Abstract

Heart disease remains the prevalent cause of premature death and accounts for a significant proportion of all hospital admissions. Molecular genetics was integrated quite late in cardiology, but introduced new concepts like sarcoplemmopathies, cytoskeletalopathies, and channelopathies useful to better understand the pathophysiology of the development of inherited cardiomyopathies (CMs). As our understanding of the cellular and molecular processes involved in the development and progression of heart disease improved, new therapeutic targets were identified, as were novel approaches such as delivery of genes to replace defective or deficient components and thereby restore structure or function in a diseased heart. We discuss gene addition strategies in the context of monogenic disorders. Moreover, a broader nucleic acid-based modulation of cardiac gene expression for the treatment of cardiac diseases might have larger clinical indications. Inadequate gene delivery remains a potential cause of negative trials. However, progress in innovative formulations and clinically relevant ways of administration should lead to significant progress in the future. Cardiac gene therapy will be integrated into the therapeutic armamentarium for CM and heart failure.

Keywords: cardiomyopathy, heart failure, genetic disease, gene therapy, cardiac structure, cardiac function, viral vector, nanoparticles, polymers

1. Introduction: cardiomyopathies

Cardiomyopathies (CMs) refer basically to diseases of the heart muscle, which can be acquired or inherited [1]. CMs can affect people of all ages. However, people in certain age groups are more likely to have certain types of CMs, as inherited forms predominate in younger individuals and acquired diseases increase with age [2–8].
Most frequently, four main clinical forms are described, meaning hypertrophic, dilated, and restrictive types as well as arrhythmogenic CM. These diseases have many causes, signs, symptoms, and treatments. We exclude ischemic cardiopathies from this overview, and focus more precisely on disorders of the heart muscle of non-ischemic origin. This does, however, not exclude anomalies of the perfusion of the myocardium, because pathophysiology of these diseases is usually complex, interleaving different mechanisms.

Diagnosis of non-ischemic CM is a challenging process that influences patient morbidity and mortality. Multiple biomarkers and imaging tools contribute to the adequate ranking of the clinical presentation of these diseases. More recently, nuclear magnetic resonance (NMR) imaging appeared as a robust diagnostic tool that offers various techniques to assess the structure, function, perfusion, and scarring of myocardial tissue, thus providing better understanding of the underlying causes of CMs [9–12]. At a molecular level, genotyping identifies precisely the causal mutations in inherited forms of CMs. Moreover, a systems biology approach can investigate more fully the molecular profiles of different phenotypic stages of CM.

From a pathophysiologic and diagnostic perspective, it might be useful to consider a stratification of CMs slightly different from the clinical classification. Considering the various genes that can trigger the development and evolution of a CM, we propose to group inherited diseases as cytoskeletal CMs or cytoskeletalopathies, sarcomeric CMs or sarcomyopathies, and finally ion channel CMs or channelopathies.

Different structural alterations of the myocardium contribute in varying degrees to the different forms of the diseases, but common features may represent as many therapeutic targets.

The focus of more extensive cellular degeneration is one of the histological hallmarks of CM [13,14]. Necrosis is not the only mechanism leading to cell death. Apoptosis, or programmed cell death, is a highly regulated and active process that contributes to the maintenance of adult cardiac tissue [15]. Myocyte cell death is implicated in the architectural rearrangement occurring in the surviving myocardium. This remodelling leads to heterogeneity in the myocardial structure, created by the altered behaviour of non-myocyte cells, particularly cardiac fibroblasts, which are responsible for myocardial collagen metabolism and fibrous tissue accumulation. It may largely explain the appearance of diastolic and/or systolic myocardial failure [15]. Adverse left ventricular remodelling leads to alteration in the structure (dimension, mass, shape) of the heart that might at the beginning of the process be considered as compensatory for the disease process, but at the end will severely impair cardiac function. Remodelling is also a prominent feature of electrophysiologic properties of the myocardium, translated as clinical presentation such as atrial fibrillation, flutter, complete heart block, ventricular ectopic pacing, and tachycardia.

Considering the remodelling process as a deleterious end effect, one can raise the question of potential reverse remodelling. Could that be an option for remission or cure of CM? It has been shown that prolonged mechanical unloading of failing hearts can preserve myocardial contractility but impairs relaxation. Could gene therapy provide new therapeutic options for those patients?
Myocardial remodelling involves not only the cardiomyocytes, but also non-myocyte cells and the extracellular matrix. Fibrosis is an essential process in the repair of damaged tissues and wounds, but its accumulation in organs and tissues can lead to scarring, organ dysfunction, and, ultimately, failure. Development of interstitial and perivascular fibrosis of varying degrees is observed in most CMs. However, in vivo diagnosis of the extent and distribution of fibrosis remains difficult. New approaches such as ultrasound elastographic and cardiac NMR techniques might provide appropriate outcome measures to monitor more specifically myocardial fibrosis, and thus potential therapeutic effects [16].

Immune mechanisms modulate interstitial fibrosis, cardiomyocyte cell death, and hypertrophy, all of which are central processes leading to maladaptive remodelling in response to a variety of stimuli. Acute inflammation, as observed in myocarditis, might be out of the scope of the present overview, and would need a dedicated review. However, in chronic heart failure (CHF) patients, a chronic inflammatory activation has long been recognized. Heart failure is associated with a wide array of mechanisms subsumed under the term “inflammation.” This chronic inflammation harms the myocardium instead of healing it. Gene therapy might find new therapeutic targets in this context.

Similarly to the structural modifications of the myocardium, functional alterations contribute to the definition of CM. From a perspective of pathophysiology, alterations of preload and afterload largely contribute to diastolic/systolic dysfunctions. Pressure–volume relationship best defines myocardium alteration beyond the hemodynamic parameters.

Moreover, a more detailed understanding of excitation–contraction coupling reveals new targets for innovative therapeutic strategies.

Furthermore, and beyond the triggering causes of CM, as heart muscle becomes weaker over time, a common clinical condition described as heart failure develops. From a pathophysiological and therapeutic perspective, heart failure could be considered as a specific disease stage, independent of the acquired or inherited origin of CM. Gene therapy could also be considered at this stage.

2. Therapeutic options: why gene therapy?

Many medicines are used to treat CM and CHF, but despite this, CM and CHF remain leading causes of morbidity and mortality even in developed countries. Correcting hemodynamic imbalances, such as fluid control (preload) or vascular resistance control (afterload), remains primordial, but cannot change the myocardial contractility per se (Figure 1). Fundamentally, determinants of cardiac output are the same as those of myocardial energy consumption. Therefore, tackling the problem of decreased contractility raises in parallel the problem of increased energy requirements. Several attempts to increase inotropism on a chronic basis led to overall negative results because energy consumption exceeded production. Gene therapy might offer new therapeutic options. The pressure–volume relationship demonstrates the contracting and relaxing portions of the cardiac cycle (Figure 2). The slope of the end-systolic
**Figure 1.** Determinants of cardiac output (CO). CO is the resultant of stroke volume, the difference between end-diastolic and end-systolic volumes times the heart rate. According to the Frank-Starling law, preload influences CO positively. With developing heart failure, CO is negatively influenced by afterload. Contractility represents the primary inotropic capacity of the myocardium.

The pressure–volume relationship represents the most objective measure of the intrinsic contractile capacity of the myocardium.

Similarly, management of CHF patients frequently takes advantage of rhythm control (pharmacologic or pacemakers/implantable cardioverter defibrillator). Gene therapy might represent a new way to address this topic by recreating new endogenous biological pacemakers rather than relying on electronic devices.

Recent clinical trials [17–19] have not only pinpointed the importance of inflammation but moreover the therapeutic potentialities of selectively targeting some cytokines. At a preclinical level, glycoprotein-130 (gp130) has been identified as a potential new target [20–22]. It is now established that with gp-130, the common receptor of IL-6 is elevated in patients with chronic heart failure. Hilfiker-Kleiner et al. have shown that mice carrying a cardio-specific mutation of gp-130 have a normal myocardial phenotype at baseline. However, induction of an experimental myocardial infarction leads to development of heart failure and increased mortality. Moreover, these observations were associated with increased expression of complement-activating mannose-binding lectin [23]. Thus, this animal model suggested a link between IL-6 and chronic myocardial injury induced by complement activation.

Cardiac myofibroblasts respond to a large number of proinflammatory cytokines (e.g. TNF-alpha, IL-1, IL-6, TGF-beta), vasoactive peptides (e.g. angiotensin II, endothelin-1, natriuretic peptides), and hormones (e.g. noradrenaline), the levels of which are increased in the remodelling heart. Reducing myocardial remodelling specifically via modulatory effects on cardiac fibroblasts might represent further new therapeutic targets.

Anticoagulants in the context of CHF are an important therapeutic class for those subgroups of patients at high risk for abnormal clotting. Anticoagulation might appear inappropriate for
gene therapy, but nucleic acids can interfere in a very selective way with proteins. Targeting proteins of the intrinsic pathway of the coagulation might achieve safe and efficient thrombus control without the usual risk of bleeding that conventional anticoagulants share. Furthermore, one should keep in mind that initiation of the intrinsic pathway is intimately linked to inflammation via kinins and complement. Nucleic acid might represent a new class of drugs in this context.

Surgery represents an important therapeutic option in the arsenal for managing CM and CHF patients. The surgical approach can consist of either specific procedures such as septal myomectomy in hypertrophic CM (HCM), coronary artery bypass graft surgery, or more generally left ventricular assist devices as a “bridge to transplant” or destination therapy [24]. Transplantation remains the reference treatment for end-stage CHF and for people who have failed other treatment options. It might be surprising to refer to surgery in the context of gene therapy, but one should not forget that most initial clinical trials have included terminally

Figure 2. Pressure–volume relationship. The pressure–volume curve for the left ventricle is represented as a pressure vs. volume plot. The slope of the end-systolic pressure–volume relationship indicates the intrinsic contractile capacity of the myocardium independently of the hemodynamic load conditions. Compared to a control curve (dark lines), cardiomyopathy (light lines) is characterized by a decreased slope among other hallmarks. Gene therapy would aim at preserving or restoring normal contractility.
ill patients. Thereby, in terms of therapeutic efficiency and risk/benefit ratio, initial evaluations will refer to outcomes of surgical procedures. The gene therapist should be aware of the competing therapeutic strategies.

So far, none of the existing treatments have definitively changed the fate of CM and CHF. There is thus space for new drug developments and gene therapy might help to solve some of the intrinsic hurdles of CM and CHF. For instance, none of the existing treatments really change myocardial contractility without excessively increasing oxygen consumption.

Advances in gene transfer vectors, development of new vector delivery methods, and discovery of new gene targets continue to fuel our motivation to use this approach in routine bedside care [25,26].

3. Gene therapy

When developing a gene therapy-based medicinal product, one should keep in mind that no active substance will become a drug product unless it can be properly formulated and administered. Compared to more conventional small molecules, gene therapy strategies based on nucleic acids are faced with new constraints linked to their chemical nature, the size of the molecule, and the coding sequence composition.

Different pharmaceutical designs for gene therapy could be considered. In the context of CM and CHF, we will focus more precisely on how to restore the functional allele in the context of inherited CM and more broadly how to restore or improve myocardial contractility.

3.1. Inherited CM

In the context of inherited CM, most frequently a monogenic transmission profile has been identified, expression profiles being either dominant or more frequently recessive. Sometimes CMs are part of a larger clinical context of a systemic myopathy, but usually cardiac and neuromuscular disorders are not proportional and thus would need separate and specific treatment, even if the genetic origin can be unique.

Considering the situation of the single causative gene acting in a recessive mode, it might be tempting to restore a normal phenotype through addition of a functional allele. So conceptually at this level, gene therapy is mainly derived from gene transfer techniques largely used in cell biology by introducing an exogenous sequence of nucleic acids into a eukaryotic cell to express new information on these cells. Over time, several independent laboratories have demonstrated that the concept of transferring an exogenous gene into the myocardium of mammals was possible, leading to the expression of a new protein not coded by the intrinsic genes. However, to transform a laboratory technique of gene transfer into a therapeutic option, additional steps had to be considered. To assess the therapeutic capabilities of gene transfer, protein expression cannot be the primary outcome measure. More subtle integration of the pathophysiology of each CM is mandatory. Considering inherited diseases where a certain phenotypic latency exists, it was possible to demonstrate that gene transfer of a functional allele
was able to delay the onset of an overt CM. More generally, when designing a gene therapy strategy, one should consider whether the defective gene should be rescued or whether other genes involved in heart failure development and progression should be targeted.

3.2. Preclinical models of CM

Delta-sarcoglycan (dSG)-deficient hamsters represent a well-characterized genetically determined model of a CM. Phenotypically these animals develop a dilated cardiomyopathy (DCM) with terminal heart failure over a rather short time span as they die as mid-aged adults [27,28]. From the perspective of gene therapy they represent a very useful model. Beyond the clear phenotype, the causal genetic mutation is known, coding sequences are readily available, and transmission is autosomal recessive. A single allele correction can correct or at least clearly improve the phenotype when administered in young animals [29]. Some authors observed even more than a simple phenotypic rescue as the lifespan of these animals seemed to increase [30,31].

However, unlike dSG, coding sequences of some normal alleles can be very extensive, the most extreme case being dystrophin with a full-length cDNA of more than 11 kb. Several strategies can be considered. Given some structural specificities, reengineering of the active pharmaceutical ingredient (API) can be performed while retaining therapeutic potential. Thus, gene therapy should not simply be considered as a substitution of defective alleles. Hence, truncated forms of dystrophin have proven to alleviate pathologic phenotypes in several experiment models [32,33].

Similarly, it was possible to show that editing the intrinsic messenger RNA can lead to coding of a functional protein. Exon skipping is used to restore the reading frame within a gene. The mechanism behind exon skipping is a mutation-specific antisense oligonucleotide. An antisense oligonucleotide is a synthesized short nucleic acid polymer that will bind to the mutation site in the pre-messenger RNA to induce exon skipping. In the context of Duchenne muscular dystrophy (DMD) the genetic mutation that leads to Becker muscular dystrophy (BMD) is an in-frame deletion. Exon skipping can induce the expression of a truncated but functional dystrophin protein and thus switch the phenotype of some DMD-type mutations to the phenotype of a BMD-type mutation [34,35].

Multiple arguments in favour of the feasibility of cardiac gene therapy have been generated over time. However, these experiments raise new questions. Most non-clinical studies were carried out on well-characterized model-rescuing defective genotypes and avoiding or delaying the development of a pathological phenotype. So referring to clinical settings, this mimics mainly presymptomatic situations.

In this setting, gene therapy would basically be a prophylactic option to avoid development of a pathological phenotype, but are we ready for a gene therapy that would be mainly preventive? What would an acceptable risk/benefit ratio be in that case? Ideally in the context of preventive medicine, gene therapy of an inherited monogenic disorder should by homologous recombination correct most if not all of the affected cells without any off-target adverse effects. Gene therapy has not yet reached this level of maturity. Nevertheless, this does not mean that such options cannot be tested in the future.
3.3. Heart failure

If gene therapy offers the possibility to interfere intimately and subtly with the molecular pathways governing the pathological processes, then introducing genetic material into cells should be able not only to compensate for abnormal genes but also to influence pathways involved in the development and progression of the disease.

In the context of inherited CMs, we postulate that several steps might occur sequentially. The causative genetic defect can be inherited or be a neo-mutation and will trigger a cascade of deleterious effects that will lead to the appearance of a patent cardiac disease. Progressively the genetic features of heart failure will dominate and one might consider that at a later stage these changes will be almost independent of the original genetic defect. Moreover, we might consider that genetic modifications at this stage are similar to those that occur in the context of acquired CM. While any disease is a potential target for gene therapy, some treatments are easier to achieve in the clinic. To test this working hypothesis experimentally, we used mainly the same dSG-deficient hamster model. However, to mimic symptomatic disease, animals were included at a later age.

Many molecular targets could be considered at this level, but several candidates might be more prominent in the present context. We have already discussed the case of gene addition of a functional allele in the context of an autosomal recessive disorder. The candidate gene will of course depend on a proper identification of the genetic disease involved in the CM. On the other side, considering the heart failure phenotype as such, multiple options appear.

Rather basically, we evaluated genes preserving myocardial structure. In the experimental setting that we considered as a model, it is known that hamsters develop a DCM. However, in some substrains carrying the very same mutation but in slightly different genetic backgrounds, animals can develop firstly a phase of HCM and have a less severe phenotype. So the question became, can we mimic this feature by introducing exogenous genetic information?

Physiologic remodelling is a compensatory change in the dimensions and function of the heart in response to physiologic stimuli such as exercise and pregnancy. The remodelling process frequently includes increases in myocardial mass. The heart can respond to environmental stimuli by growth (increased myocardial mass) or shrinkage (atrophy) with a rather large dynamic range. Remodelling is induced by changes in gene expression, which, in turn, alter the expression of key regulatory proteins, the distribution and function of subcellular organelles, the size and morphology of individual cells, the properties of the extracellular matrix, and ultimately those of the entire organ. IGF-1 is a key player in this context and prior to developing a gene therapy option we could demonstrate that administration of a recombinant IGF-1 protein can exert several beneficial effects of the cardiac phenotype of dSG-deficient hamsters [36,37]. However, a recombinant protein with pleiotropic effects will inevitably lead to extracardiac adverse effects. Therefore, a gene therapy option might offer a more targeted treatment, especially when associating local delivery with tissue-specific regulatory sequences. IGF-1 served as a role model to highlight some of the innovative differences between gene therapy and conventional treatments, but of course other APIs could be developed along a similar strategy. Various pathophysiological processes could be targeted, such as interfering with the fibrosis–cell death axis and promoting cell survival.
Besides structural changes of the myocardium, influencing cardiac function could represent further targets for gene therapy strategies. Taking advantage of the well-known hamster model, one can reformulate the clinical question as the progressive decline of contractility and development of patent heart failure. We considered animals at an early symptomatic stage to mimic as closely as possible a clinically relevant situation. We compared the efficiency of administering either a functional cDNA of dSG (rescuing the causal genetic defect) or a cDNA coding for a Ca^{2+}-handling protein, for instance SERCA2a. SERCA2a holds a key role in the development and progression of heart failure, so after the initial work by Schwartz and coworkers, it was rather obvious to test its therapeutic potential [38–40]. Briefly, we could demonstrate that from a therapeutic perspective at a clinical stage of patent heart failure, great benefits could be obtained by targeting cardiomyocyte Ca^{2+} homeostasis through SERCA2a gene expression than rescuing the initial causative genetic defect [41]. These findings as well as results from several other labs strongly support the strategy of cardiac gene therapy for heart failure based on restoring appropriate Ca^{2+} handling [42–44]. At this stage, one should cite the pioneering work led by Hajjar that led to a clinical trial (CUPID) using an expression cassette coding for SERCA2a [45]. This phase Ila study retained some intrinsic limitations due to the low number of patients. Therefore, a larger phase IIb study (CUPID2) with a double-blinded, placebo-controlled, and randomized event-driven schema and based on multinational, multicenter recruitment (n = 250) was needed to confirm the initial results described in the CUPID1 study. This phase IIb CUPID2 trial did not meet its primary and secondary endpoints. Nevertheless, multiple useful data were generated by this clinical trial. Gene therapy is a realistic therapeutic strategy in the field of CMs. Patient selection is always a difficult task in those very innovative steps, but the trial allowed refining the criteria. It also became apparent how important formulation of the API and administration are. Before discussing these aspects, one should acknowledge the research done by K. Hammond and coworkers that explored the therapeutic potential of adenyllyl-cyclase type 6 (AC6) [46–48]. These authors showed that activation of cardiac AC6 expression improves impaired function of aged hearts through improved calcium uptake. AC6 determines cAMP formation. However, favourable effects on cardiac function through abrogation of hypertrophy, increased cell survival, and improved calcium handling appear to be cAMP independent. The main goal of the trial based on AC6 administration in CHF patients is to evaluate the safety and efficacy of human AC6 gene product as a new therapeutic option. To reach this goal, 56 patients were (or have been if the trial is still ongoing - please check) included in this study, in which gene delivery was based on a drug formulation where human AC6 was carried by an adenovirus serotype 5.

Expression of a peptide inhibitor of GRK2 (βARKct) can improve the contractility of failing myocardium and promote reverse remodelling of the left ventricle.

Inhibition with antimiR-34a/antimiR-34 has emerged as a promising therapeutic strategy, as silencing of miR-34a attenuates cardiac dysfunction in a setting of moderate HCM. However, the beneficial effect does not appear in severe HCM [49]. Thus, it appears important to make appropriate staging of the clinical symptomatology, hence the cardiac phenotype. Therapies that inhibit miR-34a alone may have limited potential in settings of established cardiac pathology [50]. For instance, miR-133, which is enriched in cardiac and skeletal muscle, is involved in cell specification, differentiation, and development. Furthermore, miR-a33 is
downregulated during cardiac hypertrophy. Specific knockdown of miR-133 via antisense targeting can be sufficient for inducing cardiac hypertrophy and reinduction of the foetal gene programme [51]. In the context of DCM it might be useful to induce a compensatory mechanism by reengaging the foetal gene programme. The miR-22 should also be considered as a critical regulator of cardiomyocyte hypertrophy and cardiac remodelling [52]. Systemic inhibition of miR-21 has proven effective against myocardial fibrosis and dysfunction [53].

Substantial advances in the understanding of the cellular and molecular basis of CMs and CHF highlight the potential utility of gene therapy as a novel therapeutic approach. However, successful clinical translation is still limited by the lack of safe, efficient, and selective delivery systems.

Naked DNA has remained the preferred method of gene delivery to the myocardium and has been explored extensively in clinical trials mainly in the setting of ischemic heart disease. The results from these trials have demonstrated efficacy with regards to secondary endpoints of reduced symptomatology, but have failed to establish significant increase in angiogenesis or an improvement in myocardial function [54].

3.4. Viral vectors for cardiac gene therapy

Viruses have evolved to become highly efficient at nucleic acid delivery to specific cell types while mostly avoiding immunosurveillance by an infected host. Several types of viruses, including retrovirus, adenovirus, adeno-associated virus (AAV), and herpes simplex virus, have been modified in the laboratory for use in gene therapy applications. Adenoviruses are an efficient gene delivery system in a broad range of cell and tissue types. However, the adverse immune reactions represent an important drawback for its development. Over time, multiple viral vector systems have been tested, but more recently AAVs have become most popular. AAVs are non-enveloped paroviruses, which can rather easily be engineered to deliver DNA cargo to target cells. AAV vectors have demonstrated good potential for in vivo delivery of genetic material into various cells, thus appearing as a vector of choice for different therapeutic applications beyond cardiac diseases. Nevertheless, and even if some promising clinical outcomes have been reported, the current potential of viral vectors for gene therapy still faces significant restrictions, largely due to manufacturing challenges, including the absence of an efficient and scalable platform purification process [25, 55–58]. At least in the setting of murine models, AAV1, AAV6, AAV8, and AAV9 have been identified as the most cardiotropic serotypes after systemic delivery.

The concept of gene therapy seems straightforward, but this is clearly an oversimplification, and numerous problems and risks exist that prevent gene therapy using viral vectors. Due to the structure of the viral particles, AAV vectors retain limited DNA cargo capacity necessitating the need to optimize the therapeutic sequence. Multiple cells can be infected by AAVs, but overall the transduction efficiency remains low leading to increased multiplicities of infection, hence putting greater pressure on large-scale vector production. Moreover, AAVs’ tropism lacks cell-type selectivity resulting in off-target transduction. Regulation of the transgene expression remains difficult and frequently results in decreased expression efficiency. Hence, to achieve optimal clinical outcome, high vector doses are required, but
the presence of preexisting neutralizing antibodies precludes a number of patients from participation. Furthermore, immune elimination of infected cells often limits gene expression in vivo. Readministration remains a major challenge, because single shot solutions are counterintuitive in the era of precision or personalized medicine. Further work is therefore needed to improve viral vectors, more specifically, developing stealthier AAV vectors with the aim of optimizing vector–host interactions [59–61]. Low-grade immune stimulation by the vector system appears as an important point in terms of drug development to avoid severe adverse reactions.

3.5. Non-viral vectors for cardiac gene therapy

Optimal gene therapy vectors should meet the following criteria: retaining the safety profile of naked DNA while displaying increased efficiency and decreased variability. From this perspective, non-viral methods of transfection present certain advantages such as relative ease of large-scale production, low risk of an adaptive immune response, versatility, and high safety profile.

Most of the non-viral vectors currently developed are based on polycationic molecules, which form interpolyelectrolyte complexes with the polyanionic nucleic acids. The complexes obtained generally allow for (1) efficient condensation of nucleic acids into small particles, (2) protection against degradation from nucleases, and (3) promotion of cellular uptake. These non-viral vectors usually consist of cationic lipids/liposomes (lipoplexes), cationic polymers (polyplexes), or a combination of both lipids and polymers (lipopolyplexes) [62,63].

Among these, vectors based on lipids are especially attractive due to the biocompatibility and biodegradability of lipids and phospholipids [64]. However, the toxicity displayed by cationic lipids, as well as the rapid clearance of positively charged lipoplexes, hampers further use in vivo of first-generation lipoplexes [64]. Coating the surface of lipoplexes, with hydrophilic polymers such as polyethylene glycol (PEG) can efficiently decrease their toxicity while increasing their circulation half-life [65]. Nonetheless, PEGylated lipoplexes often display reduced transfection efficiency due to diminished cellular uptake and can trigger anti-PEG IgM production, thus leading to accelerated blood clearance after readministration [66].

The tremendous diversity of shape, composition, and charge ratio of cationic polymers is a great asset when formulating polyplexes. Cationic polymers, which have been most widely used for cardiac gene delivery, include polyethylenimine, poly-(l-lysine), and dendrimers [67]. Despite their capacity to efficiently condense nucleic acids while preventing their degradation by nucleases and improving endosomal escape, the resulting in vivo gene expression remains too low and, for some of them, cytotoxic effects are detected [62,67,68].

Although non-viral vectors have dramatically improved over the past decades, they remain underrepresented for cardiac gene delivery. Further improvements to increase transfection efficiency while reducing their cytotoxicity are much needed.

From this perspective, polymers displaying few or no positive charges could be the much needed formulation for cardiac gene therapy [69]. Poloxamers, which are non-ionic amphiphilic
block copolymers, were first reported by Lemieux et al. [70] as efficient formulations for muscle gene delivery. Contrary to polycationic molecules, these delivery systems do not condense DNA into small particles and display no or weak interactions with nucleic acids [69,71]. Direct intramyocardial injection of poloxamer/DNA formulations showed no toxic effect towards the myocardium although gene expression remained limited and restricted to the injection site [71]. To increase the diffusion of poloxamer/DNA formulations into the myocardium, further experiments conducted in vivo on larger animals, through a clinically relevant administration route, were performed. As seen in Figure 3, this resulted in similar gene expression rate compared to that of the same transgene delivered using an AAV1 vector. To provide more insight into poloxamer-based delivery systems, further studies addressing their mechanism of action as well as experiments evaluating the possibility to readminister these formulations should be carried out.

The principal limitation of most non-clinical studies and some clinical trials was the inability to efficiently transfer genes to the cardiac ventricles. Although in vivo experiments using small animals may show efficient gene transfer, many fundamental differences exist between small animal and human hearts. Large animal studies are best suited for comprehensive evaluation at the preclinical stages of therapeutic development. It might seem obvious that delivery methods should meet all criteria of clinically relevant practices. Nevertheless, some preclinical methods seem to lack this realism.

3.6. Administration strategies

With regards to the first step to translate in vivo gene transfer into clinically relevant gene therapy and based at least partly on the use of naked DNA, physical methods like direct intra-myocardial injections have demonstrated feasibility, but also limited efficiency. Derived from these pioneering steps, several refinements have been introduced over time. In the context of rhythm control, one should look with interest to techniques like gene painting [72]. Gene painting refers basically to an innovative technique aimed at a very

Figure 3. Preclinical evaluation of intracoronary vector administration in large animals, for instance Beagle dogs. Similar amounts of cDNA were formulated differently. (A) Sample of a coronary contrast injection of the left main coronary artery in a dog heart highlighting the route of administration. (B) Mid-ventricular cross-section after AAV1 vectorization of a lacZ coding cDNA. (C) Mid-ventricular cross-section after polymer P85 vectorization of a lacZ coding cDNA. X-gal staining reveals lacZ gene expression (unpublished results).
local gene transfer by a topical administration. Proof-of-concept studies have shown the efficiency of this approach in atrial fibrillation. Strategies based on ultrasound-targeted microbubble destruction could be a promising method for gene delivery [73]. Microbubbles are small (<5 μm) gas-filled voids that are generally stabilized by phospholipids or synthetic polymers. The use of microbubbles as gene vectors is based on the paradigm that destruction of the DNA-loaded microbubbles by ultrasounds will result in local transduction and still spare non-target areas. Percutaneous antegrade coronary injections are among the least invasive cardiac selective gene delivery methods and are rather broadly available. Intracoronary delivery allows diffuse transduction throughout the myocardium, but as such it is a highly inefficient process. However, dense regional gene transfer (>80% of myocytes in the target territory) is possible. Pharmacological manipulations to induce vasodilation and maximize vascular permeability in a specific coronary perfusion territory can greatly improve transfection efficiency [74]. Given the high perfusion velocity and the submaximal extent of the vascular bed, one has to maximize the duration of vector exposure to the local vasculature while minimizing the systemic distribution. Several options have been tested such as pharmacologically induced coronary artery dilation, blocking the venous return or developing a cardiac recirculation approach. Delivery methods based on cardiopulmonary bypass (CPB) with a closed-loop system can be used for cardiac gene therapy [75,76]. It might seem excessive to selectively prescribe CPB for gene delivery given the clearly invasive nature of such a procedure. Nevertheless, one should not forget that many of the CM/heart failure patients might need invasive procedures due to their clinical condition. Gene therapy should also be evaluated in the context of combination therapies. CM/heart failure presents as a syndrome with multiple pathophysiological facets. Early treatment of some specific aspects like atrial fibrillation by gene therapy might be as efficient as conventional cardioversion. Targeting the autonomous nervous system through gene therapy should be evaluated with reference to current beta-blockers. Inotropism might be improved by means of additive gene therapy, for example. However, beyond the diversity of gene therapy targets, combination with more conventional drugs might be improved by reinforcing the target pathways.

4. Conclusions

Gene therapy is emerging as a suitable alternative, with substantial progress in preclinical models of cardiovascular disorders. Despite the fact that none of the clinical trials, which investigated new treatments for CMs, has met their primary efficacy endpoints, subanalysis, however, has demonstrated potential efficacy. Inadequate gene delivery remains one of the underlying causes behind failures seen in clinical trials. Higher transduction efficiency is needed to achieve therapeutic effects. Use of block copolymers in gene delivery is a promising area of research, in which new and important developments are expected.

CMs can serve as a disease model for several aspects when it comes to the development of gene therapy strategies in the context of cardiac diseases, since they also engulf inherited diseases like acquired disorders.
The emphasis on gene therapies was initially focused on inherited diseases notably rescuing cardiac phenotype by introducing a functional allele in the context of recessive disorders. Even gene therapies that would only help a couple of thousand people would be a remarkable achievement.

More recently the concept of gene therapy has been extended to a larger perspective, including the reprogramming of failing myocardial cells beyond inherited diseases. Several non-clinical studies have supported the concept, but the true challenge of gene therapy for CM remains translation into the clinic. Sticking to the old paradigm that a drug substance can only become a medicinal drug product, if one is able to formulate and administer it, it seems more obvious that gene therapy has to be clinically oriented. Treating the failing heart implies several strong constraints linked to the anatomy and physiology of the heart. Successful gene therapy approaches in other diseases support the notion, but cannot fully address the underlying specific challenges facing cardiac gene delivery.

The development of robust administration techniques and improved formulations are therefore needed before cardiac gene therapy can be integrated into the therapeutic armamentarium.

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Conflict of interest

The authors declare no conflict of interest.

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