diastolic dysfunction disease model *in vitro* and in an *in vivo* mouse model of familial hypertrophic cardiomyopathy (restrictive cardiomyopathy) [46]. In a dog model of coronary microembolization, ParvE101Q transduction caused acceleration of the relaxation speed on isolated cardiomyocytes [46]. These characteristics make parvalbumin a novel and attractive target for gene therapy in post-MI diastolic dysfunction.

4. Angiogenesis

Increasing capillary density is a valid strategy to restore pseudo-physiological blood flow in ischemic areas either through angiogenesis or vasculogenesis [47]. Since angiogenic growth factors administration alone would require multiple administrations given their short lives, gene therapy approach represents a very attractive methodology. Vascular endothelial growth factor (VEGF) has been the most studied and tested growth factor in large animal gene transfer studies [48–51], where enhanced collateral formation and myocardial perfusion in chronic ischemia models and post-ischemic HF models were found. In some studies cardiomyocytes mitotic events secondary to increased angiogenesis were claimed [52]. Alternatively, the fibroblast growth factor (FGF) family and hepatocyte growth factor (HGF) can act as pluripotent mitogens for a variety of cell types, and have potential pleiotropic and angiogenic properties. These growth factors have also been suggested to upregulate VEGF, which explains their angiogenic effects. When plasmid FGF-2 was

injected intramyocardially in a pig model of left anterior descending artery stenosis resulted in improved regional contractility and perfusion [53,54]. Gene therapy of HGF and its variant has demonstrated efficacy in chronic ischemic myocardium in dogs [55,56] and pigs [57,58]. Most studies succeeded in showing that the improved cardiac function was due to an increased perfusion. The angiogenic-like chemokine, stromal derived factor (SDF-1), is already in clinical development after having shown a very promising effect in large animal models of HF. A Phase I clinical trial [59] has assessed the safety of SDF-1 in 17 patients with ischemic cardiomyopathy injected as a plasmid (JVS-100) via endomyocardial injection. These data suggest that overexpression of SDF-1 via gene therapy is a strategy for improving angiogenesis in patients with ischemic cardiomyopathy.

5. Gene transfer for cardiac regeneration post-MI

Cardiac stem cells represent a potential therapy for MI. Together with the possibility to inject progenitor cardiac precursors, the cardiac regeneration field can now take advantage of recent discovery [60] of induced pluripotent stem cells. Cardiac fibroblasts can be directly reprogrammed to adult cardiomyocytes like cells *in vitro* by the addition of three transcription factors Gata4, Mef2c and Tbx5 (GMT) [61] and four transcription factors GATA4, HAND2, MEF2C and TBX5 [62], and *in vivo* by local delivery of GMT using a retroviral system after coronary ligation. This strategy decreased infarct size and modestly attenuated cardiac dysfunction up to 3 months after coronary ligation.

Another group has identified a combination of microRNAs (miRs) 1, 133, 208 and 499 capable of inducing direct cellular reprogramming of fibroblasts to cardiomyocyte-like cells *in vitro* [63]. Using a single transient transfection of the miRs, the authors could direct a switch in cell fate as documented by expression of hallmarks of mature cardiomyocytes, exhibition of spontaneous calcium flux and characteristic sarcomeric organization of a cardiomyocytes-like phenotype [63]. Importantly, administration of miRs into ischemic mouse myocardium resulted in evidence of direct conversion of cardiac fibroblasts to cardiomyocytes *in situ* [63]. These findings demonstrate that cardiac fibroblasts can be reprogrammed into cardiomyocyte-like cells in their native environment for regenerative purposes using either transcription factors or miRs and, more importantly, related to the scope of this review, they indicate an additional potential usage of viral delivery approach: *in vivo* transduction of transcription factors rather than genes encoding proteins. When safer vectors (please see safety paragraph) will be employed, this therapy will be tested in large animals and hopefully translated into human testing.

6. Targeting cardiac arrhythmias

6.1 Ventricular arrhythmias

MI-derived scar slows down conduction, due to intercellular uncoupling and discontinuities in tissue architecture causing ventricular arrhythmias which represent the number one cause of mortality in patients affected by HF [64]. The major contributors in controlling coupling between cells are gap junctions and Connexin43 (Cx43) [65]. Cx43 gene transfer using adenovirus in a model of pigs increased conduction velocity and reduced susceptibility to tachycardia after MI [65].

6.2 Atrial fibrillation

Another consequence of MI can be atrial fibrillation (AFib). It can be caused by direct ischemic injury of the sinus atrial node or due to increased left atrial pressure secondary to diminished LV compliance. Gene transfer approaches were performed in a burst-pacing AFib model. Adenoviral gene transfer using KCNH2-G628S successfully delayed the onset of AFib in a swine model [66,67]. Although this approach has shown efficacy in suppressing AFib, a more clinically relevant disease model needs to be investigated before undertaking any experimentation in humans.

7. Gene therapy directed toward the etiology of myocardial infarction: coronary vessel atherosclerosis, in-stent restenosis, by-pass venous graft disease

In a broader sense, gene therapy directed toward genetic causes of hypercholesterolemia (familial hypercholesterolemia, FH) can be considered as a therapeutic approach toward causes of MI. Lowering high cholesterol levels is indeed one of the main goals of the therapy for cardiovascular disease. Animal models are available for the study of FH, which include the Watanabe heritable hyperlipidemic (WHHL) rabbit [68], rhesus monkeys [69], the apoE-knockout (apoE^{-/-}) mouse and the LDLR-knockout (LDLR^{-/-}) mouse models [70]. In experimental animal models of FH using WHHL rabbit, LDLR overexpression following viral vector-based gene transfer has been shown to be associated with long-term stable correction of hyperlipidemia, with attenuation of atherosclerosis progression, and in certain cases even with lesion regression [71].

Revascularization with *vein grafts* is a standard surgical therapy for coronary artery disease. Unfortunately, up to 50% of saphenous vein grafts will be occluded during the first decade after surgery [72]. Vein graft occlusion occurs as a result of neo-intimal hyperplasia, which takes place in response to hemodynamic changes and vessel wall injury, and is characterized by the migration and proliferation of vascular smooth muscle cells. Intimal hyperplasia is further complicated by the concomitant development of atherosclerosis and thrombosis. Cell-cycle blockade by *ex vivo* gene therapy using E2F-decoy oligodeoxynucleotide (Edifoligide) acting against E2F, which plays a critical role in the *trans*-activation of several genes involved in cell-cycle regulation on experimental vein grafts, inhibited the neointimal hyperplasia and subsequent accelerated atherosclerosis. Unfortunately, a comparison of 5-year clinical outcomes of patients enrolled in PREVENT IV treated with Edifoligide and placebo did not show any advantage of *ex vivo* gene transfer compared to the placebo in terms of death, MI, repeated revascularization and rehospitalization [73].

In-stent restenosis post-angioplasty might result from neointimal hyperplasia due to mechanical injury and a foreign-body response to the prosthesis. Inflammation mediated by monocyte chemoattractant protein-1 (MCP-1) is a potential player in this process. Anti-MCP-1 gene therapy has been tried in hypercholesterolemic rabbits and monkeys, showing a suppressed monocyte infiltration/activation in the stented arterial wall and markedly reduced the development of neointimal hyperplasia. This strategy also suppressed local expression of MCP-1 and inflammatory cytokines [74]. Therefore, inhibition of MCP-1-mediated

inflammation is effective in reducing experimental in-stent restenosis. This approach might be a useful form of gene therapy against human in-stent restenosis [74].

8. Safety of gene therapy

The main concern of any new investigational product trying to enter into clinical development is safety. The death of Jesse Gelsinger [75,76] because of a massive immunoresponse 4 h after adenoviral gene transfer, treatment-induced cancer [75,77–79] and the occurrence of leukemia in three X-linked severe combined immunodeficiency (X-SCID) patients treated in France [80] have raised awareness about potential risks of gene therapy. Despite these tragic events, the scientific community has intensified research [75] and, more importantly, the dialog between scientists, developers and regulatory agencies has greatly improved. These are the major concerns related to the use of gene product: i) viral integration and potential insertional mutagenesis (IM); ii) immunogenicity-neutralizing antibodies due to natural and acquired immunity (Nab); and iii) shedding.

8.1 Viral integration

The risk related to viral integration is determined by the choice of viral vectors. Viral vectors from the family of Retroviridae (retrovirus and lentivirus) can integrate into DNA. Integration of retrovirus follows a pseudorandom mechanism, and when insertions occur into promoter and enhancer regions, it can be oncogenic as it happens in the trial of gene therapy for X-SCID [80]. Lentiviral vectors (HIV virus based) do not activate proto-oncogenes, as retroviruses do, but can transduce mitotically inactive cells as cardiomyocytes allowing a long-term expression. After the demonstration that HIV vectors integrated near the T-cell proto-oncogene *Lim-only 2* promoter [81–83], appropriate methods have been established for the risk assessment of IM, and to identify safe vectors, factors and conditions determining pro-viral integration into a given site of the genome [84]. Despite the development of non-primate lentiviral vectors that do not induce seroconversion in humans and their employment in clinical trial for the treatment of ADA-deficient SCID and HIV infection [85–87], caution is used in employing these viruses in cardiovascular research.

8.2 Neutralizing antibodies

Adenovirus vectors have shown considerable promise in animal models and are currently being used in numerous clinical trials, especially for the therapy of cancer. Their use is more and more reduced in cardiovascular gene therapy because of their short expression (2 weeks [88,89], please see paragraph under ‘expression’) and the possibility to be neutralized by systemic antibodies and their high immunogenic potential. The death of Jesse Gelsinger suffering from partial ornithine transcarbamylase (OTC) deficiency in a Phase I study occurred after adenoviral administration containing OTC gene [90]. This experience pointed to the limitations of animal studies in predicting human responses, the steep toxicity curve for replication-defective adenovirus vectors, substantial subject-to-subject variation in host responses to systemically administered vectors, and the need for further study of the immune response to these vectors [90]. Because of these limitations, adenoviral vectors are not used in cardiovascular gene therapy and are nowadays employed only in small animal studies. In that regard, AAVs elicit a reduced adaptive and innate immune response as compared to

adenoviral vectors [91–93]. One of the biggest challenges in optimizing viral vectors for gene therapy relates to the immune response of the host [93]. AAV vectors are able to transduce a wide variety of tissues, associated with low immunogenicity and toxicity, resulting in vector persistence and long-term transgene expression [94–96]. AAV-mediated gene therapy, however, can be impaired by antibodies against the vector capsid. Anti-AAV antibodies have neutralizing effects that decrease the efficiency of *in vivo* gene therapy and can prevent vector re-administration. Indeed, in the hemophilia B trial, upon AAV gene transfer to the liver, two subjects have displayed transient elevation of liver enzymes, probably as a response operated by CD8⁺ T cells likely derived by wild-type AAV infections. Both cell-mediated response and humoral response to the delivered gene depend on a number of variables, including the nature of the transgene, the promoter used, the route and site of administration, vector dose and host factors. The response of the host to the vector, in terms of antigen-specific immunity, will play a substantial role in clinical outcome [97,98]. It has been found that anti-AAV neutralizing antibodies are highly prevalent in humans, and the frequency of subjects with detectable titers can reach up to two third of the population. The approach to the problem of preexisting humoral immunity to AAV so far has been the exclusion of seropositive subjects [26,27,99,100], limiting the large-scale possibility of gene therapy for the majority of the population. Several solutions have been proposed: Monteilhet [101] showed that repeated cycles of plasmapheresis are effective in lowering anti-AAV NAb. An alternative tested strategy is to induce transient immunosuppression for the duration of the intervention [102,103]. Mimuro *et al.* recently tested the efficacy of saline flushing, with or without balloon catheter isolation of the hepatic circulation, to minimize the inhibitory effect of NABs. Administration of an AAV8 vector encoding for Factor IX using saline flushing technique increased transgene expression levels between 4 and 10% of the normal, even in the presence of detectable NABs. They show that accessing the portal vein and purging the liver of venous blood with saline can minimize contact of the gene transfer vectors with NABs and can enhance the efficiency of vector transfer [104].

8.3 Shedding

Another safety concern, often ignored, is the possibility of environmental spreading of gene products through excreta, namely *shedding*. There is, indeed, a risk of dissemination of a viral vector into the environment. Unfortunately, data on shedding are not reported in the majority of publications of gene therapy trials [105]. The evaluation of the potential impact and consequences of the viral vectors is complicated by the high degree of variety in the experimental design of shedding analysis between trials, by the lack of a validated, sensitive and uniform protocol across laboratories. It is important to monitor about the possibility that personnel close to patients subjected to gene therapy come in close contact to viral vectors. Interestingly, AAV9 used for the treatment of hemophilia B has been found in 6 out of 7 treated patient's semen up to 16 weeks after therapy, raising the unexplored issue of sexual transmission [106]. This evidence alone informs about the potential risk related to vector dissemination.

9. Expression

Gene expression can be achieved in many different ways. Modality of delivery is a very important aspect: These include intramyocardial injection, coronary perfusion (antegrade and retrograde), pericardial delivery and epicardial painting. All these techniques are moderately successful when adopted for the right purpose, but each one is hindered by efficacy, tolerability, or access. Myocardial injection has been mostly used in the field of angiogenesis. In the experimental setting using small and large animals, the virus has been injected from a syringe through a needle embedded in the myocardium. The local area around the injection site has a high density of gene transfer, but even 5–10 mm from the needle, gene expression is negligible. Given this limitation, a single injection can transduce the majority of a mouse LV, but multiple injection sites are required for adequate coverage in larger mammals. In addition, studies have reported acute inflammation at the injection site because of tissue disruption, which needs to be kept in mind when considering this type of gene delivery [107,108]. Intracoronary perfusion of gene transfer vectors has been reported by percutaneous catheterization [109], open-chest aortic cross-clamp with LV cavity infusion [110], and cardiopulmonary bypass with direct coronary arterial perfusion [110]. Preclinical studies have shown that treatment with vasodilatory and vascular permeability-enhancing agents increase gene transfer [109]. A number of agents have been used: nitroglycerin, nitroprusside, serotonin, bradykinin, histamine, VEGF and reduced perfusate calcium [61] concentration, among others [111]. An advantage of intracoronary perfusion is the ability to deliver more globally across the myocardium than that is achievable with intramyocardial injection, allowing gene transfer to occur across the entire perfusion territory of the targeted coronary vessel.

A promising delivery technique for cardiac atrial gene therapy is epicardial painting, in which the viral vector is literally painted on to the epicardial surface suspended in a gelatinous polymer. Preclinical data show complete transmural transmission of reporter genes [65]. The downside to this method is that it currently requires an open chest for access to the epicardium; therefore, minimally invasive ‘brush devices’ might be needed. Monitoring the expression of therapeutic genes in targeted tissues in disease models is important to assess the effectiveness and safety of systems of gene therapy delivery [112].

Quantitative and qualitative monitoring of gene delivery and expression after its administration are paramount to develop new strategies and assess the progress and prospects of the treatment. Both *in vitro* and *in vivo* strategies are essential, especially the ones that allow for noninvasive evaluations of gene therapy in living subjects.

9.1 GFP-based imaging

From its discovery in the early 1990s, GFP and its variants have become invaluable markers for monitoring protein localization and gene expression *in vivo* [113] and have allowed in the field of gene therapy to detect protein expression in *in vitro* studies using immunofluorescence technique or probing by western blotting GFP protein content within tissue lysates. Although GFP penetrates tissue poorly, its expression can also be visualized noninvasively in intact animals [114,115].

9.2 Tomographic imaging of gene expression

Aside from the various strategies for imaging gene expression in live cells and tissues by fluorescence, fluorescence diffuse tomography has been developed recently for small animal imaging [116] to generate a more quantitative fluorescence readout. The animal is scanned in the transilluminative configuration by a single source and a detector pair, and a reconstruction algorithm estimates the fluorophore distribution. Another technique, fluorescence-mediated tomography, can also provide important insights into *in vivo* gene expression within deep tissues [117].

Another optical imaging technique, bioluminescence imaging (BLI), is an attractive alternative to fluorescence for optical imaging of gene expression. Bioluminescence is the emission of light from biochemical reactions that occur within a living organism. Although bioluminescence has little, if any, clinical potential, it is virtually free of any background signal, an ideal property for sensitive imaging of gene expression in cells and small animals. The luciferases are a family of photoproteins that can be isolated from a variety of insects, marine organisms and prokaryotes [118]. Luciferase has been used as a reporter gene in transgenic mice, but, until the instrumentation was created for BLI, the detection of luciferase activity required either sectioning the animal or excising the tissue and homogenizing it to measure the luciferase activity in a luminometer [119]. BLI has proven to be a very powerful method for detecting luciferase activity in intact small animal models. It is noninvasive, convenient and relatively inexpensive, thus making it an excellent method for elucidating expression of the gene of interest. To date, firefly luciferase and Renilla luciferase are the most widely used reporter proteins for BLI of gene expression in living animals. These tools can be used to continuously and noninvasively monitor the location and amount of gene delivery as well as the functional outcome. Wu *et al.* [120] showed for the first time that gene expression in the myocardium of living subjects can be monitored non-invasively. After injection of adenovirus-expressing firefly Luciferase directly into the myocardium they were able to detect Fluc activity peaks for 2 weeks. Therefore optical imaging has many applications for cardiac gene therapy studies. Its noninvasive feature nature makes it ideal to monitor cardiac gene expression quickly and accurately.

Diagnostic imaging tools such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have been employed to allow for visualization of the location of radiolabeled vector, immune cell dispersal and to assess transgene expression for gene therapy [121]. These techniques use reporter genes and reporter probes. Introduction of a reporter gene into a target tissue by various methods such as viral and nonviral vectors may lead to transcription of the gene and translation of the protein of interest, which in turn interact with the imaging tracer probe, administered in trace amounts for PET/SPECT [122,123].

Molecular imaging is becoming an extremely powerful technique that has the potential of analyzing gene expression noninvasively, repetitively and quantitatively in an intact, living whole-body system, and represents a useful tool to employ in preclinical studies. Its applicability, though, is limited to animal studies, and there are no techniques capable of monitoring gene expression in humans.

10. Specificity and tropism for cardiac gene delivery

Cardiac-specific delivery of a transgene is an important prerequisite for gene therapy of cardiac diseases, especially if the transduction of extracardiac tissue may be a potential risk of side effects. Several studies have tried to answer this question. A breakthrough in cardiac gene transfer was achieved with the finding that AAV vectors based on AAV serotypes 6 and 9 allow an efficient cardiac transduction in rodents [37,124–126]. Transcriptional targeting with cardiac-specific promoters has been described as another valuable strategy to improve specificity of cardiac transgene delivery. Muller *et al.* found that one way to improve specificity of cardiac vector delivery can be obtained by combining transcriptional and cell-surface targeting [127]. They showed that expression of a transgene driven by a CMV-MLC1.5-hybrid promoter is suitable to increase specificity of cardiac transgene expression of AAV vectors [127]. Their results in a dog model demonstrate that a heterologous promoter consisting of the 1.5-kb MLC2v promoter fused to a CMV enhancer enables a predominantly cardiac reporter gene expression in dogs after systemic administration of AAV2 vectors, combined to a reduced hepatic transduction [127]. However, despite significant improvements by employment of cardiotropic AAV vectors and use of heart-specific promoters, residual expression of transgenes stayed still detectable in several nontargeted tissues following AAV vector application [127].

In an interesting paper, the specificity of AAV9 was improved using a vector-mediated cardiac gene transfer by miR-dependent control of transgene expression [128]. Intravenous injections of AAV9 vectors bearing three repeats of miR122 TS in the 3'UTR of an enhanced green fluorescent protein (EGFP) expression cassette resulted in the loss of EGFP expression in the liver of adult mice, whereas the control vectors without miR TS displayed significant hepatic EGFP expression. Importantly, miR122-dependent hepatic de-targeting of AAV9 vectors seems to be per se superior to transcriptional targeting of the vector to myocardium using the cardiac CMV-MLC0.26 promoter [128].

11. Efficacy

Efficacy is probably the most important parameter to achieve. It is the result of the choice of i) the appropriate delivery method that allows expression; ii) a promising target for that disease; iii) the right amount of gene expression in the desired tissue and cell types; and iv) of course, the right clinical endpoint to measure it. It has to be distinguished between efficacy in preclinical studies and efficacy in clinical trials. Usually, in preclinical development, the tested product is compared to placebo and very rarely we have encountered in revising the literature studies comparing the investigational gene product to the current standard of therapy [129]. Although more expensive, we believe that these types of studies might allow to predict the outcome of an experimental product on a clinical development setting and even identify potential synergistic relationship with current drugs. Definition of efficacy in clinical trials is far more restrictive because an investigational product is compared for obvious ethical reasons to the best standard of care.

12. Conclusions

Like Fitzgerald's Jay Gatsby, we believe in a green light – in the present case, the green light of the GFP expression of our gene of interest holds much promise for the future of therapy in cardiology. The results of CUPID, MYOHEART and adenoviral AC VI trials are encouraging and they indicate that gene therapy in cardiac disease represents a realistic and feasible avenue.

13. Expert opinion

In 1963, Nobel laureate Joshua Lederberg said that 'The ultimate application of molecular biology would be the direct control of nucleotide sequences in human chromosomes, coupled with recognition, selection and integration of desired genes.' Gene therapy, with its current clinical achievements for treatment of monogenic disease [75], embodies Dr. Lederberg's concept. Far from considering gene therapy as a panacea for all the human diseases, we envision a future where gene therapy is one of the tools that will flank drug, stem cell and device therapies. From our perspective, there are two major issues that are delaying the progression of the gene therapy field: i) a delay in shifting from small to large animal studies; and ii) the long process of filing regulatory documents required from FDA to start human experimentation. Gene targeting discovery has allowed basic scientists to study the role of virtually any gene, facilitating the process of understanding the role of each individual gene in many pathologies. KO mice models have, on one hand, permitted researchers to elucidate disease mechanisms and instruct the gene therapy field, especially the field of monogenic disease; on the other hand, this has polarized the attention of many scientists and reduced the number of them working on large animal experiments. Nowadays, few scientists are employed on large animal studies, and the number of facilities where these studies can be performed is limited. When the efficacy of replacing a gene has been demonstrated in mice, it becomes challenging to find teams of experts who can perform disease model experiments on large animals and administer a gene product. In our opinion, these challenges can be overcome by establishing preclinical development academic centers. Here, small and large animal studies can be performed simultaneously, setting a new research standard, standing as examples, and offering service as core facilities for large animal studies to other academic entities. This can be possible when multidisciplinary expertise coexists, which nurtures interaction of scientists, physicians and physician-scientists. Once the efficacy of a gene target is ascertained in large animal models, universities and academic centers should be able to provide scientists with 'in house' support service in order to file regulatory documents and help expedite the IND process through FDA.

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Article highlights

- Gene therapy is one of the tools that will flank drug, stem cell and device therapies.
- A gene therapy product has to be evaluated at the pre-clinical stage on 4 parameters: safety, expression, specificity and efficacy (SESE).
- Preclinical development of gene therapy can be achieved and speeded up through the institution of academic centers where small and large animal studies can be performed and where interaction between scientists, physicians and physician–scientists coexists.

This box summarizes key points contained in the article.

Target towards clinical consequences of MI	Etiological causes of MI	Safety	Expression	Specificity	Efficacy
Adrenergic manipulation	Atherosclerosis	Viral integration and potential insertional mutagenesis (IM)	Modality of delivery –intramyocardial injection –coronary perfusion (antegrade and retrograde, with or without balloon) –pericardial delivery –epicardial painting	Choice of the right vector	Appropriate delivery method
Inhibition of G protein-coupled receptor kinase 2 (GPRK2)	LDLr			Transcriptional targeting with cardiac-specific promoters	Promising target for disease
Adenylyl cyclase VI				microRNA (miR)-dependent control of transgene expression	Gene expression
Ca cycling proteins	By-pass venous graft disease	Immunogenicity	Quantification of expression		Definition of clinical efficacy in clinical trial and preclinical studies (small and large animals)
SERCA2a					
Phospholamban	Using E2F-decoy oligodeoxynucleotide (edifoligide)		GFP		
S100A1			Fluorescence-mediated tomography (FMT)		
Parvalbumin					
Angiogenesis	In stent restenosis	Neutralizing antibodies (Nab)	Bioluminescence imaging (BLI)		
VEGF			Firefly luciferase (Fluc)		
SDF-1	Monocyte chemoattractant protein-1 (MCP-1)		Renilla luciferase (Rluc)		
Cardiac regeneration		Shedding	Positron emission tomography (PET)		
Transcription factors			Single-photon emission computed tomography (SPECT)		
miRNAs					
Cardiac arrhythmias					
Cx43					
KCNH2-G628S					

Figure 1.

Summary of the scheme of the review: gene therapy for causes and consequences of MI and the requisites of gene therapy: safety, expression, specificity, efficacy (SESE).

Table 1The history of β ARKct.

β ARKct life events	Refs.
Evidences of decreased catecholamine sensitivity and β AR density in failing human hearts	[130]
Elevation of GRK2 has been demonstrated in several models of cardiovascular disease, including hypertension, myocardial ischemia, ventricular hypertrophy and chronic HF	[131–133]
β ARKct overexpression <i>in vivo</i> can block GRK2 activity and restore β -adrenergic signaling in a mouse <i>in vivo</i> model	[8]
β ARKct mice could rescue the phenotype of the KO mouse of the muscle LIM protein (MLP ^{-/-}) and of the transgenic mice overexpressing the sarcoplasmic reticulum Ca ²⁺ -binding protein calsequestrin	[134,135]
GRK2 KO mice with 50% less GRK2 expressed in myocytes (and all cells) resulted in enhanced cardiac function <i>in vivo</i>	[136]
Conditional KO lines were developed, α MHC-Cre/GRK2 ^{fllox/fllox} and MerCreMer/GRK2 ^{fllox/fllox} , and prevented and reversed HF after MI	[4]
GRK2 KO lines <i>in vivo</i> exhibited improved cardiac function and activation of pro-survival pathways (Akt, Bcl-2, Bcl-xl) post-MI injury	[137]
AAV6- β ARKct gene therapy improves cardiac function and normalizes the neurohormonal axis in chronic HF in a rat model	[129]
Large swine preclinical model of HF in the pig showed that AAV6 β ARKct reversed ventricular dysfunction	[10]