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Review

Nanotechnology-driven heart targeting to fulfil the potential of cardiac microRNA therapies

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ABSTRACT

MicroRNAs play a crucial role in regulating gene expression in most biological processes, including cardiac disease, regeneration and aging. They are therefore an attractive and novel therapeutic option, and extensive research in small animal models has demonstrated this potential in the heart. While the therapeutic outcomes obtained in large animal models are also promising, far fewer studies have been conducted in this species. Even fewer microRNA therapeutics have reached clinical trials, only CDR132L and MRG-110. This highlights a bottleneck in their development primarily associated with challenges in their *in vivo* delivery, specifically due to their limited stability and ability to penetrate the heart tissue.

This review aims to describe the current state of development of cardiac microRNA therapies in relation to these challenges and to provide a comparative overview of the progress made in overcoming them. A special focus is set on the application of pharmaceutical nanotechnology and the development of targeted delivery strategies to the heart. These approaches have been tested primarily in small animal models and have shown to improve the cardiac penetration, accumulation and/or stability and of microRNAs and, therefore, their therapeutic efficacy compared to naked delivery. Notably, the latter is typically the method of choice in late preclinical research and early clinical trials. Consequently, targeted microRNA nanotherapies could be pivotal in overcoming the current bottleneck and realizing the therapeutic potential of microRNAs in the heart.

In conclusion, this review offers an outlook of the future applicability of microRNA therapies leveraging the latest advances in non-viral targeted delivery with nanocarriers.

1. Introduction: microRNAs in cardiac pathophysiology and therapeutic potential

MicroRNAs (miRs) are evolutionarily conserved small non-coding RNAs that regulate gene expression post-transcriptionally. miRs play a role in cardiac development and function. Importantly, they have been described to be dysregulated in heart disease (HD)-associated processes or ageing (Sheikh & Salma, 2024) and have a role in regeneration (Eulalio et al., 2012). Preclinical *in vitro* and *in vivo* research has demonstrated the contribution of specific miRs to cardiac hypertrophy, fibrosis, apoptosis or proliferation, among other pathological processes, in the context of cardiac disease, regeneration or aging. *Ex vivo,* a number of dysregulated miRNAs have also been reported in diseased or aged human cardiac tissue (Fig. 1a). Examples include upregulation of miR-34a (Boon et al., 2013) and MIR-24 (Ramos-Marquès et al., 2021) in aging; miR-132 in heart failure (HF) (Thum et al., 2007), miR-328, miR-24 and miR-206 in atrial fibrillation (AF) (Lu et al., 2010; Van Den Berg et al., 2023; Wang et al., 2019), and downregulation of miR-125-5p in the infarcted ventricle (Boštjančič et al., 2012).

The interest in the therapeutic potential of miRs has grown substantially as they offer a whole new spectrum of therapeutic possibilities to treat cardiac processes that have unmet medical needs, such as

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cardiac regeneration. The miR therapies can have two purposes: miR suppression, using anti-miR sequences, or miR replacement, using miR mimic sequences, to either inhibit or increase their levels, respectively (Fig. 1b). However, a number of factors related to the intrinsic molecular nature of miRs and their mechanisms of action have raised several hurdles to the development of effective miR therapies. Ensuring the miR therapies stability *in vivo* and delivering them not only to a given organ but also to a specific cell type of interest are major challenges in the field. Exploration and further development of recent advances in targeted drug delivery are likely to provide key opportunities to overcome these obstacles and unlock the full therapeutic potential of miR treatments.

This review aims to provide a comprehensive overview of the current state of cardiac miR therapy development. It focuses on the challenges encountered in the late preclinical research involving large animals and the progression of this research into clinical trials. Importantly, it carefully explores the application of advances in cardiac-targeted delivery with nanocarriers (NCs) to understand how to overcome current limitations and fulfil the therapeutic potential of miRs.

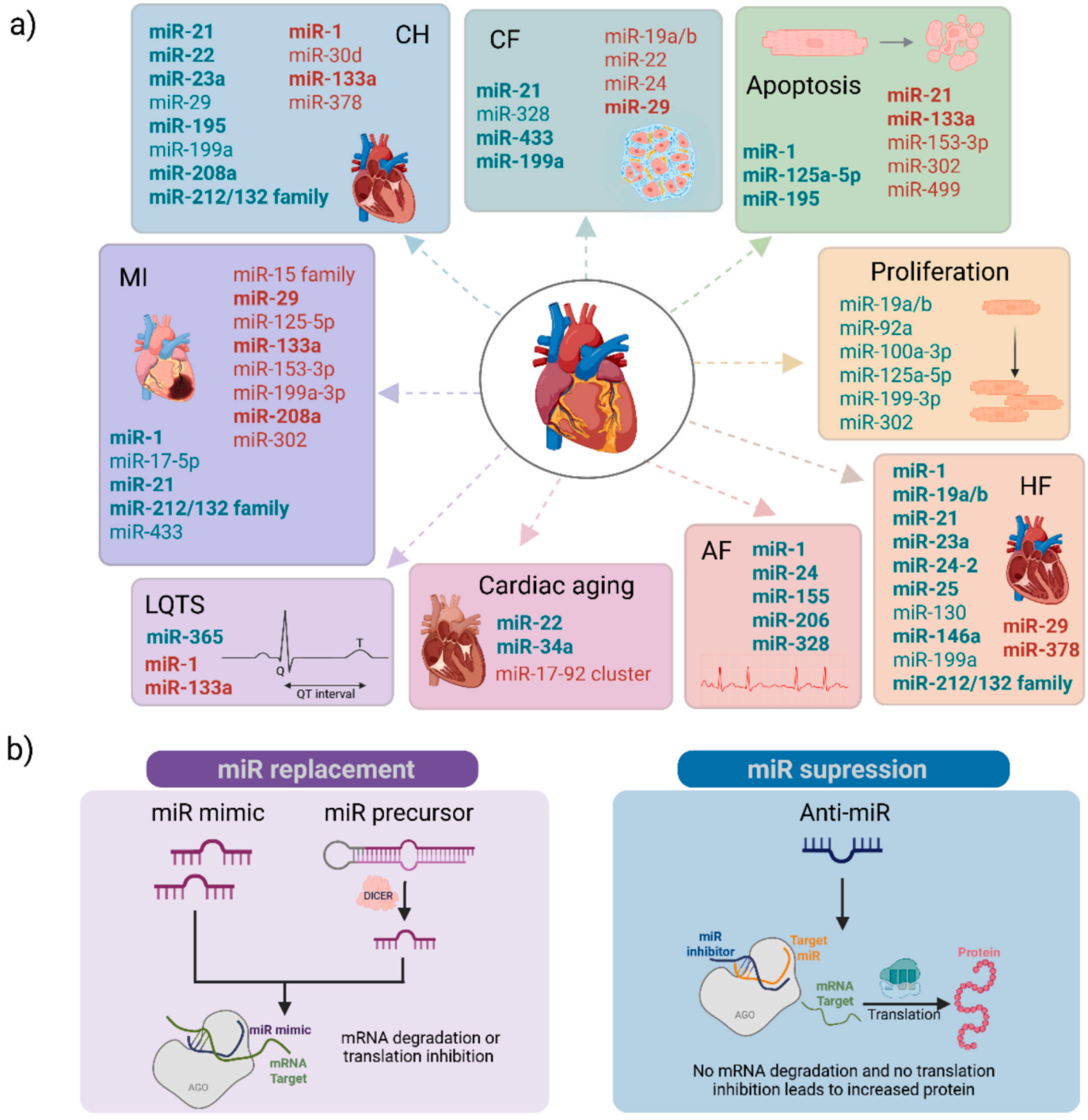


Fig. 1. Overview of role of miRs in cardiac disease and aging. (a) miRs involved in pathological processes, diseases and in cardiac aging in animal models and in humans. Upregulated and downregulated miRs are color coded in green and red letters, respectively. Bold letters represent those dysregulated in human tissue. CH: cardiac hypertrophy, CF: cardiac fibrosis, HF: heart failure, AF: atrial fibrillation, LQTS: long QT syndrome, MI: myocardial infarction, miR: miRNA. b) Representation of the types of miR therapies including miR replacement (left) and miR suppression therapies (right). Created in BioRender. https://BioRender.com/k4fge52.

2. Challenges of cardiac therapy with miRs

Despite its potential, cardiac miR therapeutics is still in its infancy. To understand this scenario (described in next sections) it is important to comprehend the main bottlenecks in their development (Fig. 2).

To be effective, miR therapies must overcome a number of biological barriers. They must resist nuclease degradation in the blood and extracellular media, avoid rapid renal clearance and immune elimination, be able to cross the capillary endothelium of the target organ, and finally cross the plasma membrane of the target cell to reach the cytoplasm, where they must be present in sufficient amount to specifically exert their regulatory function. The intrinsic physicochemical and biological properties of miRs pose difficulties throughout this process, leading to defective intracellular delivery and thus lack of efficacy, the most common cause of clinical trial termination of RNA therapies (Winkle et al., 2021).

The persistence of miR therapies in the organism is determined by their stability and rate of clearance. Their negative charge and hydrophilicity limit the efficiency of the vascular extravasation and cellular uptake at the anionic phospholipid bilayer of the cell membrane. Taken together, the cellular uptake ratio of miR therapies is low and the circulating half-life is short. Thus, systemic delivery of miR therapies faces a major obstacle in tissue penetration and achieving a sufficient on-target dose. In addition, the continuous vasculature of the heart makes the efficiency of cardiac uptake lower than in other organs with fenestrated or discontinuous vessels, such as the kidney, liver, or spleen. These hurdles cannot simply be overcome by using higher doses of miR, as this could lead to renal or hepatic toxicity (Bernardo et al., 2015) or other so far unknown side effects.

Side effects could be triggered by (i) saturation of the endogenous RNA interference (RNAi) processing machinery, altering the normal function of endogenous miRs (Grimm et al., 2006; Khan et al., 2009); (ii) off-target activity and/or (iii) on-target activity in undesired organs. The intracellular dose of the miR therapy may be critical to avoid undesired perturbations of its natural targetome. Preclinical studies to assess the optimal effective doses without undesired alterations of the miR targetome seem to be fundamental to ensure future clinical translation. Sequence-mediated off-targeting, the ability of miRs to simultaneously regulate hundreds to thousands of genes, has been suggested as a possible cause of side effects observed in clinical trials of discontinued miR therapeutics (Zhang et al., 2021).However, miR therapies are particularly interesting for those conditions with complex mechanisms where exploiting the miR pleiotropy may offer an advantage over conventional drugs with a single molecular target. Specifically, a given miR can regulate multiple genes and thus potentially control multiple functional processes. For example, it has been reported that the miR-15a-16-1 cluster, directly or indirectly, can regulate approximately 14% of the total transcriptome in leukemic cells (Calin et al., 2008). Therefore, leveraging their ability to regulate entire pathways and a variety of processes with a single intervention is highly valuable for treating intricate pathological processes. Undesired on-targeting occurs when a miR-based treatment simultaneously acts through its specific targets in unwanted organs or in non-target cell types within the desired organ. For example, a liposomal miR-34a mimic for cancer therapy (MRX34) showed undesired on-target effects in immune cells. It led to severe immune-related adverse effects and to the termination of clinical trials (Hong et al., 2020). In the heart, miRs can also have cell type-specific effects and responses (Dai et al., 2020; Liu et al., 2021). For instance, miR-29 has been described as anti-fibrotic in cardiac fibroblasts (Van Rooij et al., 2008), but pro-hypertrophic and pro-fibrotic in cardiomyocytes (CMs) (Sassi et al., 2017). To split hairs, even the 5p and 3p strands of a given miR can have opposite effects, one being associated with therapeutic effects and the other with pathological effects (i.e., miR-199a discussed in Section 3.2.1).

To diminish the potential side effects and enhance efficacy, local delivery of miR therapies has been applied successfully compared to systemic injection (Chen et al., 2015). This holds true also for the heart via intramyocardial (IM) or intracoronary (IC) administration (Hinkel et al., 2013). However, local administration did not prevent the miR therapies from being effective in extracardiac organs (Hinkel et al., 2020). In view of all the hurdles identified, strategies aimed at optimizing the delivery of the therapeutic miR in the heart/cell type of

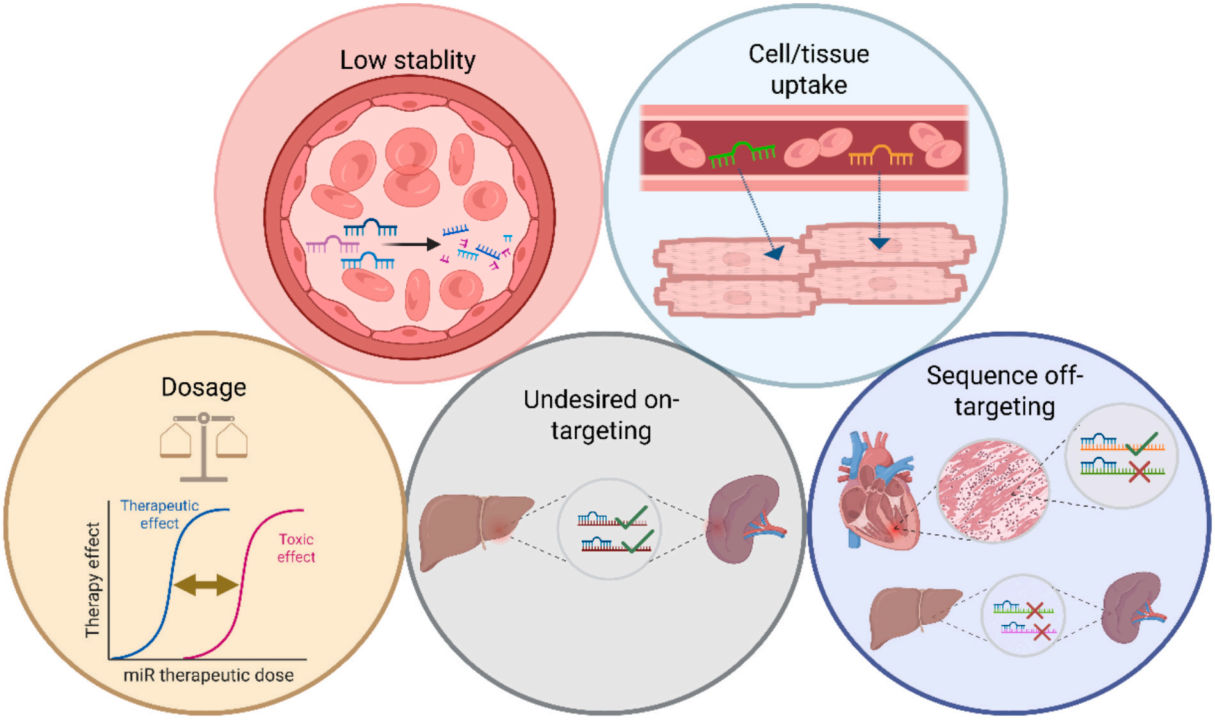


Fig. 2. Challenges of cardiac miR therapies. On-targeting and off-targeting are represented with a green tick and red cross, respectively. Created in BioRender. https://BioRender.com/g5ojun7.

interest, combined with strategies to improve their specificity (i.e. by promoting RNA-induced silencing complex (RISC) loading of the guide strand or applying chemical modifications to minimize sequence-mediated off-targeting (Bereczki et al., 2025)), are currently in development. In this review, we focus in the challenge of delivery and the progresses made to overcome it, including the encapsulation of miR therapies in NCs and developing strategies promoting targeted delivery to the heart.

3. Current developmental status of cardiac miRs therapy: late preclinical trials

Since the first reports in the first decade of the 2000 s investigating the role of miRs in HD (i.e., HF (Van Rooij et al., 2006; Yang et al., 2007)), their potential as therapeutic targets has been attractive (Van Rooij & Olson, 2007). After 20 years of research, the body of evidence is large enough to warrant recognition of their active contribution to HD, but the translation of this knowledge into clinical applications has yet to occur (Fig. 3). The aforementioned challenges together with interspecies differences most likely explain why the percentage of miR drugs reaching clinical trials is minimal compared to those that have shown therapeutic effects in preclinical studies. In fact, none have reached phase III clinical trials (S. Zhang et al., 2021). Currently, miR therapies are mainly at the discovery or preclinical testing phases, with most studies conducted so far in small animal models. Relatively few are evaluating their therapeutic efficacy in large animal models and, to date, only two miR therapies have entered clinical trials for the treatment of HD (CDR132L and MRG-110).

Next, we describe key miRs involved in HD and summarize their specific stages of development. The focus is set on those with a higher preclinical level of development (efficacy assessed in large animals), highlighting important aspects related to the challenges faced by the miR therapies (Table 1, Section 3.1). In addition, we review the miR therapies that have entered clinical trials for HD, but also those for non-cardiac pathologies with potential application in HD (Table 2, Section 3.2).

3.1. miR therapies assessed in large animal models (Table 1)

The miR-92a, a member of the mir-17–92 cluster, is upregulated in ischemic mouse heart tissue. Its inhibition demonstrated its capacity to regulate the ischemia-induced angiogenesis *in vivo* in mice (Bonauer et al., 2009). However, the cell compartment involved in this effect is unclear since, in agreement with its cell-type specific effect (Rogg et al., 2018), the inhibition of miR-92a was not only detected in endothelial cells (Bonauer et al., 2009). In large animal models of cardiac ischemia, miR-92a inhibition promoted neovascularization through its proangiogenic effect leading to cardiac functional and structural improvements (Bellera et al., 2014; Hinkel et al., 2013). Locked nucleic acid (LNA)-anti-miR-92a administered intravenously (IV) or IC showed efficacy only with IC (Hinkel et al., 2013), providing the first evidence of the higher miR therapeutic efficacy of local over systemic administration. However, undesired on-targeting in extracardiac organs was still present in both administration routes. Instead, a single IC injection of the anti-miR-92a encapsulated in albumin-polylactic-co-glycolic acid (PLGA) microspheres in ischemic pigs avoided the organ off-targeting while it maintained efficacy by restoring the LV ejection fraction (LVEF) up to 51.4% compared to 36.7% in untreated animals (Bellera et al., 2014).

The miR-21 pathological effect is complex due to its cell-type and CVD-dependent effect (Dai et al., 2020; Surina et al., 2021). miR-21 is described as pro-fibrotic and pro-hypertrophic in CVD (Cheng & Zhang, 2010) and to be upregulated in the LV myocardium in patients of HF (Thum et al., 2008) or myocardial infarction (MI) (Van Rooij et al., 2008). However, it has also been reported to exert cardioprotective effects in ischemia by enhancing angiogenesis and myocardial remodeling (Bejerano et al., 2018; Cheng & Zhang, 2010; Liu et al., 2015). Repeated IC infusion of LNA-anti-miR-21 in pig models of ischemic HF was anti-fibrotic and anti-hypertrophic and improved the cardiac function by enhancing the LVEF up to ~35% compared to untreated animals (~25%) (Hinkel et al., 2020). However, there was also downregulation of miR-21 in kidney and lung, raising safety concerns related to organ off-targeting even with local administration. In mimic approaches, ultrasound-targeted microbubble destruction (UTMD) with pre-mir-21-

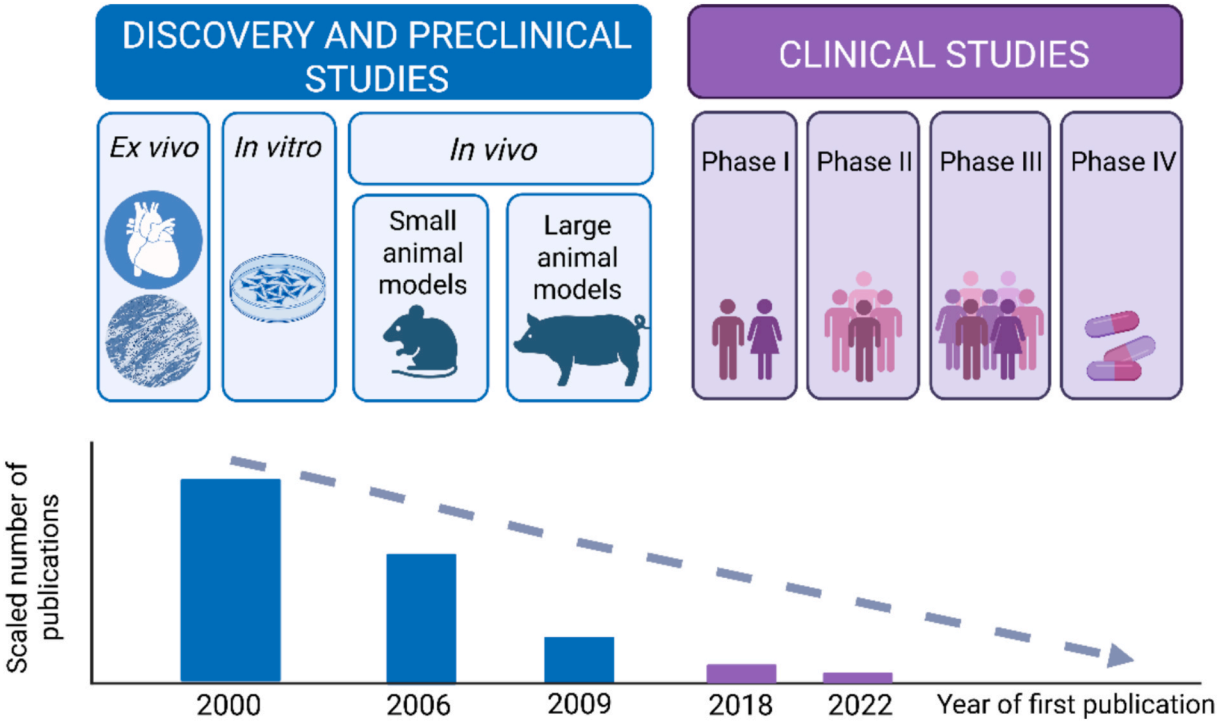


Fig. 3. Representation of the course of miR-related research in HD and its progress into clinical investigations. The bar graph represents a figurative scaled number of publications and the year of the first publication on each phase. Created in BioRender. https://BioRender.com/ohtbdzt.

Table 1
Preclinical efficacy evaluation of miR therapies in large animal models.

miR	Sp.	Disease model	Therapeutic molecule	Vehicle	Administration route/time course	Main findings	Undesired side effects	Ref.
miR-92a	Pig	Ischemia (LAD occlusion 60 min)	LNA-anti-miR-92a	Naked	IV, IC, 5 min before reperfusion	Structural and functional improvement only with IC	miR-92a expression decreased in liver, spleen and kidney (lower by IC administration)	(Hinkel et al., 2013)
	Pig	Ischemia (LAD occlusion 49 and 60 min)	Anti-miR-92a	Albumin-PLGA microspheres	IC, 5 min after reperfusion in 2 injections	Structural and functional improvement	Microspheres not present in spleen, lung and liver, no suppression of miR-92 in remote organs No proarrhythmic effect	(Bellera et al., 2014)
miR-21	Pig	Ischemia (LAD occlusion, 60 min)	LNA anti-miR-21	Naked	IC, d5 and d19 after reperfusion	Structural and functional improvement	Downregulation of miR-21 in kidney and lung	(Hinkel et al., 2020)
	Pig	CME model	Pre-miR-21 plasmid	Microbubbles for UTMD	IV, d4 before CME treatment	Functional improvement Size of CME-induced infarct not greatly reduced	Not assessed	(Su et al., 2015)
	Pig	Ischemia (OM1 and OM2 of the circumflex artery ligation)	miR-21 mimic	MSN and injectable hydrogel matrix	IM, immediately post-MI in 6 injection sites	Local, controlled and long half-time delivery (28 d) Long-term safety risk Structural and functional improvement	Not assessed	(Li et al., 2021)
miR-132	Pig	Ischemia (LAD occlusion 90 min)	CDR132L	Naked	IV, monthly during 3 or 5 months starting 1 month after ischemia	Safe and well-tolerated and structural and functional improvement	No therapy-related adverse events (body weight, electrocardiographic or hematology or laboratory chemistry parameters)	(Batkai et al., 2020)
	Pig	Ischemia (LAD occlusion 90 min)	Anti-miR-132	Naked	IC or IV d3 post-MI and IV d28	Safe, well-tolerated and favorable PK. Dose-dependent PK/PD relationships Structural and functional improvement	No therapy-related adverse effects (hematology or laboratory chemistry parameters and myocardial damage)	(Foinquinos et al., 2020)
	Pig	HF (pTAC stent model, LV pressure overload)	Anti-miR-132	Naked	IC, d0 and d28 after aortic stent implantation	Structural and functional improvement	Not assessed	(Hinkel et al., 2021)
miR-199a-3p	Pig	Ischemia (LAD occlusion 90 min)	pri-mir-199a-1 gene	AAV	IM, 10 min after reperfusion	Structural and functional improvement	Not assessed	(Gabisonia et al., 2019)
miR-125a-5p	Pig	Ischemia (LAD occlusion 60 min)	miR-125a-5p mimic	Naked	IM, at the onset of reperfusion	Persistence of miR-125a-5p for up to 28 days after cardiac I/R Structural and functional improvement	No hepatic, renal or cardiac toxicity (blood chemistry indicators) Not increase the burden of arrhythmia	(Gao et al., 2023)
miR-19a/b	Pig	Ischemia (LAD occlusion, 10 min)	miR-19a/b mimic	ROS-scavenging hydrogel	IM, immediately post-MI in 9 injection sites	Structural and functional improvement	Not assessed	(Wang et al., 2025)
miR-146b-5p	Pig	Ischemia (left circumflex artery ligation, time not specified)	Anti-miR-146b-5p	Naked	IM, after ligation	Functional improvement Short term inhibition of targets	Not assessed	(Liao et al., 2021)
miR-328	Dog	AF (right A-TP during 8 weeks)	Anti-miR-328	AAV	Intramuscular, after A-TP in 10 injection sites	Structural and functional improvement	Not assessed	(Lu et al., 2010)
miR-206	Dog	AF (right A-TP during 4 weeks)	Anti-miR-206	Lentivirus	Intramuscular after A-TP in 8 injection sites	Structural and functional improvement	Not assessed	(Zhang et al., 2015)

AAV: adeno-associated virus, AF: atrial fibrillation, A-TP: atrial tachypacing, CME: coronary microembolization, d: day, IC: intracoronary, HF: heart failure, I/R:ischemia/repefusion, IC: intracoronary; IM: intramyocardial, IV: intravenous, LAD: left anterior descending artery, LNA: locked nucleic acid, LV: left ventricle, min: minutes, N/A: not applicable, OM: obtuse marginal, PK/PD: pharmacokinetics/pharmadynamics, PLGA: Poly(lactic-co-glycolic) acid, pTAC: Percutaneous transverse aortic constriction, ROS: reactive oxygen species, Sp.: species, Ref.: reference, UTMD: ultrasound-targeted microbubble destruction, w:week.

Table 2
MiR therapies in clinical trials with potential application in cardiac pathologies.

	Preclinical phase			Clinical phase					
	Specie	Indication	Refs.	Therapeutic molecule	Sponsor	Admin./ Vehicle	Trial phase, subjects and NCT number	Status and main reported results	Cardiac indication
miR-92	Mouse Pig	Cardiac ischemia	(Bellera et al., 2014; Bonauer et al., 2009; Hinkel et al., 2013)	LNA-anti-miR-92a (MRG-110, S95010)	miRagen Therapeutics and Servier	IV, naked	Phase I, healthy volunteers (NCT03494712)	Completed: safe, tolerable and molecularly effective (Abplanalp et al., 2020)	Cardiac ischemia (Bellera et al., 2014; Hinkel et al., 2013)
	Mouse Pig	Skin wound healing	(Gallant-Behm et al., 2018; Lucas et al., 2017)	LNA-anti-miR-92a (MRG-110, S95010)	miRagen Therapeutics and Servier	ID, naked	Phase I, healthy volunteers (NCT03603431)	Completed: safe, promoted skin angiogenesis and wound healing (Viridian Therapeutics Inc, 2019)	
miR-21	Mouse	Alport nephropathy	(Chau et al., 2012; Gomez et al., 2014; Rubel et al., 2022)	Anti-miR-21 modified with sugar moieties and phosphorothioate (RG012, Lademirsen, SAR339375)	Genzyme, a Sanofi Company	SC, naked	Phase I, Alport syndrome patients (NCT03373786)	Completed: without results	Ischemic HF (Hinkel et al., 2020; Thum et al., 2008) MI (Li et al., 2021; Van Rooij et al., 2008) and myocarditis (Mirna et al., 2022)
							Phase II, Alport syndrome patients (NCT02855268)	Terminated (Gale et al., 2024)	
miR-132	Pig	HF	(Batkai et al., 2020; Foinquinos et al., 2020; Hinkel et al., 2021)	LNA-anti-miR-132 (CDR132L)	Cardior Pharmaceuticals GmbH	IV, naked	Phase I, ischemic HF patients with reduced LVEF (NCT04045405)	Completed: safe, tolerable, favourable PK and moderate effect (Taubel et al., 2021)	HF (Batkai et al., 2020; Foinquinos et al., 2020; Hinkel et al., 2020; Thum et al., 2007)
							Phase II, HF patients with preserved LVEF (NCT05350969)	Active (not recruiting)	
							Phase II, HF patients with mild reduced/preserved LVEF (NCT05953831)	Withdrawn	
miR-29	Mouse Rat Rabbit	Fibrotic scar (keloid)	(Gallant-Behm et al., 2019)	Cholesterol-conjugated miR-29 mimic (MRG-201, Remlarsen)	miRagen Therapeutics	ID, naked	Phase I, healthy volunteers (NCT02603224)	Completed: safe and tolerable (Gallant-Behm et al., 2019)	MI (Van Rooij et al., 2008)
	Mouse	Pulmonary fibrosis	(Montgomery et al., 2014)				Phase II, keloid patients (NCT03601052)	Completed: reduced fibroplasia with normal wound healing (miRagen Therapeutics Inc, 2018)	
miR-17-5p	Mouse	ADPKD	(Hajarnis et al., 2017)	LNA-anti-miR-17-5p (RGLS4326)	Regulus Therapeutics	SC, naked	Phase Ib, APKD patients (NCT04536688)	Completed: clinical proof of mechanism, but neurological toxicity	I/R (Du et al., 2014), MI (Chen et al., 2022) and HCM (Shi et al., 2019)
				LNA-anti-miR-17-5p (RGLS8429, Farabuantirsen)		SC, naked	Phase I, healthy volunteers (NCT05429073)	Completed: without results	
							Phase Ib, ADPKD patients (NCT05521191)	Recruiting, preliminary results announce therapeutic effect (Regulus Therapeutics, 2025)	
miR-155	Mouse	DLBCL	(Anastasiadou et al., 2021)	LNA-ant-miR-155 (MRG-106, Cobomarsen)	miRagen therapeutics	SC and IV, naked	Phase I, lymphoma and leukemia patients (NCT02580552)	Completed: safe, therapeutic effect (Foss et al., 2018; Querfeld et al., 2018)	HF (Zhang et al., 2025), AF (Wang et al., 2015; Zhang et al., 2025) and HCM (Shi et al., 2019)
						IV, naked	Phase II, cutaneous T-cell Lymphoma/mycosis fungoides patients (NCT03713320)	Terminated	
						IV, naked	Phase II, cutaneous T-cell Lymphoma/mycosis fungoides patients (NCT03837457)	Terminated	

Admin.: administration, ADPKD: Autosomal Dominant Polycystic Kidney Disease, AF: atrial fibrillation; CHO: cholesterol; DLBCL: Diffuse large B-cell lymphoma, HCM: hypertrophic cardiomyopathy, HF: heart failure, I/R: ischemia/reperfusion, ID: intradermal, IV: intravenous, LNA: locked nucleic acid, LVEF: left ventricle ejection fraction, MI: myocardial infarction, PK: pharmacokinetic, Refs: references, SC: subcutaneous.

loaded microbubbles in pig models of coronary microembolization (CME)-induced inflammation, showed functional improvement without structural recovery with systemic treatment (Q. Su et al., 2015). IM-injected hydrogels harboring miR-21-loaded mesoporous silica NCs (MSN) in ischemic pigs demonstrated the cardioregenerative and cardioprotective role of the miR by increasing the LVEF up to ~50% compared to untreated MI animals (~40%) (Li et al., 2021). Therefore, the dual role of miR-21 in cardiac pathophysiology demands a careful therapeutic use of its related therapies in very well-defined pathological conditions.

HF patients presented upregulated myocardial expression of miR-132 (Thum et al., 2007). The role of miR-132 in cardiac hypertrophy and adverse myocardial remodeling along with its therapeutic potential was then demonstrated in mouse models of MI and HF (Chen et al., 2019; Foinquinos et al., 2020; Ucar et al., 2012). In pig models of ischemia, the anti-miR-132 treatment led to tolerability and safety as well as to cardiac functional and structural improvements up to 43.8% of LVEF (over 35% in untreated animals) after IV or IC followed by IV administrations using different dosing regimens of up to 5 months (Batkai et al., 2020; Foinquinos et al., 2020). In this case, the administration pathway resulted in comparable therapeutic effect, validating the IV administration scheme. No therapy-related adverse effects were reported, but the expression levels of miR-132 were not investigated in other tissues despite of miR-132 having a well-known role in neurological disorders (i.e., Alzheimer disease (Walgrave et al., 2023) or cancer (Rafat et al., 2021). Comparable therapeutic effects were observed in a porcine model of LV pressure overload-induced HF after 8 weeks, where the anti-miR CDR132L was administered twice IC with 4-weeks interval between administrations, achieving 48.9% LVEF over 36.1% in untreated HF animals (Hinkel et al., 2021). Despite of the promising beneficial effects, and the lack of apparent side effects, full recovery was not achieved (52% LVEF in sham operated animals) independently of the administration route or dosing program, suggesting that prolonged treatments may be needed.

Following a high-throughput functional screen, miR-199a-3p was found to promote murine CM proliferation *in vitro* and to induce cardiac regeneration with functional and structural improvement after infarction in mice (Eulalio et al., 2012). Similar therapeutic outcomes were reported in infarcted rats and pigs with miR-199a-3p treatment (Gabisonia et al., 2019; Yang et al., 2019) holding promise for the cardiac regeneration in humans. In pigs, LVEF was restored up to ~65%, closer to untreated healthy animals (~72%) after single IM injection of an adenoassociated virus (AAV6)-delivered pri-miR-199a-1 gene. However, these results must be taken with caution, as the persistent expression of the pre-miR led to arrhythmic events and sudden cardiac death in pigs (Gabisonia et al., 2019). Whether this lethal outcome is explained by the persistent expression of the pre-miR-199a and/or the co-expression of the miR-199a-5p strand, remains unknown. In support of the temporal hypothesis and the potentially safer profile of transient miR-based regenerative treatments, a methodologically comparable study with miR-125-5p mimic (Boštjančič et al., 2012) obtained similar beneficial effects without therapy-related arrhythmogenic events (Gao et al., 2023). On the other hand, the effect of the miR-199a-5p passenger strand in the heart is unclear. It has been associated with pathological processes where it is both upregulated, i.e., in murine models of cardiac hypertrophy and fibrosis and MI (Asensio-Lopez et al., 2021; Song et al., 2010; Yan et al., 2017; Zeng et al., 2021), and downregulated, in murine models of MI and patients of DCM and other cardiac pathologies (Chen et al., 2024). Indeed, anti-miR-199a-5p hard decoys revealed its important role in cardiac hypertrophy in mice (Yan et al., 2021), while IM supplementation with miR-199a-5p mimic promoted cardiac regeneration in infarcted rats (Chen et al., 2024). In any case, these studies highlight the relevance of transient and strand-specific delivery of miR mimic therapies to minimize potential side effects due to persistent exposure to the therapy and/or active passenger strands.

All these studies, mostly focusing on cardiac regeneration, provide robust evidence for the efficacy of the miR therapies in translational models of cardiac disease. However, they also highlight the potential disadvantages of miR therapies and the remaining challenges to overcome, mainly those related to safety, controlled and targeted delivery and dosage. Despite the apparent superiority of IC infusion or IM injection in terms of efficacy and reduced adverse effects when compared with systemic administration, these routes are limited in their clinical applicability since multiple administrations to achieve sustained therapeutic effects and full recovery may be needed. The serious adverse effects observed for the AAV-delivered miR-199a-3p suggest that the therapeutic potential of miRs will require meticulous regulation of the local dose and duration of the treatment, as well as the provision of only the therapeutic strand of the miR, ideally with a cell-type-specific delivery strategy. However, the delivery of miRs in a heart-, cell-, transient- and/or strand-specific manner is beyond the capabilities of naked or viral delivery. As outlined in Section 4, recent advancements in targeted drug delivery employing non-viral vectors hold great potential for the advancement of miR therapies into clinical phases, with the prospect of achieving efficacy and safety.

3.2. *miR therapies in clinical trials with potential in cardiac therapy (Table 2)*

The development status of cardiac miR therapies is still concentrated in the preclinical phase, but a few miR therapies for cardiac and non-cardiac diseases have entered clinical trials (Table 2). Next, we will describe them including those for non-cardiac diseases that may be applicable to HD upon adequate cardiac delivery, namely miR-92, miR-21, miR-17-5p and miR-155.

One of the miR therapies developed for the treatment of HD that entered clinical trials is CDR132L, an anti-miR-132 that was developed for the treatment of ischemic HF (Batkai et al., 2020). CDR132L has completed a phase I clinical trial (NCT04045405) with patients of ischemic HF and its systemic administration has shown good tolerability and safety, favorable pharmacokinetics (PK) and preliminary evidence of therapeutic efficacy (Täubel et al., 2021). A Phase II trial evaluating efficacy and safety in HF with reduced LVEF after MI (NCT05350969, HF-REVERT) is currently active, but not recruiting (Bauersachs et al., 2024). Another Phase II trial evaluating the efficacy and safety of CDR132L in patients with HF with mildly reduced or preserved LVEF and cardiac hypertrophy (NCT05953831, REMOD-REVERT) has been withdrawn due to transfer of study responsibility and planning.

miR-92 has a role in promoting wound healing (Gallant-Behm et al., 2018; Lucas et al., 2017) and in endothelial dysfunction and atherosclerosis (Loyer et al., 2014), suggesting that its inhibition may promote angiogenesis/revascularization in the CV system and skin (Gallant-Behm et al., 2018). Other miR therapy developed for HD treatment that entered in clinical trials is MRG-110 or S95010. Two Phase I clinical trials in healthy volunteers (NCT03494712 and NCT03603431) were designed to test the safety and efficacy of this LNA-anti-miR-92a for the treatment of ischemic conditions such as HF and skin wound healing, respectively. In NCT03494712, MRG-110 was administered IV in healthy volunteers in single ascending doses, demonstrating safety and tolerability (Abplanalp et al., 2020). For its potential application in skin wound healing, NCT03603431 was also carried out in healthy volunteers but administered by intradermal injection at the site of small skin wounds for 3 weeks. The treatment was safe and preliminary evidence of therapeutic effects related to the promotion of angiogenesis and improvement of wound healing were obtained (Viridian Therapeutics Inc., 2019). Altogether, MRG-110, due to its systemic safety (NCT03494712) and pro-angiogenic effect (NCT03603431) in humans demonstrated by preclinical evidence demonstrated by anti-miR-92 efficacy in small and large animal models of MI (Bellera et al., 2014; Bonauer et al., 2009; Hinkel et al., 2013), could be further considered a candidate for the regeneration of the ischemic heart.

The miR-21 is pro-fibrotic in lung (Liu et al., 2010), kidney (Chau

et al., 2012; Gomez et al., 2014; Rubel et al., 2022) and in liver diseases (Zhang et al., 2020), has a pro-inflammatory role in immune-related diseases (Dong et al., 2014) and a pro-proliferative and anti-apoptotic role in cancer (onco-miR) (Iorio & Croce, 2009). An anti-miR-21 modified with sugar moieties and phosphorotioate (RG-012, Lademirsen or SAR339375) that showed preclinical efficacy in Alport nephropathy models (Gomez et al., 2014), entered Phase I (NCT03373786) and Phase II (NCT02855268) clinical trials in patients with Alport syndrome. Phase I results were not reported, but subcutaneous administration in Phase II showed an acceptable safety profile with no significant improvement in renal function (Gale et al., 2024). Whether the reported plasma concentrations of RG-012, which accumulated less than 2-fold over the duration of the trial, were insufficient to achieve therapeutic effects remains unknown. However, based on the safety profile in NCT02855268, the preclinical efficacy results in large animals (Hinkel et al., 2020) and, supposing that the functional role of miR-21 in HD is properly elucidated, RG-012 could be a candidate to treat ischemic HF (Hinkel et al., 2020; Thum et al., 2008), MI (Li et al., 2021; Van Rooij et al., 2008) or myocarditis (Mirna et al., 2022).

The miR-29 is downregulated during lung, liver and kidney fibrosis (Deng et al., 2017). A cholesterol conjugated miR-29 mimic (MRG-201, Remlarsen) effective in preclinical trials (Gallant-Behm et al., 2019; Montgomery et al., 2014), completed a Phase I clinical trial (NCT02603224). Single or multiple ascending doses of miR-29 mimic were injected intradermally into healthy volunteers with and without skin incisions, demonstrating safety and tolerability (Gallant-Behm et al., 2019). A subsequent Phase II study in patients with a history of keloid (NCT03601052) showed fibroplasia reduction compared to placebo, but no effect on normal wound healing (miRagen Therapeutics Inc., 2018). In the context of HD, miR-29 has been described as an anti-fibrotic miR that is downregulated after MI due to cardiac injury, particularly in the region adjacent to the infarct zone in human LV myocardial samples (Van Rooij et al., 2008). Therefore, miR-29 supplementation therapy could be a candidate miR therapy with application in the treatment of MI-related cardiac fibrosis. However, as for miR-92 and miR-21 (Dai et al., 2020; Rogg et al., 2018; Surina et al., 2021), its application requires thorough evaluation considering the cell-specific effects described in preclinical (Sassi et al., 2017; Van Rooij et al., 2008).

The miR-17-5p was upregulated in renal cysts of both mice and humans with autosomal dominant polycystic kidney disease (ADPKD) (Hajarnis et al., 2017). A LNA-anti-miR-17-5p (RGLS4326 or RG4326) evaluated preclinically in ADPKD models, completed a Phase I clinical trial (NCT04536688) with ADPKD patients. The trial provided clinical proof of functionality, but also of toxicity to the central nervous system at higher doses (Xue et al., 2025). Subsequently, a second-generation LNA-anti-miR-17-5p (Farabursen or RGLS8429) also showed efficacy, but without neurological adverse effects (Regulus Therapeutics, 2021) and Phase I (NCT054269073) or Ib (NCT05521191) trials in healthy volunteers or ADPKD patients, respectively, started. The company announced that preliminary results found RGLS8429 efficient in NCT05521191 (Regulus Therapeutics, 2025). In the context of HD, miR-17-5p is upregulated in mouse models of ischemia/reperfusion (I/R)-induced cardiac injury (Du et al., 2014), MI (Chen et al., 2022) and HCM (Shi et al., 2019), and its downregulation has demonstrated therapeutic effects in both ischemia models. Thus, RGLS8429 could potentially be a candidate treatment for these cardiac pathologies (Du et al., 2014; Shi et al., 2019).

The miR-155 is involved in the pathogenesis of many types of hematological malignancies, but it has a strong causative role in the development of diffuse large B-cell lymphoma (Ranganath, 2015). A LNA-anti-miR-155, (Cobomarsen or MRG-106) (Seto et al., 2018) safe and effective in preclinical models of lymphoma (Anastasiadou et al., 2021), completed a Phase I trial with lymphoma and leukemia patients (NCT02580552). The subcutaneous or systemic injection of the LNA-anti-miR-155 showed therapeutic efficacy without serious adverse effects (Foss et al., 2018; Querfeld et al., 2018). Subsequently, two Phase II clinical trials in cutaneous T-cell lymphoma/mycosis fungoides subtype were terminated: The SOLAR trial (NCT03713320) because of business reasons although the results showed safety and low efficacy, and the PRISM trial (NCT03837457) because of a crossover with an arm of the SOLAR clinical trial. Noteworthy, the miR-155 has been described to be upregulated during inflammation, atherosclerosis and AF in animal models (reviewed in (Zhang et al., 2025)) and in the atria of AF patients (Wang et al., 2015). Therefore, clinical advancements in the development of Cobomarsen could potentially have an application in the treatment of these CV pathologies.

To sum up, if compared to the extensive preclinical research *in vivo*, only a limited number of miR-based clinical trials have been conducted for cardiac and non-cardiac diseases. Consistent with the preclinical research in large animals (Table 1), but even more pronounced, anti-miR therapies predominate over the miR mimic-based therapies in the clinical phase of development (Table 2), which in turn indicates that the miR supplementation therapies are lagging behind. Overall, all but one of the clinical trials demonstrate safety and tolerability of miR therapies. However, they also show limited therapeutic effect. Considering the unfavorable PK profiles imposed by the intrinsic properties of miR therapies (characterized by rapid systemic clearance, limited tissue retention, and inefficient cellular uptake), it is unclear whether this general clinical outcome, with all treatments delivered as naked miR/anti-miR molecules, could result from subtherapeutic intracellular doses and limited pharmacodynamic (PD) effects in target organs. It is also unclear how the route of administration and/or the delivery method could address these aspects in humans. Nevertheless, as suggested by the efficacy improvements of local and/or encapsulated delivery in large animal models (Table 1), the advances of targeted miR delivery are likely to have a positive impact on the clinical outcome of HD miR therapies.

4. The challenge of *in vivo* miR delivery

The *in vivo* delivery method most commonly used so far in late preclinical or clinical stages of miR therapeutics is naked delivery, with some studies using viral vectors (Tables 1 and 2). Drug encapsulation in nanostructures emerged as a promising approach to overcome most of the limitations of conventional gene therapies and their side effects caused by unfavorable systemic distribution and toxicity. Particularly, NCs using engineered macromolecules and biomaterials have advanced drug delivery by leveraging a combination of capabilities (i.e. biocompatibility, tunable in size/shape/surface properties) that improve their PK and PD. This improves circulation and retention time of the drug by protecting it from degradation and inactivation, controllable drug release profiles, low immunogenicity or amenability to targeted delivery with appropriate functionalization. Thanks to these properties, many lipophilic and biological drugs such as proteins or peptides can be delivered avoiding solubility limitations, enhancing their efficacy and promoting their prolonged and sustained release at the desired site of action (Din et al., 2017; Kalepu & Nekkanti, 2015). miR therapies can also leverage these properties for restoring impaired cardiac function and promote cardiac regeneration. Specifically, as just mentioned, naked delivery of miR therapies typically exhibits poor bioavailability, rapid degradation by nucleases, limited tissue distribution, and short-lived PD effects, likely resulting in insufficient intracellular concentrations in target organs. Instead, nanosystems for nucleic acid delivery (including miR therapies), provide protection from enzymatic degradation, improved cellular uptake, and targeted biodistribution, resulting in more durable pharmacological effects (Walia and Mehta, 2024; Marcato, 2013). Moreover, rational NC design can balance systemic circulation and intracellular delivery: for instance, controlled surface de PEGylation has been shown to prolong nanoparticle circulation while enabling efficient intracellular siRNA delivery, demonstrating how surface chemistry modifications can optimize both PK and cellular uptake (Zhu et al., 2017). Next, we will comprehensively review the

different materials used as NCs for the delivery of cardiac miR therapies (Table 3).

4.1. Polymeric NCs

Natural polymers such as heparin (Hong et al., 2020; Nie et al., 2018) or semi-synthetic, such as hyaluronan sulfate (Bejerano et al., 2018), have been used to deliver cardiac miR therapies. Among biopolymers, nucleic acids have also been used as scaffolds to construct emerging nanomaterials with unique properties, particularly in terms of programmability and biodegradability (Hu et al., 2019; Mathur and Medintz, 2019). In addition, their preparation reproducibility, design versatility and suitability for functionalization make them amenable vehicles for gene therapy based on nucleic acid base complementarity (Huang et al., 2020; Zhu et al., 2023). However, nucleic acid nanotechnology is nearly unexplored to develop cardiac therapies. Chapter 4 of this thesis, describes the development of an anti-mir-24-2 therapy based on DNA nanotechnology.

So far, most of the used polymeric NCs are based on synthetic polymers, such as ethanolamine-aminated polyglycidyl methacrylate (PGEA), PLGA, dendrigraft poly-L-lysine (DGL), polyethylene imine (PEI), polyethylene glycol (PEG)-polylactic acid (PLA) and PEG-DGL derivatives (Bellera et al., 2014; Hong et al., 2020; Mirna et al., 2022; Nie et al., 2018; Sun et al., 2020; Xue et al., 2018; Zhi et al., 2019).

Compared to naked delivery, polymeric NCs significantly accumulated the miR therapeutics in the heart after systemic administration in murine models of MI (Hong et al., 2020; Nie et al., 2018; Zhi et al., 2019) (i.e., ~2.3-fold increase of cardiac content (Zhi et al., 2019)). Also, NC-based delivery led to improved therapeutic effect over naked delivery, i.e., shorter ST segment in the ECG or restoring the LVEF (Sun et al., 2020). All miRs packaged in polymeric NCs, mostly administered systemically for MI treatment, exhibited therapeutic effect.

When assessed, functionalized polymeric NCs with non-cardiac specific ligands such as the arginine-glycine-aspartic acid (RGD) penetrating peptide (Sun et al., 2020) or angiotensin II type 1 (AT_1) peptide (X. Xue et al., 2018) demonstrated higher cardiac accumulation and heart therapy retention *in vivo* compared to non-functionalized NCs (Sun et al., 2020; Xue et al., 2018) with no significant toxic effects in off-target organs and high effects in hypoxic cells (Xue et al., 2018). Thus, functionalization of the polymeric NCs administered systemically for MI treatment led to superior therapeutic efficacy compared to their non-functionalized counterparts (Sun et al., 2020; Xue et al., 2018).

4.2. Lipid-based NCs

Different lipid-based NCs (LNCs) have been developed for drug delivery, including nanoemulsions, liposomes, solid lipid nanoparticles, nanostructured lipid carriers or lipid-polymer hybrid NCs. The first LNCs-based drug, Doxil, was approved by FDA more than a decade ago (Barenholz, 2012). LNCs have been widely used for nucleic acid delivery *in vivo* due to their easy adaptability and their efficient cellular delivery (Mehta et al., 2023). In particular cationic and ionizable LNCs, have received much attention due to the strong electrostatic interactions between negatively charged miR and positive lipids, resulting in an efficient complexation that protects miR from rapid degradation and inactivation while enhancing their cellular uptake by improved interaction with the cell membrane (Zhao & Huang, 2014). In addition, their popularity increased in the last years during the development of the mRNA COVID-19 vaccine (Wang et al., 2022; Warne et al., 2023).

In MI, modified cationic LNCs have been used to deliver miR therapies in murine models of MI (Li et al., 2020; Liu et al., 2014; Tan et al., 2021; Zhou et al., 2022). Compared to naked delivery, one study shows that repeated dosing of miR-21-loaded LNCs restored miR-21 expression levels in the infarcted myocardium of mice, even beyond those found in healthy controls, while with the naked therapy remained unchanged (Li et al., 2020). MiR therapeutics-loaded LNCs showed efficacy in terms of improved cardiac remodeling and function (e.g., with improvements in the percentage of LVEF) over untreated MI animals up to 4 weeks using a single-dose IM administration of miR-153-3p-loaded liposomes (Zhou et al., 2022), after repeated IV doses of miR-21-loaded liposomes (Li et al., 2020) or after a single-dose IV injection of miR-21-loaded LNCs (Tan et al., 2021). Single-dose IV injection of AMO-1 also improved cardiac function with shorter ST segment in the ECG over naked delivery up to 3 days (Liu et al., 2014). This suggests that, even if LNC encapsulation improves delivery, the therapy efficacy may still depend on any of these other factors, such as the dosing scheme, administration pathway or the miR-specific activity.

Functionalization with either cell camouflage (coating approaches to evade immune recognition), with hyaluronic acid, or with cardiac-specific targeting molecules such as anti-cardiac troponin I (cTnI) antibodies, increased the accumulation of LNCs in the heart after systemic administration over their non-functionalized configurations (Li et al., 2020; Liu et al., 2014; Tan et al., 2021; Zhou et al., 2022) improving the therapeutic effects (Li et al., 2020; Tan et al., 2021) irrespective of the evaluation at short (Tan et al., 2021) or long (Li et al., 2020) time points. For example, treatment with miR-21-loaded anti-cTnI antibody-functionalized LNCs restored the LVEF from ~45% in untreated MI animals up to ~75% (~65% without functionalization), which is near the ~80% value at baseline in untreated healthy controls (Li et al., 2020).

4.3. Inorganic NCs

Among all the inorganic materials proposed as NCs for the delivery of miR therapies, MSN have received attention for clinical applications due to their large surface area, high pore volume, tunable structure, high biocompatibility, and thermal stability (Cha et al., 2017; Fu et al., 2019).

In relation to HD, MSNs have been used for miR delivery via systemic administration in murine models of MI and have clearly contributed to both functional and morphological recovery compared to untreated animals (Chen et al., 2024; Wang et al., 2021; Yao et al., 2020). In agreement with polymeric or LNCs particles, encapsulation outperformed naked delivery as proven by superior therapeutic effect, i.e., improved cardiac function, reduction of myocardial apoptosis, and increased CM proliferation (Yao et al., 2020).

Cellular camouflage (Yao et al., 2020), cardiac-specific peptides (Chen et al., 2024; Kanki et al., 2011), or even the combination of both functionalization approaches (Kim et al., 2012; Wang et al., 2021), were applied to successfully increase cardiac targeting, which improved functional and structural recovery mediated by the cargo over their non-functionalized versions.

4.4. Hydrogels

Hydrogels have emerged as versatile delivery systems for the local controlled and sustained release of therapeutic agents or drugs, including miRs (Yang et al., 2025), thanks to their tunable physicochemical properties, controlled degradation, biocompatibility and ability to prevent degradation of labile drugs (Jindal et al., 2022).

In HD, therapeutic miR-loaded hydrogels, alone or in combination with NCs, acting as scaffolds, have demonstrated, along with the aforementioned properties, efficacy when applied locally in murine and porcine models of MI or I/R (Eding et al., 2024; Li et al., 2021; Wang et al., 2025; Wang et al., 2017; Yang et al., 2019). In addition, in pigs, hydrogel-encapsulation of miR-21 mimic has demonstrated advantages over naked delivery (Li et al., 2021) accomplishing increased myocardial levels of the miR, improved LVEF and reduced scar size in infarcted pigs (Li et al., 2021).

Functionalization with non-cardiac specific ligands, such as *trans*-activating transcriptional activator (TAT) peptide (Yang et al., 2019), or the use of bioactive hydrogels, such as ROS-scavenging materials (Wang et al., 2025), have been used with success to enhance miR delivery and

Table 3
Nanocarriers for miR delivery for cardiac therapeutic purposes.

NC type	Material	Specie	HD	Administration route and time course	Cargo	*In vivo* targeted delivery		*In vivo* therapeutic effect		Ref.
						Functionalization or targeting ligand	Targeting efficacy of F vs NF or ND	General outcome	Effect of F over NF and/or over ND delivery	
Polymeric NCs	Heparin-PGEA	Mouse	MI	IV, daily during 3d pre and post MI	miR-499 mimic	Heparin	Cardiac accumulation of F over ND	Improved cardiac function and tissue remodeling 2w post-MI	Not assessed	(Nie et al., 2018)
Polymeric NCs	Hyaluronan-sulfate – Ca^{2+}	Mouse	MI	IV, daily during 3d post-MI, starting 24 h post-MI	miR-21 mimic	N/A	N/A	Improved cardiac function and tissue remodeling 30d post-MI	Not assessed	(Bejerano et al., 2018)
Polymeric NCs	Cholesterol-PGEA	Mouse	CH	IV, from 3rd to 9th week post-TAC, one time during 3d	Anti-miR-182	Cholesterol	Cardiac accumulation of F over ND	Improved cardiac function 9w post-TAC	Not assessed	(Zhi et al., 2019)
Polymeric NCs	PLGA microspheres	Pig	MI	IC, 5 min post-MI, single dose	Anti-miR-92	N/A	N/A	Improved cardiac tissue remodeling 30d post-MI	Not assessed	(Bellera et al., 2014)
Polymeric NCs	LMW Heparin-DGL	Rat	MI	IV, 2 h post-MI, single dose	Anti-miR-1 (AMO-1)	LMW Heparin	Cardiac accumulation of F over ND and NF	Improved cardiac function 3w post-MI	Not assessed	(Hong et al., 2020)
Polymeric NCs	PEI F25-LMW	Mouse	EAM	IV + IP 1d pre-EAM and IP 6d post-EAM	Anti-miR-21a-5p	N/A	N/A	Improved cardiac remodeling 7w post-EAM	Not assessed	(Mirna et al., 2022)
Polymeric NCs	RGD-PEG-PLA	Rat	MI	IV, post-MI, single	miR-133 mimic	RGD tripeptide (Wang et al., 2018a)	Cardiac accumulation of F over NF	Improved cardiac function and tissue remodeling 1w post-MI	▪ Improved electrophysiological (ST segment) and functional parameters with F (i.e., LVEF ~ 55%), NF (i.e., LVEF ~ 47%), and ND (i.e., LVEF ~ 37%) vs untreated MI animals (i.e., LVEF ~ 29%) ▪ Improved serum levels of cardiac damage markers and inflammatory profile markers, mitochondrial oxidative stress markers, vascular endothelial cell damage markers in F and NF vs ND ▪ Improving myocardial remodeling (improved cellular organization and reduced fibrosis) and reduced apoptosis in F and NF vs ND	(Sun et al., 2020)
Polymeric NCs	PEG-DGL	Mouse	MI	IV, 24 h post-MI, single	Anti-miR-1 (AMO-1)	AT_1	Cardiac accumulation of F over NF	Improved cardiac function and tissue remodeling 48 h post-MI	▪ Reduced miR-1 with F (0.7-fold) vs NF (1.9-fold) and untreated (2.3-fold) relative to sham (1-fold). ▪ Improved functional parameters with F (i.e., LVEF = 35.9%), NF (i.e., LVEF = 30%) vs untreated MI animals (i.e., LVEF = 13.9%) ▪ Reduced infarct size with F (16.6%), NF (28.9%) vs untreated (46.2%) ▪ Improving myocardial remodeling (reduced fibrosis) and reduced apoptosis (2-fold) with F vs NF	(Xue et al., 2018)
Lipid NCs	Hyaluronic- cationic liposomes	Rat	MI	IM, 3d pre-MI, single	miR-153-3p mimic	Hyaluronic acid	Cardiac accumulation of F over NF	Improved cardiac tissue remodeling (not specified day of measurement)	Not assessed	(Zhou et al., 2022)
Lipid NCs	Platelet like-fusogenic liposomes	Mouse	I/R	IV, 3d post-MI, single	miR-21 mimic	Platelet membranes camouflage	Cardiac accumulation of F over NF	Improved cardiac function 4w post-MI and cardiac tissue remodeling 5d post-MI	▪ Promoted reparative polarization of inflamed macrophages with F vs NF ▪ Improved inflammatory cytokines profile in heart in F vs NF ▪ Improved function with F (i.e., LVEF ~ 38%) vs NF (i.e., LVEF ~ 32%) and untreated MI (i.e., LVEF ~ 32%) ▪ Improving myocardial remodeling (increased left ventricular anterior wall (LVAW) thickness, reduced fibrosis and promoted angiogenesis) with F vs NF	(Tan et al., 2021)

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Table 3 (*continued*)

NC type	Material	Specie	HD	Administration route and time course	Cargo	*In vivo* targeted delivery		*In vivo* therapeutic effect		Ref.
						Functionalization or targeting ligand	Targeting efficacy of F vs NF or ND	General outcome	Effect of F over NF and/or over ND delivery	
Lipid NCs	Liposomes (DSPE-PEG-Maleimide)	Rat	MI	IV, post-MI, single	Anti-miR-1 (AMO-1)	Anti-cTnI antibody	Cardiac accumulation of F over NF	Improved cardiac function 3d post-MI	▪ Reduced miR-1 with F (~ 0.5-fold) vs NF (~ 3.2-fold), untreated MI (~ 1.7-fold) relative to untreated healthy animals (1-fold) ▪ Improved ECG parameters (ST segment) with F vs ND	(Liu et al., 2014)
Lipid NCs	Liposomes (DSPE-PEG-Maleimide)	Rat	AMI	IV, 12 h, 3d and 7d post-MI	miR-21 mimic	Anti-cTnI antibody	Cardiac accumulation of F over NF	Improved cardiac function and tissue remodeling 15d post-MI	▪ Increased miR-21 with F (3.48-fold) vs NF (2.15-fold), ND (~ 0.5-fold) and untreated MI (0.53-fold) relative to untreated healthy animals (1-fold) ▪ Reduced expression of pro-apoptotic factors in F and NF vs ND. ▪ Improved function with F (i.e., LVEF ~ 75%) and NF (i.e., LVEF ~ 65%) vs untreated MI animals (i.e., LVEF ~ 45%) ▪ Reduced infarct size with F (16.4%), NF (27.9%) vs untreated MI (38.9%) relative to untreated healthy animals	(Li et al., 2020)
Inorganic NCs	MSN	Mouse	MI	IV, 24 h post-MI, single	miR-1, miR-133, miR-208 and miR-499 mimic	FH peptide (Kim et al., 2012) and neutrophil membrane camouflage	Ligand and cell camouflage favor F accumulation over NF	Improved cardiac function and tissue remodeling 4w post-MI	▪ Increased miR combo with F (> ~ 7.5-fold) vs NF (~ 1-fold) relative to untreated MI ▪ Functional test in F and no detected in NF ▪ Improved function with F (i.e., LVEF ~ 70%) vs NF (i.e., LVEF ~ 47%) and untreated MI (i.e., LEVF ~ 27%) ▪ Reduced infarct size with F (~ 21%), NF (~ 47%) vs untreated MI (~ 60%)	(Wang et al., 2021)
Inorganic NCs	MS membranes-PEI	Rat	MI	IV, following MI, single	miR-199a-5p mimic	IMTP (Kanki et al., 2011)	Cardiac accumulation of F over NF	Improved cardiac function and tissue remodeling 4w post-MI	▪ Improved function with F (i.e., LVEF ~ 24.5%), NF (i.e., LVEF ~ 22.3%) vs untreated (i.e., LVEF ~ 14.0%) ▪ Reduced infarct size with F (~ 11%), NF (~ 25%) vs untreated MI (~ 43%)	(Chen et al., 2024)
Inorganic NCs	MSN	Rat	MI	IV, daily during 3d, starting 24 h post-MI	miR-21 mimic	MSC membrane camouflage	Cardiac accumulation of F over NF and ND	Improved cardiac function and tissue remodeling 4w post-MI	▪ Improved function with F (i.e., LVEF = 63.2%), NF (i.e., LVEF = 54.9%) vs ND (i.e., LVEF = 31.6%) and untreated MI (i.e., LVEF = 28.0%) ▪ Improving myocardial remodeling (reduced CM apoptosis and enhanced CM proliferation) with F vs NF and both vs ND	(Yao et al., 2020)
Hydrogels	Hyaluronic acid hydrogel	Mouse	MI	IM, post-MI, single	miR-302 mimic	N/A	N/A	Improved cardiac function and tissue remodeling 4w post-MI	Not assessed	(Wang et al., 2017)
Hydrogels	PFBT-DSPE-PEG injected with elastin-like protein-hyaluronic acid hydrogel	Rat	MI	IM, post-MI in 3 injection sites	miR-199a-3p mimic	TAT	Not assessed	Improved cardiac function 1- and 3-months post-MI and tissue remodeling 28d post-MI	▪ Improved function at 3 months with F-hydrogel (i.e., LVEF = 64%) vs NF (i.e., LVEF ~ 45%) and untreated MI (i.e., LVEF = 45%)	(Yang et al., 2019)
Hydrogels	MSN loaded in hydrogel (Ciclodextrin-PEG)	Pig	MI	IM, following MI in 6 injection sites	miR-21-5p mimic	N/A	N/A	Improved cardiac function 2w and 4w post-MI and tissue remodeling 4w post-MI	▪ Increased miR-21 with NF-hydrogel (~ 2.5-fold) vs ND (~ 1.2-fold) and untreated MI (~1-fold) relative to sham (1-fold) ▪ Improved function at 4w with NF-hydrogel (i.e., LVEF ~ 50%), ND (i.e., LVEF ~ 42%) vs untreated MI (i.e., LVEF ~ 40%) and sham (i.e., LVEF ~ 65%) ▪ Reduced infarct size with NF-hydrogel (~ 15%), ND (~ 27%) vs untreated MI (~ 35%) relative to sham ▪ Improving myocardial remodelling (reduced fibrosis, promoted angiogenesis and reduced inflammation mediators expression) in NF-hydrogel compared to ND	(Li et al., 2021)

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Table 3 (*continued*)

NC type	Material	Specie	HD	Administration route and time course	Cargo	*In vivo* targeted delivery		*In vivo* therapeutic effect		Ref.
						Functionalization or targeting ligand	Targeting efficacy of F vs NF or ND	General outcome	Effect of F over NF and/or over ND delivery	
Hydrogels	ROS-scavenging hydrogel	Rat Pig	MI	IM, 10 min post-MI, single	miR-19a/b mimic	N/A	N/A	Improved cardiac function 4w post-MI and tissue remodeling 50d post-MI	Not assessed	(Wang et al., 2025)
EVs	Engineered exosomes derived from bone marrow mesenchymal stem cells	Mouse	I/R	IV, every 2d for 4w, post-I/R	miR-302 mimic	Modified PCM peptide (McGuire, Samli, Johnston, & Brown, 2004)	Not assessed	Improved cardiac function and tissue remodeling 4w post-MI	▪ Increased miR-302 with F (~ 1.6-fold), ND (~ 1.1-fold) vs untreated I/R (0.75-fold) relative to sham (1-fold) ▪ Decreased necrosis and apoptosis markers, myocardial injury markers, proinflammatory factors with F vs ND ▪ Improved function with F (i.e., LVEF ~ 43%), ND (i.e., LVEF ~ 39%) vs untreated I/R (i.e., LVEF ~ 32%) and sham (i.e., LVEF ~ 48%)	(Gu et al., 2024)
EVs	Milk-derived EVs	Mouse	Hypertrophic HF	IV, 3 times per week for 4w	miR-30d mimic	IMTP (Kanki et al., 2011)	Cardiac accumulation of F over NF	Improved cardiac function and tissue remodeling 4w post-MI	▪ Improved cardiac remodeling (reduced cardiac hypertrophy and fibrosis) with F vs NF ▪ Improved cardiac function with F (i.e., LVEF ~ 65%) vs NF (i.e., LVEF ~ 50%), untreated hypertrophic mouse (i.e., LVEF ~ 50%) and healthy animals (i.e., LVEF ~ 65%)	(Tong et al., 2025)
EVs	HEK293-derived EVs	Mouse	MI	IM, following IM, single	miR-21 mimic	N/A	N/A	Improved cardiac function and tissue remodeling 4w post-MI	Not assessed	(Song et al., 2019)

AMI: acute myocardial infarction, AT_1: angiotensin II type 1, BMSCs: bone marrow mesenchymal stem cells, CD: cyclodextrin, CHO: cholesterol, cTnI: cardiac troponin, d: day, DGL: dendrigraft poly-l-lysine; DSPE-PEG: Distearoylphosphosphatidyl-ethanolamine, EAM: experimental autoimmune myocarditis, EC: endothelial cell, ECG: electrocardiogram, ELP: elastin-like protein, EV: extracellular vesicle, F: functionalized, h: hours, HA: hyaluronic acid, HD: heart disease, Hep: heparin, HF: heart failure, I/R: ischemia/reperfusion, IMPT: ischaemic myocardium peptide targeting, IP: intraperitoneal, LMW: low molecular weight, LVAW: left ventricle anterior wall, LVEF: left ventricle ejection fraction, MAL: maleimide, MI: myocardial infarction, MSCs: mesenchymal stem cells, MSN: Mesoporous silica NCs, N/A: not applicable, NC: nanocarrier, ND: naked delivery, NF: non-functionalized, PCM: primary cardiomyocyte peptide, PEG: polyethylene glycol, PEI: polyethylene imine, PBFT: poly(9,9-dioctylfluorene-alt-benzothiadiazole), PGEA: ethanolamine-aminated polyglycidyl methacrylate, PLA: polylactic acid, PLGA: poly(lactic-co-glycolic) acid, Ref.: reference, RGD: arginine-glycine-aspartic acid tripeptide, ROS: reactive oxygen species, TAC: transversal aortic constriction, TAT: *trans*-activating transcriptional activator, w: week.

therapeutic effect in both small and large animal models of MI, with sustained therapeutic effects observed up to three months after a single administration (Yang et al., 2019).

4.5. Extracellular vesicles (EVs)

EVs, small membrane vesicles enriched in a variety of biomolecules including miRs, have been explored to deliver miR therapies due to their circulatory stability, biological barrier permeability, low immunogenicity and high delivery efficiency, among other properties (Henning, 2020). EVs from different biological origins have been engineered to enrich their content in specific miR mimics to treat HD observing therapeutic effects (Gu et al., 2024; Song et al., 2019; Tong et al., 2025).

Current EVs engineering efforts are mainly focused on strategies to enhance site-directed delivery (Liang et al., 2021; Salunkhe et al., 2020). These include the functionalization of EVs and/or surface modification (Gu et al., 2024; Tong et al., 2025) to improve targeting regardless of the administration route and to overcome the limitation of exosome intracellular recruitment to RNA substrates for degradation. MiR-loaded EVs functionalized with cardiac-specific peptides were reported to improve their accumulation in the heart and therapeutic effect over non-functionalized EVs or naked miR following multiple doses of systemic administration (Gu et al., 2024; Tong et al., 2025).

In summary, several types of NCs have demonstrated their ability to deliver miR therapeutics to the heart and promote therapeutic effects in preclinical studies, mainly cardiac regeneration in small animal models of MI, but also in large animal models (Table 1 and 3). Even if only a limited number of studies have compared the performance of naked and encapsulated delivery of miR therapies, all, regardless of the nature of the used NC material, animal species, functional readout or miR cargo (among other variables across studies) and mostly using systemic administration, have independently demonstrated improved stability and/or therapeutic efficacy over naked delivery. This is remarkable as most of the studies in large animal models and clinical trials rely on naked miR therapeutics, which according to preclinical data limits their therapeutic potential. However, systematic head-to-head comparisons of the administration route and/or the different NC-based formulations are lacking to fully understand the capabilities of each material to enhance the therapeutic effect of a given miR. Thus, it remains unclear whether the advantages offered by the systemic administration of NCs over the naked delivery exceeds the benefits of naked/encapsulated local administration. However, the clinical applicability of the systemic pathway remains convenient for those pathologies where repeated therapy administrations may be needed. In addition, functionalization of NCs to enhance cardiac tissue retention has consistently been shown to improve the therapeutic efficacy of miR therapeutics in terms of cardiac function repair and regeneration.

5. The challenge of targeted delivery to the heart

Targeting a specific organ, tissue and/or cell remains an independent hurdle of utmost relevance in terms of reducing off-tissue/cell toxicity and increasing on-tissue/cell drug availability. NC-based delivery systems not only improve the PK and PD profiles of miR therapies, but also broaden their therapeutic window. By enhancing targeting efficiency to cardiac tissue, NCs could reduce off-target accumulation in organs such as the liver and kidney, and minimize immune activation, thereby decreasing the risk of systemic toxicity. This improved safety profile would allow for the administration of higher effective doses of miRs, which could enhance therapeutic efficacy while maintaining tolerability. Different approaches or even their combination are available to achieve targeted drug delivery such as UTMD, which efficiently delivered both conventional (Chen et al., 2011; Saliba et al., 2012; Vancraeynest et al., 2006) and cardiac miR therapeutics *in vivo* (Liu et al., 2015; Su et al., 2015) as well as cellular camouflage, that has been explored for cardiac targeting delivery (Tan et al., 2021; Wang et al., 2021). Another strategy consists in targeting LNCs with modified lipids (Vasileva et al., 2024), which however remains unexplored for cardiac targeting.

However, most frequent targeting strategy of NCs rely on the use of targeting moieties. These are natural, rationally designed or functionally-selected ligands that aim to achieve specific interactions between NCs and target cells via cell surface molecules. Next, we will describe these kinds of targeting strategies used to address the challenge of targeted delivery to the heart, including those used for miR therapies (Table 3).

5.1. Non-cardiac-specific targeting

Non-cardiac cell-specific surface molecules include those that are upregulated in response to disease. For example, functionalization with ligands of AT_1 receptor (AT_1R), which is upregulated in the CM of the infarcted myocardium (Dvir et al., 2011; Xue et al., 2018), increased the accumulation of NCs in the infarcted heart compared to non-functionalized NCs (Dvir et al., 2011; Xue et al., 2018). However, AT_1R is also expressed in other organs (Gasc et al., 1994), such as the liver (Dvir et al., 2011), although no liver side effects have been reported (Xue et al., 2018). Ligands of adhesion molecules, such as P-selectin, ICAM-1 or E-selectin, which are upregulated on the endothelium in response to ischemia or inflammation (Scott et al., 2008), have been also used (Scott et al., 2009, 2007). However, differences in the temporal expression of the disease-response specific molecules (Scott et al., 2009, 2007) as well as changes related to inflammation, perfusion, and vascularization in the affected area (Dvir et al., 2011) require fine-tuning of the injection time to ensure effective targeting and retention in the infarcted myocardium. In addition, the use of disease-response-specific molecules for targeting may limit their applicability to acute conditions.

Cell-penetrating peptides (CPPs), short peptides with amphipathic helixes or arginine-rich sequences, are often used to increase the cellular permeability of NCs, and so the cargo delivery, although without cellular selectivity (Dowaidar, 2024). For example, the TAT peptide (Frankel & Pabo, 1988; Vivès et al., 1997) has been used to deliver miR to infarcted mouse tissue demonstrating its beneficial effect on promoting the therapeutic effect of the miR over the non-functionalized configuration (Yang et al., 2019). The RGD tripeptide (Sun et al., 2020) or FH peptide (Wang et al., 2021) enhanced the cardiac accumulation and therapeutic effects of miR-loaded NCs.

5.2. Cardiac-specific targeting

A number of cardiac-specific targeting ligands have been described (Table 4). Among the different molecules used as ligands to target the heart with NCs, cardiac cell-specific antibodies have been used. Remarkably, and in contrast to the traditional approach of targeting cell-surface molecules, NCs decorated with intracellular antibodies such as anti-myosin- and anti-cTnI- have successfully targeted the myocardium in small animal models (Ko et al., 2009; Li et al., 2020; Liu et al., 2014; Torchilin et al., 1992; Torchilin et al., 1996; Verma et al., 2006) Functionalization of LNCs with Anti-cTnI antibodies improved cardiac accumulation and the functional effect of cardiac miR therapeutics over their non-functionalized versions (Li et al., 2020; Liu et al., 2014). Whether this increased delivery results from intracellular targeting alone or from a combination with the lipophilic properties of LNCs remains unclear. However, this strategy is rarely used these days.

Cardiac cell-specific penetrating peptides (cardiac CPPs) have been identified through *in vitro* (McGuire et al., 2004) and *in vivo* (Dasa et al., 2015; Kanki et al., 2011; Zahid et al., 2010) affinity screens (phage display biopanning). They have been used to specifically deliver miR therapies to CMs and other cardiac cells (endothelial cells, cardiac fibroblasts, c-Kit+ cells) *in vivo* (Table 4). The primary-CM (PCM) peptide, selected *in vitro* to bind to CM (McGuire et al., 2004), demonstrated CM

Table 4
Cardiac-specific targeting ligands.

Cardiac-specific targeting ligands	Denomination	Identification	Reference	NC-based delivery to the heart (all types of cargoes, miR-related in bold letters)	Translation to human cardiac cells
Antibodies	Anti-myosin	N/A	N/A	(Ko et al., 2009; Torchilin et al., 1992; Torchilin et al., 1996; Verma et al., 2006)	Not assessed
	Anti-cTnI	N/A	N/A	(Li et al., 2020)	Not assessed
	Anti-cTnI	N/A	N/A	(Liu et al., 2014)	Not assessed
CPPs	PCM	*In vitro* phage display (mice)	(McGuire et al., 2004)	(Gu et al., 2024; Mentkowski & Lang, 2019; Nam, Kim, Kim, & Bull, 2012; Rana et al., 2015; ao et al., 2024; Zhao et al., 2018)	Not assessed
	CM, endothelial and cardiac fibroblasts CPPs	*In vivo* phage display (mice)	(Dasa et al., 2015)	(Dasa et al., 2015)	Not assessed
	IMTP	*In vivo* phage display (rat)	(Kanki et al., 2011)	(Chen et al., 2024; Tong et al., 2025; Vandergriff et al., 2018; Wang et al., 2018b)	(Tong et al., 2025)
	CTP	*In vitro* (H9C2 rat cells) and *in vivo* (mice) phage display	(Zahid et al., 2010)	(Kim et al., 2018)	(Zahid et al., 2018)
Aptamers	Anti-PLN	SELEX	(Sakai et al., 2014)	Not used for this purpose	Not assessed
	Anti-PLN	SELEX	(Tanaka, Honda, Matsuura, Kimura, & Inui, 2009)	Not used for this purpose	Not assessed
	CM-specific binding	*In vivo* SELEX (mice)	(Philippou et al., 2023)	Not used for this purpose	Not assessed
	CM-specific internalizing	*In vivo* SELEX (mice)	(Narayan et al., 2024)	Not used for this purpose	Not assessed

targeting *in vitro* and heart targeting *in vivo* in a variety of NCs loaded with different kinds of cargoes (Mentkowski & Lang, 2019; Nam et al., 2012; Rana et al., 2015; Zhao et al., 2024; Zhao et al., 2018), including miR-302 enriched EVs (Gu et al., 2024). *In vivo* PCM-based targeting showed limited presence of NCs in other organs and consequently absence of side effects (Rana et al., 2015; Zhao et al., 2024; Zhao et al., 2018). The cardiac targeting peptide (CTP) (Zahid et al., 2010) (US9249184B2), selected *in vivo* to bind to CM, demonstrated *in vivo* CM targeting of NCs (Zahid et al., 2023). The CTP linked to a naked miR also demonstrated specific delivery *in vitro* to human CMs compared to other cardiac (cardiac fibroblasts or endothelial cells) and non-cardiac cells (HEK293) (Gallicano et al., 2022). This peptide has been also used for the NC-based delivery of drugs showing the cardiac-specific delivery to cardiac cells *in vitro* and to the heart *in vivo* (Kim et al., 2018). NCs' functionalization with the *in vivo*-identified ischemic myocardial targeting peptide (IMTP) (Kanki et al., 2011), selected for its binding capacity to ischemic LV and that showed higher targeting capacity in ischemic heart than in normal heart (Kanki et al., 2011; Won et al., 2013) or in other organs, enhanced the therapeutic efficacy of the cargo over non-functionalized NCs (Vandergriff et al., 2018; Wang et al., 2018b). IMTP-functionalized NCs have been used to deliver miR therapeutics improving cardiac function and remodelling over non-decorated NCs (Chen et al., 2024; Tong et al., 2025). Finally, other cardiac cell-specific peptides for CM, endothelial cells, cardiac fibroblasts and c-Kit+ cells in the infarcted/border zones were identified *in vivo* (Dasa et al., 2015). They were validated for targeted delivery with NCs *in vivo* and also the therapeutic efficacy was assessed *in vivo* for the specific targeting of CM with the I-1 peptide (Dasa et al., 2015). Although the capabilities of CPPs for *in vivo* heart targeted delivery of NCs have been explored, the cell-type specific targeting of PCM and CTP remains unreported.

Aptamers are synthetic short DNA or RNA sequences selected by *in vitro* or *in vivo* affinity screens, namely Systematic Evolution of Ligands by Exponential Enrichment (SELEX). They are ligands with high specificity and affinity for a given target molecule that have low molecular weight, leading to enhanced and rapid tissue penetration, high chemical and thermal stability, low or non-immunogenicity and toxicity, high biodegradability and biocompatibility and low-cost production (Tan et al., 2019). Specific aptamer-based functionalization of NCs for target delivery has been explored, even to promote their active transport across the blood–brain barrier, which acts as a bottleneck in the treatment of brain diseases (Lombardo et al., 2020; Su et al., 2023). Aptamers have been used to decorate NCs for the targeted delivery of miR or short interference RNA (siRNA) therapies in tumoral and non-tumoral tissues, demonstrating high cell and/or tissue specificity and significant therapeutic efficacy *in vitro* and *in vivo* (Liang et al., 2015; Yin et al., 2019). For example, osteoblast-specific aptamer-functionalized LNCs harboring a bone anabolic siRNA targeted rat osteoblasts over 20-fold more efficiently than the non-functionalized NC *in vitro,* which was translated in the targeted therapy promoting bone formation in osteopenic and healthy rodents (Liang et al., 2015). Aptamers recognizing cardiac cells have been identified by SELEX for phospholamban (PLN)-binding (Sakai et al., 2014; Tanaka et al., 2009) that were later found to have theragnostic purposes (Honda, Sakai, & Inui, 2024; Zhan et al., 2019). More recently, CM-specific binding (Philippou et al., 2023) and internalizing (Narayan et al., 2024) RNA aptamers have been obtained by *in vivo* SELEX. However, their capabilities for *in vivo* heart targeting of NCs remains to be explored.

In general, the different targeting strategies increased the cardiac accumulation of NCs and the effect of their cargo. This holds true for the miR targeted delivery to the heart with non-cardiac- (Sun et al., 2020; Wang et al., 2021; Xue et al., 2018; Yang et al., 2019) and cardiac-specific ligands (Chen et al., 2024; Li et al., 2020; Liu et al., 2014; Tong et al., 2025) as compared to non-functionalized NCs, highlighting the therapeutic improvement achieved with functionalization (Table 3 and 4). However, the gain in cellular selectivity in the delivery of NCs provided by the cardio-specific ligands, which is fundamental for the cell-type specific targeted delivery, has been mostly demonstrated *in vitro.* More importantly, different studies evidence how the ligand-mediated targeted delivery, either in heart (Dasa et al., 2015; Rana et al., 2015; Tong et al., 2025; Vandergriff et al., 2018; Zhao et al., 2024) or other tissues (Liang et al., 2015), leads to active liver (and other organs) detargeting, measured between 4 to 24 h post-NC systemic administration. This proves the active contribution of the tissue/cell-specific ligands to reduce extrahepatic delivery, one of the major hurdles of drug delivery. It is likely that increased drug safety and compatibility of the systemic administration of cardiac (miR) therapies (important for repeated dosing regimens) benefits from this effect.

Because targeting ligands are important for unlocking the therapeutic potential of miR in combination with NCs, identifying ligands that recognize human cardiac cells is essential for advancing these therapies for clinical use. However, there is a notable lack of studies selecting ligands specific to human cardiac cells (i.e., those derived from human iPSC). This is especially concerning given the consistent intra-species translational value of *in vitro*-developed ligands (Liang et al., 2015; McGuire et al., 2004) that enable *in vivo* targeted drug delivery of NCs within the same species (Gu et al., 2024; Hong et al., 2025; Mentkowski & Lang, 2019; Nam et al., 2012; Rana et al., 2015; Ren et al., 2021; Xu et al., 2024; Zhao et al., 2024; Zhao et al., 2018; Zheng et al., 2023) (Table 4). Similarly, the limited number of studies assessing the specificity of murine-raised cardiac-specific ligands in human cardiac cells (Tong et al., 2025; Zahid et al., 2018) is notable (Table 4), as this would establish their intraspecies translational value for NC-based targeted delivery. Therefore, there are currently very few cardiac-specific ligands with translational relevance. However, the small number of reported cardiac-specific targeting ligands and the recent application of the cutting-edge aptamer technology to generate them (Narayan et al., 2024; Philippou et al., 2023) creates the perception in the field that cardiac-specific ligand development is currently flourishing. Nevertheless, the true translational value of those raised *in vitro* (human/animal cardiac cells) or *in vivo* (small animal models), can only be empirically determined in real scale, clinically relevant models (large animal models) before their clinical assessment. Careful validations as well as early-phase PK/PD studies will be needed to ensure effective and safe targeting in humans.

6. Pharmaceutical translational barriers of NC-based miR therapies

Despite promising preclinical data in animal models, several pharmaceutical translational barriers have slowed down the progression of NC-based miR therapies to clinical trials:

i. PK and PD challenges. Differences in systemic clearance, intracellular delivery, and tissue distribution between preclinical models and humans can reduce therapeutic efficacy of miR therapy-loaded NCs. In addition, translationally relevant cardiac-specific ligands are currently scarce and have not been tested in clinically relevant species. This emphasizes the need for careful validation of target-directed NC-based miR therapies in large animal models, including early-phase PK/PD studies and exposure–response studies, to support further clinical development.
ii. Immunogenicity. NCs and repeated dosing can elicit innate and adaptive immune responses, including complement activation, cytokine release, and anti-carrier antibody formation (Ray et al., 2021). These responses may alter PK, restrict repeated administration, alter biodistribution, and induce off-target toxicity, representing a major barrier to long-term clinical use. In addition, different NC platforms, exhibit distinct immunological profiles (Ray et al., 2021). Several materials such as PLGA, PEG, and LNCs are FDA-approved or used in approved formulations, supporting their biocompatibility; nevertheless, repeated dosing may still elicit immune responses that limit long-term administration.
iii. Manufacturing and scalability challenges are also critical. Production of miR-loaded NCs under Good Manufacturing Practices (GMP) conditions is technically demanding and costly (Herdiana, 2025; Martino et al., 2025). Achieving batch-to-batch consistency in particle size, polydispersity index, surface properties, miR loading, and long-term stability is essential, as even minor variations can significantly impact pharmacological performance and safety. These constraints, particularly for multi-component systems, limit clinical advancement despite promising preclinical data.
iv. Regulatory requirements for nanomedicines and nucleic acid therapies, including extensive Chemistry, Manufacturing, and Controls (CMC) documentation and co-development of companion diagnostics (Herdiana, 2025), add complexity to clinical translation. Comprehensive characterization of biodistribution, PK/PD, immunogenicity, and off-target toxicity is required, while standardized regulatory guidelines are still evolving.

Taken together, the limited clinical progression of NC-based miR therapies reflects risk–benefit and development feasibility considerations rather than insufficient preclinical efficacy. While preclinical studies often emphasize efficacy, clinical application requires balancing high intracellular delivery with systemic safety. Optimized NCs can broaden the therapeutic window by enhancing targeting efficiency to cardiac tissue, reducing off-target accumulation in liver or kidneys, and mitigating systemic immune responses, enabling higher effective doses without compromising safety.

Thus, pharmaceutical design considerations as well as immunogenicity, manufacturing and scalability and regulatory compliance, remain central to successful translation. Therefore, careful selection of NC composition, surface properties, and dosing regimens is required to minimize immune activation while preserving therapeutic activity. Integration of PK/PD modeling, rational ligand selection, robust CMC strategies, and early regulatory alignment will be critical to overcoming these barriers. Addressing these challenges in a systematic and translationally relevant manner can expand the therapeutic window, enhance efficacy, and enable the safe and effective clinical development of NC-based miR therapies for HD.

7. Conclusion

MiRs emerged as key regulators in cardiac pathophysiology, making them attractive therapeutic targets to treat heart repair and regeneration during HD. While preclinical research in small animal models confirms this potential, their development has reached a bottleneck in the late preclinical phase using large animal models. This is primarily due to limitations in their delivery imposed by the biological nature of miRs. To date, only two miR-based therapies for HD have advanced to early-phase clinical trials, which clearly reflects this bottleneck. However, while most of the studies in large animal models and clinical trials are conducted with naked miR therapeutics, this review highlights how encapsulating them in NCs functionalized for cardiac-targeted delivery can improve their beneficial effects on the heart compared to naked delivery. It is important to highlight the potential of ligand-directed targeting to improve undesired organ detargeting of therapeutic NCs, providing future directions for the development of translational cardiac targeting strategies for regenerative medicine. From a pharmaceutical perspective, NC-based delivery may broaden the therapeutic window of miR therapies by improving PK stability, enhancing intracellular delivery to cardiac tissue, and reducing off-target accumulation and systemic immune responses. Successful clinical translation, however, will require addressing key pharmaceutical translational barriers, including immunogenicity upon repeated dosing, scalable manufacturing under GMP conditions, batch-to-batch consistency, and evolving regulatory requirements for nanomedicines and nucleic acid therapeutics. Overall, despite the remaining substantial technical and biological hurdles, progress in overcoming the pharmaceutical translational barriers of RNA nanotherapies (i.e. COVID-19 vaccine) and advances in miR biology and delivery with targeted nanotechnology suggest that miR-based therapies could realistically be part of future treatments for HD.

CRediT authorship contribution statement

Natalia Hernández-Bellido: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Silvia Hernández-Ainsa:** Writing – review & editing, Investigation,

Funding acquisition. **Ralf Köhler:** Writing – review & editing, Investigation. **Laura Ordovás:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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