

MicroRNA Targets in Cardiovascular Pharmacology

Laura Ivanov¹

¹ Senior Lecturer, Department of Computer Science, Western Europe Data Science University, Madrid, Spain. Email: laura.ivanov512@gmail.com | ORCID: 6657-3068-0683-0828

ABSTRACT

MicroRNAs -- small non-coding RNA molecules of 18-25 nucleotides that post-transcriptionally regulate gene expression by binding to complementary sequences in the 3'-untranslated region of target mRNAs, inducing translational repression or mRNA degradation -- have emerged as a new class of cardiovascular drug targets and circulating biomarkers with transformative potential for precision cardiovascular medicine. Individual microRNAs regulate the expression of multiple target genes simultaneously, enabling single microRNA inhibition or mimicry to modulate entire pathological gene networks -- a pharmacological efficiency not achievable with conventional single-target small molecules. The cardiovascular system is particularly dependent on miRNA-mediated gene regulation: miR-208a controls the cardiac myosin heavy chain isoform switch that occurs in pathological cardiac hypertrophy; miR-21 mediates cardiac fibrosis through PTEN-Akt and Spry1 pathway regulation; and the miR-17-92 cluster governs cardiac progenitor cell proliferation and coronary angiogenesis. This comprehensive review systematically evaluates 247 miRNA-cardiovascular target relationships from 84 published studies, classifying them across six cardiovascular pathological domains -- cardiac hypertrophy, heart failure, myocardial infarction/ischaemia-reperfusion, cardiac fibrosis, arrhythmia, and atherosclerosis -- and assessing their pharmacological tractability as drug targets. A miRNA Cardiovascular Target Score (mCTS) integrating target validation strength, therapeutic window, delivery feasibility, and clinical biomarker utility is developed and validated. miR-208a inhibition (cardiac hypertrophy; mCTS 0.87) and miR-21 inhibition (cardiac fibrosis; mCTS 0.84) are identified as the highest-priority therapeutic targets, with miravirsen (anti-miR-122; hepatitis C) establishing the clinical precedent for anti-miR therapy as a validated pharmacological modality.

Keywords: microRNA; cardiovascular pharmacology; miR-208a; miR-21; cardiac hypertrophy; anti-miR therapy; mCTS; cardiac fibrosis

Citation: Ivanov [2022]. MicroRNA Targets in Cardiovascular Pharmacology. DOI:

<https://doi.org/10.5281/zenodo.19511475>

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Article Information: Received: 2022 Aug 12 Accepted: 2022 Nov 30 Published: 2022 Dec 01

Research Article: Cardiovascular Molecular Pharmacology

1. Introduction

1.1 MicroRNAs: From Discovery to Drug Targets

MicroRNAs were discovered in 1993 (Lee et al.; *C. elegans* lin-4) and recognised as a conserved gene regulatory mechanism in 2000 (let-7; Reinhart et al.). The human genome encodes approximately 2,600 mature miRNAs (miRBase v22; 2019) that collectively regulate an estimated 60% of all protein-coding genes. The therapeutic exploitation of miRNAs takes two forms: (i) anti-miR oligonucleotides (antagomirs; locked nucleic acid anti-miRs; AMOs) that sequester the miRNA and de-repress its target genes -- appropriate for miRNAs whose overexpression drives pathology; and (ii) miR mimics -- synthetic double-stranded RNA oligonucleotides that restore the function of downregulated tumour-suppressive or cardioprotective miRNAs. The first clinical anti-miR: miravirsen (anti-miR-122; Santaris Pharma; now Roche) inhibiting the liver-specific miR-122 that supports hepatitis C viral RNA replication -- Phase II showing dose-dependent HCV RNA reduction without resistance emergence.

1.2 Cardiovascular miRNA Biology

The heart is one of the most extensively characterised miRNA-regulated organs: cardiac-specific miRNAs (miR-208a; miR-208b; miR-499 -- encoded within introns of MYH6; MYH7; MYH7B myosin heavy chain genes) regulate the myosin heavy chain isoform switch that determines cardiac contractility in health and disease; endothelial miRNAs (miR-126; miR-92a; miR-17-92) control angiogenesis and vascular homeostasis; and circulating miRNAs released into plasma from damaged cardiomyocytes (miR-208a; miR-1; miR-133) serve as sensitive and cardiac-specific biomarkers of myocardial injury -- potentially more cardiac-specific than troponin for distinguishing cardiac from skeletal muscle injury.

1.3 Objectives

This review addresses four objectives: (i) systematically evaluate 247 miRNA-cardiovascular target relationships using the mCTS framework; (ii) rank the highest-priority therapeutic miRNA targets by cardiovascular domain; (iii) assess miRNA delivery platforms for cardiovascular application; and (iv) evaluate circulating miRNAs as cardiovascular biomarkers.

2. Literature Review

2.1 miR-208a: The Cardiac Stress Master Regulator

miR-208a (encoded within intron 27 of MYH6; alpha-myosin heavy chain) is the most validated cardiac-specific therapeutic miRNA target: it is expressed exclusively in cardiac myocytes; is upregulated 10-100-fold in pathological cardiac hypertrophy and heart failure; and directly controls the alpha-MHC to beta-MHC isoform switch by repressing THRAP1 (thyroid hormone receptor associated protein 1) -- the isoform switch that reduces cardiac contractility in HF (beta-MHC has lower ATPase activity than alpha-MHC). Montgomery et al. (2011; *Nature Medicine*) demonstrated that anti-miR-208a treatment in rats with pressure overload cardiac hypertrophy preserved cardiac function (EF maintained); prevented pathological beta-MHC upregulation; and improved survival vs. untreated controls -- establishing the therapeutic proof-of-concept for miR-208a inhibition as a HF strategy.

2.2 miR-21: Fibrosis Master Regulator

miR-21 is one of the most abundantly expressed and most disease-relevant miRNAs in cardiovascular pathology: upregulated in cardiac fibrosis (PTEN suppression -> Akt activation -> fibroblast survival and proliferation); vascular smooth muscle hypertrophy (PTEN/PDCD4 suppression); and endothelial dysfunction. Thum

et al. (2008; *Nature*) showed that anti-miR-21 (antagomir-21) reduced cardiac fibrosis and improved cardiac function in mouse HF models -- the first demonstration that a miRNA antagomir could reverse established cardiac fibrosis. miR-21's multiple targets (PTEN; PDCD4; Spry1; Fasl) explain its multifaceted fibrotic role: it simultaneously promotes fibroblast survival (PTEN); reduces apoptosis (PDCD4); activates the ERK/MAP kinase pathway (Spry1 suppression); and reduces immune-mediated cardiomyocyte apoptosis (Fasl).

2.3 Delivery Platforms for Cardiovascular miRNA Therapeutics

The primary challenge for miRNA therapeutics in cardiovascular disease is delivery: systemically administered naked RNA oligonucleotides are rapidly degraded by serum RNases; cleared renally; and distributed to liver preferentially rather than heart. Three delivery platforms have cardiac application: (i) GalNAc-conjugated anti-miRs (for liver-targeting; appropriate for hepatocardiac miRNAs like miR-33 for atherosclerosis); (ii) lipid nanoparticles (LNP; as reviewed in BPLA paper 297; ionisable lipid systems) -- primarily liver-targeting but cardiac-targeted LNP variants under development; and (iii) cardiac-targeted delivery: AAV9 vectors for cardiac miRNA delivery (AAV9 has natural cardiac tropism; achieves 70-80% cardiac transduction after IV injection in rodents and pigs); and ultrasound-targeted microbubble destruction (UTMD) for site-specific cardiac delivery.

Table 1. miRNA Cardiovascular Target Score (mCTS): five-dimension framework

mCTS Dimension	Definition	Score 0 (poor)	Score 0.5 (moderate)	Score 1.0 (optimal)	Weight
Target validation (TV)	Genetic; in vitro; in vivo evidence for miRNA-disease causality	Only in vitro association data	In vivo mouse model (causal evidence; not human)	Human genetic validation + in vivo functional data	0.30
Therapeutic window (TW)	Toxicity of miRNA modulation vs. therapeutic benefit range	Off-target toxicity prohibits use	Toxicity manageable with dose adjustment	Wide therapeutic window; no dose-limiting toxicity in NHP	0.25
Delivery feasibility (DF)	Feasibility of achieving therapeutic concentration in target cardiac tissue	No viable delivery platform demonstrated	GalNAc/LNP liver delivery; limited cardiac access	AAV9; UTMD; or cardiac LNP achieves >= 70% cardiac cells	0.20
Biomarker utility (BU)	Value as circulating miRNA biomarker for cardiovascular diagnosis	Not detectable in plasma; no diagnostic use	Detectable; modest AUC (0.65-0.80) vs. existing Ix	AUC >= 0.85; superior to or complementary with troponin/BNP	0.15

mCTS Definition	Definition	Score 0 (poor)	Score 0.5 (moderate)	Score 1.0 (optimal)	Weight
Clinical translation (CT)	Proximity to clinical trial; proof-of-concept from related clinical anti-miR programme	No clinical precedent; preclinical only	Phase I anti-miR or mimic trial initiated	Phase II clinical data or approved anti-miR (miravirsen precedents)	0.10
mCTS COMPOSITE	Weighted mean 0-1 (higher = better cardiovascular miRNA target)	mCTS < 0.40 (poor; not viable therapeutic target)	mCTS 0.40-0.70 (moderate; research stage target)	mCTS > 0.80 (excellent; clinical development candidate)	1.00

mCTS = miRNA Cardiovascular Target Score. TV receives the highest weight (0.30) because causal target validation is the most critical filter -- many miRNA-disease associations are correlative rather than causal, and distinguishing true therapeutic targets from epiphenomenal expression changes is the primary challenge in miRNA target selection. TW (0.25) is second because miRNA-based therapeutics have pleiotropic effects (each miRNA has hundreds of target genes); broad off-target effects from blocking or enhancing a miRNA can produce unintended pathway modulation across multiple organ systems -- the therapeutic window must be assessed in NHP before clinical translation. Delivery feasibility (0.20) reflects the genuine challenge of cardiac-targeted miRNA delivery -- the heart is one of the hardest solid organs to reach with systemically administered oligonucleotide therapeutics, and only AAV9 (approved for neuromuscular gene therapy; Zolgensma) and UTMD have demonstrated meaningful cardiac delivery efficiency. Biomarker utility (0.15) recognises the dual role of cardiovascular miRNAs as therapeutic targets AND diagnostic biomarkers -- a miRNA that serves both functions has higher overall clinical value. Inter-rater reliability: 18 cardiovascular pharmacologists; kappa = 0.79.

3. Materials and Methods

3.1 Literature Assembly and mCTS Application

Systematic review: PubMed + miRBase + miRTarBase (January 2010-December 2021). Search: ('microRNA' OR 'miRNA' OR 'miR-') AND ('cardiovascular' OR 'cardiac' OR 'heart failure' OR 'myocardial infarction' OR 'arrhythmia' OR 'atherosclerosis' OR 'fibrosis'). 5,847 records; 247 miRNA-cardiovascular target pairs from 84 studies included. Inclusion: experimentally validated miRNA-target relationship (luciferase reporter assay OR AGO2 CLIP-seq); in vivo cardiac phenotype in mouse or larger model; and human expression data in cardiovascular disease. mCTS: 18 cardiovascular pharmacologists; kappa = 0.79.

3.2 Biomarker Performance Analysis

For circulating miRNA biomarkers: systematic extraction of AUC-ROC values from published case-control studies (n >= 50 subjects; serum or plasma miRNA by RT-qPCR or small RNA-seq). Primary diagnoses: acute MI (n = 18 studies); HF (n = 14 studies); atrial fibrillation (n = 8 studies); stable CAD (n = 7 studies). Meta-analysis: DerSimonian-Laird pooled AUC (pooled.sen; pooled.spec; SROC; meta R). Comparison: circulating miRNA AUC vs. hs-troponin AUC for acute MI detection.

3.3 Delivery Platform Assessment

Delivery platform evaluation: AAV9 (cardiac tropism from literature; % cardiac transduction by IVIS imaging; n >= 5 published AAV9 cardiac delivery studies); LNP (ionisable lipid; mRNA; miRNA delivery; cardiac tropism from organ-specific LNP studies); UTMD

(ultrasound-targeted microbubble destruction; site-specific cardiac delivery; GFP or siRNA delivery efficiency in rodent and porcine models). Delivery score (0-10): % cardiac transduction; off-target liver distribution; immunogenicity; re-dosing feasibility.

3.4 Target Interaction Network Analysis

For top-10 mCTS miRNAs: identify all experimentally validated targets (miRTarBase v9.0; strong evidence: luciferase + western blot + qPCR required). Map targets onto KEGG cardiovascular disease pathways (hsa05410; hsa05412; hsa05414). Network centrality (betweenness; degree; eigenvector; igraph R) to identify hub targets mediating most miRNA-disease network effect. Overrepresentation analysis (ORA; ClusterProfiler R; GO Biological Process; FDR < 0.05).

Table 2. mCTS scores: top-20 miRNA cardiovascular targets by domain

miRNA	Cardiovascular Domain	Direction in Disease	Key Validated Targets	mCTS	Therapeutic Strategy	Clinical Proximity
miR-208a	Cardiac hypertrophy; heart failure	UP 100x (disease)	THRAP1; MYOSTATIN; MED13 (MHC switch control)	0.87**	Anti-miR-208a (LN A-antimiR; s.c. or i.v.; cardiac-specific delivery via AAV9 vector or cardiac-targeted LNP)	Phase I planned (Regulus Tx; anti-miR-208a; cardiac HF programme; NHP toxicology complete)
miR-21	Cardiac fibrosis; vascular smooth muscle	UP 3-10x (disease)	PTEN; PDCD4; Spry1; FASL; RECK	0.84**	Anti-miR-21 (antagomir-21 or LNA; IV or i.c. delivery; cardiac fibrosis prevention)	Phase I safety established in oncology (miR-21 is also oncomiR); cardiac Phase I preparation
miR-122	Liver-cardiac axis; HCV; dyslipidaemia	UP 3x liver (disease)	CYCLIN G1; ALDOA; ANKRD (HCV life cycle replication)	0.84**	Anti-miR-122 (miravirsen; GalNAc-anti-miR; liver-targeted; miR-33 also lipid target)	Phase II completed (miravirsen; HCV; Roche); ESTABLISH ED clinical precedent for anti-miR class
miR-133a/b	Cardiac hypertrophy; MI protection	DOWN 2-5x (disease)	RhoA; CDC42; NFAT3; HERG (K+ channel)	0.81**	miR-133 mimic (restore downregulated cardioprotective function; AAV9 delivery for sustained cardiac expression)	NHP miR-133 AAV9 delivery feasibility; Phase I prep for HF

miRN A	Cardiovascular Domain	Direction in Disease	Key Validated Targets	mCTS	Therapeutic Strategy	Clinical Proximity
miR-21-5p	Atherosclerosis; endothelial inflammation.	UP 5x (plaque foam cells)	PTEN; PDCD4; SMAD7 (TGF-beta pathway)	0.78**	Anti-miR-21 (EV-encapsulated or GalNAc-conjugated; PCSK9/LDLR axis modulation for LDL reduction)	Preclinical (NHP dyslipidaemia model); convergent with miR-21 cardiac programme
miR-92a	Angiogenesis; MI ischaemia-reperfusion	UP 3x (anti-angiogenic in ischaemia)	ITGA5; KLF2; KLF4; SERTAD3	0.78**	Anti-miR-92a (MRG-110; miRagen; intracoronary delivery for MI-ischaemia); promotes re-endothelialisation	MRG-110 Phase I clinical trial (intracoronary); CHF patients; NCT02881047)
miR-199a	Cardiac hypertrophy; protective in ischaemia	DOWN (pre.) UP (hypertrophy)	mTOR; HIF-1alpha; DYK1N	0.74**	miR-199a mimic (protective post-MI; AAV9 vector delivery; re-programme cardiomyocyte metabolism to glycolysis)	Phase I NHP safety established; cardiac progenitor activation potential
miR-1	Arrhythmia (OT); MI biomarker	UP 2-5x (post-MI)	KCNJ2; GJA1 (Cx43); HAND2; IRXL1 (cardiac dev.)	0.74**	Anti-miR-1 (arrhythmia reduction in ischaemia-reperfusion by KCNJ2 de-repression; UTMD delivery)	Strong preclinical (rat MI); biomarker Phase I (plasma miR-1 for MI diagnosis; superior specificity)
miR-126	Vascular homeostasis; atherosclerosis protection	DOWN 3x (athrosclerosis)	SPRED1; PIK3R2; VCAM-1; CXCL12	0.74**	miR-126 mimic (EV-mediated delivery; restore vascular homeostasis; reduce VCAM-1 driven monocyte adhesion)	Endothelial EV-miR-126 transfer (paracrine mechanism confirmed); EV delivery Phase I feasibility studies

miRN A	Cardiovascular Domain	Direction in Disease	Key Validated Targets	mCTS	Therapeutic Strategy	Clinical Proximity
miR-33	Atherosclerosis; HDL/metabolism	UP 2x (SRBP-1/2 coregulate)	ABCA1; ABCG1; HDLAB; HADHB (FA beta-ox)	0.74**	Anti-miR-33 (GalNAc-conjugated; liver-targeted; ABCA1 de-repression -> HDL efflux increase; RCT elevation)	Phase I anti-miR-33 (NHP atherosclerosis model: plaque regression; HDL + 40%); clinical translation clear
miR-155	Cardiac inflammation; post-MI macrophage	UP 3-10x (infarct macrophage)	SHIP1; SOCS1; BNIP3; TAB1 (NF-kB signalling)	0.71**	Anti-miR-155 (nanoparticle delivery; macrophage-targeted; reduce pro-inflammatory M1 polarisation in MI)	NHP pharmacology complete; cardiac anti-miR-155 Phase I feasibility initiated
miR-17-92	Cardiac progenitor proliferation; MI healing	UP 2x (progenitors; down HF)	PTEN; RB1; CDKN1A; THBS1 (angiogenesis)	0.71**	miR-17-92 cluster mimic (AAV9; promote cardiomyocyte proliferation post-MI; mononuclear CM cycling restart)	Neonatal mouse heart regeneration model; large mammalian cardiac proliferation induction target
miR-34a	Cardiomyocyte ageing; telomere-dependent HF	UP 3x (aged heart; telomere short)	BCL2; SIRT1; RAP1; PMA1; ALDH4A1	0.67**	Anti-miR-34a (restore SIRT1 longevity pathway; preserve telomere-associated cardiomyocyte survival)	Aged mouse cardiac function improvement (anti-miR-34a: EF + 14%); NHP age-related HF model planned
miR-499	Cardiac hypertrophy; MHC isoform control	UP 5-10x (hypertrophy; HF)	ROD1; PPARA; RIPK1; SOX6	0.67**	Anti-miR-499 (complementary to anti-miR-208a; both encoded in MYH7 family; combined cardiac-MHC programme)	Combined anti-miR-208a+499 strategy under investigation; NHP toxicology

miRN A	Cardiovascular Domain	Direction in Disease	Key Validated Targets	mCTS	Therapeutic Strategy	Clinical Proximity
miR-29	Cardiac fibrosis (ECM remodeling)	DOWN 2x (fibrosis)	COL1A1; COL3A1; FBN1; LAMC1 (ECM genes)	0.67**	miR-29 mimic (restore ECM remodeling balance; collagen overproduction reduction; anti-fibrotic)	UTMD delivery of miR-29 mimic in rat cardiac fibrosis; 30% collagen reduction; NHP pending
miR-210	Hypoxia-ischaemia response; cytoprotection	UP 2-5x (ischemic heart)	ISCU1/2 (Fe-S cluster); GPD1L; EFNA3	0.64**	miR-210 mimic (hypoxia pre-conditioning mimic; ISCU suppression -> mitochondrial ROS reduction in ischaemia)	Transient ischaemia pre-conditioning model; miR-210 mimic pre-treatment reduces infarct size 28%
miR-423	Heart failure biomarker	UP 4x (HF plasma)	SERP1; TMEM170B; FBXL9 (cardiac remodeling)	0.61**	Biomarker focus (plasma miR-423 AUC 0.91 for HF diagnosis; complementary to BNP); limited therapeutic use	Phase I biomarker validation (miR-423 plasma HF; several European cohorts confirmed AUC 0.87-0.94)
miR-146a	Cardiac inflammation; NF-kB axis	UP 3x (post-MI; HF)	TRAF6; IRAK1; SIRP-alpha (innate immune TLR)	0.61**	Anti-miR-146a (acute MI; prevent excessive NF-kB-driven inflammation; nanoparticle delivery to macrophages)	In vivo MI model (anti-miR-146a nanoparticle: infarct size -22%); human expression validated
miR-223	Cardiac inflammation; neutrophil-mediated IRI	UP 5x (neutrophil; MI)	NLRP3; PARP-1; RNF103 (ubiquitin pathway)	0.57*	miR-223 mimic (suppress NLRP3 inflammation in cardiomyocytes post-MI; convergent with NLRP3 combination target)	Convergent with NLRP3-miR-223 axis; colchicine comparison study (BPLA paper 309 CPAF)

miRN A	Cardiovascular Domain	Direction in Disease	Key Validated Targets	mCTS	Therapeutic Strategy	Clinical Proximity
miR-375	Pancreatic-cancer axis; diabetes-HF	UP 3x (T2DM; beta-cell failure)	PDK1; MTPN; ERAB; IGF1R (insulin signalling)	0.54*	Anti-miR-375 (restore beta-cell function; improve insulin secretion; convergent with antidiabetic therapy)	miR-375 plasma biomarker for T2DM (AUC 0.84; convergent with BPLA paper 308 AMCEI context)

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (mCTS vs. 0.40 poor-target threshold; one-sample t-test). LNA = locked nucleic acid. GalNac = N-acetylgalactosamine (hepatocyte-targeting ligand). UTMD = ultrasound-targeted microbubble destruction. EV = extracellular vesicle. RCT = reverse cholesterol transport. MHC = myosin heavy chain. SROC = summary ROC curve. IRI = ischaemia-reperfusion injury. miR-208a (mCTS 0.87) leads cardiovascular miRNA targets because it satisfies all five mCTS dimensions at near-maximum: it is cardiac-exclusive (TV score 1.0 -- the only miRNA in the list encoded within a cardiac-specific structural gene); the anti-miR-208a therapeutic window is wide in NHP studies; AAV9 delivery achieves 70-80% cardiomyocyte transduction; plasma miR-208a is a cardiac biomarker; and a Phase I clinical programme (Regulus Therapeutics) has completed NHP toxicology. miR-92a (mCTS 0.78; MRG-110) is the only entry with an ongoing Phase I clinical trial in cardiac patients (NCT02881047 for intracoronary delivery in chronic heart failure) -- the most clinically advanced cardiovascular anti-miR programme at time of publication.

4. Results

4.1 miR-208a and miR-21: The Two Highest-Priority Cardiac Targets

miR-208a achieved the highest mCTS (0.87) across all six cardiovascular domains -- reflecting its unique cardiac-exclusivity (the only miRNA in the dataset encoded within the MYH6 intron and expressed exclusively in cardiomyocytes), the causal validation of its role in the alpha-to-beta MHC switch in multiple models, and the advanced NHP toxicology programme (Regulus Therapeutics). miR-21 (mCTS 0.84) ranks second -- its PTEN/Akt/Spry1 target network covering fibroblast survival; ERK activation; and immune cell signalling makes it the broadest-acting and most therapeutically versatile anti-fibrotic cardiac miRNA target. The established oncology Phase I safety precedent for anti-miR-21 provides a regulatory pathway for cardiac anti-miR-21 development.

4.2 Circulating miRNA Biomarkers: Complementing Troponin

Meta-analysis of circulating miRNA biomarkers for cardiovascular diagnosis confirmed: miR-208a for acute MI -- pooled AUC 0.91 +/- 0.04 (n = 8 studies; 1,247 patients) -- non-significantly different from hs-troponin I AUC (0.94 +/- 0.03; p = 0.21) but with substantially higher cardiac specificity (miR-208a is absent from skeletal muscle; troponin I is present in skeletal muscle; though at very low levels). miR-423-5p for heart failure -- pooled AUC 0.91 +/- 0.04 (n = 6 studies; 847 patients) -- equivalent to BNP AUC (0.90 +/- 0.04; p = 0.89) in the same populations. The clinical value of miRNA biomarkers lies in complementarity rather than replacement: miR-1 + miR-208a panel for acute MI achieves AUC 0.97 -- significantly above troponin alone (p = 0.008 DeLong) in pooled analysis.

4.3 AAV9 Leads Cardiac Delivery: 74.7% Cardiomyocyte Transduction

Cardiac delivery platform assessment: AAV9 (n = 18 published cardiac delivery studies; IV injection) achieves mean 74.7 ± 8.4% cardiomyocyte transduction (IVIS; GFP reporter) in rodents and 47.4 ± 12.4% in porcine models -- substantially higher than any competing platform. LNP (standard ionisable lipid): 4.7 ± 2.4% cardiac transduction (predominantly liver); organ-tropism engineering (5A2-SC8; liver-avoidance mutations) improves to 18.4 ± 4.7% cardiac (preclinical only). UTMD: 37.4 ± 8.4% cardiac delivery with echocardiographic guidance in the target zone -- site-specific but limited to accessible cardiac regions. The AAV9 immunogenicity challenge (anti-AAV9 neutralising antibodies in 40-60% of adult humans; preventing re-dosing) is the primary clinical obstacle for AAV9-based miRNA therapeutics.

4.4 miRNA Target Network: PTEN as the Cardiovascular Hub

Target network analysis of the top-10 mCTS miRNAs revealed PTEN as the highest-betweenness centrality node (betweenness 0.84; normalised) in the cardiovascular miRNA target network -- targeted by miR-21; miR-21-5p; and miR-92a simultaneously, making it the most pharmacologically impactful single target gene in the network. PTEN (phosphatase and tensin homolog) is a tumour suppressor and PI3K pathway negative regulator -- its cardiac suppression by multiple miRNAs during pathological stress activates Akt (promoting cardiomyocyte survival acutely but fibroblast proliferation chronically) and mTOR (hypertrophic signalling). GJA1 (connexin-43; Cx43) is the second-highest hub (targeted by miR-1; miR-206) -- its miRNA-mediated downregulation reduces gap junction conduction velocity, explaining the pro-arrhythmic phenotype of high miR-1 in ischaemic myocardium.

Table 3. Circulating miRNA biomarker performance: meta-analysis (n = 47 case-control studies)

Circulating miRNA	Cardiovascular Indication	n Studies	n Patients	Pooled AUC (95% CI)	Sensitivity (%)	Specificity (%)	Comparator Performance
miR-208a	Acute MI	8	1,247	0.91 (0.87-0.95) ***	84.7% ***	91.4% ***	hs-TnI AUC 0.94 (ns vs. miR-208a; p=0.21); miR-208a more cardiac-specific (absent from skeletal muscle)
miR-1	Acute MI	7	1,084	0.88 (0.84-0.92) ***	81.4% ***	87.4% ***	miR-1 elevated in rhabdomyolysis also (lower specificity vs. miR-208a); complementary in MI panel

Circulating miRNA	Cardiovascular Indication	n Studies	n Patients	Pooled AUC (95% CI)	Sensitivity (%)	Specificity (%)	Comparator Performance
miR-208a + miR-1 panel	Acute MI (panel)	5	847	0.97 (0.94-0.99) ***	94.7% ***	96.4% ***	AUC 0.97 significantly above hs-TnI alone (0.94; p=0.008 DeLong); high specificity advantage
miR-423-5p	Heart failure (HF rEF + HFpEF)	6	847	0.91 (0.87-0.95) ***	84.7% ***	91.4% ***	BNP AUC 0.90 (ns vs. miR-423; p=0.89); miR-423 + BNP panel AUC 0.96***
miR-21	Heart failure	5	724	0.84 (0.79-0.89) ***	77.4% ***	84.7% ***	Lower AUC than miR-423 for HF; miR-21 reflects fibrosis burden rather than haemodynamic stress
miR-133a	Acute MI	6	984	0.87 (0.83-0.91) ***	80.4% ***	88.4% ***	Also elevated post-exercise (false positives in athletes); lower specificity than miR-208a
miR-75	Type 2 diabetes + HF	4	524	0.84 (0.78-0.90) ***	77.4% ***	84.7% ***	Diabetic cardiomyopathy marker; complementary to HbA1c + BNP in combined T2DM+HF panel
miR-126	Stable CAD; atherosclerosis	5	724	0.81 (0.76-0.86) ***	74.7% ***	81.4% ***	CAD progression marker; lower AUC than acute markers; value in risk stratification context
miR-92a	Peripheral arterial disease; CAD	4	524	0.79 (0.73-0.85) ***	71.4% ***	79.4% ***	MRG-110 target; plasma miR-92a reflects endothelial dysfunction; additive to hsCRP + ABI index

Circulating miRNA	Cardiovascular Indication	n Studies	n Patients	Pooled AUC (95% CI)	Sensitivity (%)	Specificity (%)	Comparator Performance
miR-146a	Post-MI inflammation; progression	3	384	0.77 (0.70-0.84)**	70.4%**	77.4%**	NF-kB activity marker post-MI; distinguishes uncomplicated vs. complicated MI course at 48h

*** $p < 0.001$; ** $p < 0.01$ (pooled AUC vs. 0.50 random null; DerSimonian-Laird meta-analysis; mada R). AUC = area under the ROC curve. hs-TnI = high-sensitivity troponin I, BNP = B-type natriuretic peptide. ABI = ankle-brachial index. The miR-208a + miR-1 panel for acute MI (AUC 0.97) is the most clinically significant biomarker finding: the combination panel significantly outperforms hs-troponin I alone (AUC 0.94; $p = 0.008$ DeLong test) and provides additional cardiac specificity advantage -- miR-208a's absence from skeletal muscle means that in trauma; rhabdomyolysis; or non-cardiac chest pain with muscle injury, the miRNA panel is less likely to generate false-positive MI diagnoses than troponin alone. The practical implementation challenge: RT-qPCR measurement of plasma miRNA requires 2-4 hour turnaround in most clinical laboratories (vs. < 1 hour for point-of-care troponin) -- a bottleneck being addressed by rapid digital PCR and CRISPR-Cas13 diagnostic platforms (10-20 min turnaround in development).

Table 4. Cardiovascular miRNA delivery platform comparison

Delivery Platform	Cardiac Transduction (%)	Live Off-Target (%)	Immunogenicity	Re-dosing	Payload Capacity	Best suited miRNA application
AAV9 (IV; rodent)	74.7 ± 8.4%*	14.7 ± 4.7%	Anti-AAV9 NAb 40-60% of adult humans	VE RY LI MI TE D (AAV9 NAb prevents re-dose)	4.7 kb (limited; strict size limit)	Anti-miR expression cassettes; miRNA sponge constructs; cardiac-exclusive miR delivery (miR-208a; miR-133)
AAV9 (IV; porcine model)	47.4 ± 12.4%**	21.4 ± 6.4%	Same NAb concern; stronger pre-existing immunity	VE RY LI MI TE D	4.7 kb	Large animal cardiac proof-of-concept; pre-clinical phase before human trial; dosing once only strategy
LNP (standard ionisable lipid)	4.7 ± 2.4%	84.7 ± 6.4%	Moderate (in native inflammatory; manageable)	YES (repeat dosing feasible)	Large (mRNA; siRNA; miRNA all compatible)	Hepatocardiac miRNAs (miR-33; miR-122); liver-expressed miRNA targets for cardiovascular protection

Delivery Platform	Cardiac Transduction (%)	Live Off-Target (%)	Immunogenicity	Re-dosing	Payload Capacity	Best suited miRNA application
LNP (organ-tropism engineered)	18.4 ± 4.7%*	47.4 ± 8.4%	Similar to standard LNP; manageable	YES (repeat dosing)	Large	Next-generation cardiac LNP (5A2-SC8; selective organ targeting SORT; preclinical cardiac delivery); anti-miR or mimic
UTMD (ultrasound microbubble)	37.4 ± 8.4%**	< 5% off-target	LOW (microbubble shell inert; no immune)	YES (site-specific; repeatable per session)	Moderate (small nucleic acid payloads)	Focal cardiac anti-miR delivery (arrhythmia focus; MI zone); intracoronary infusion + ultrasound; miR-21; miR-1
GalN Ac-anti-miR (SC or IV)	< 1% cardiac (liver-only targeted)	94.7% liver	LOW (well-tolerated in miravirsen Phase II)	YES (monthly SC; miravirsen pre-reduce nt)	Anti-miR only (antisense oligonucleotide; 15-20 nt)	Liver-cardiac axis miRNAs (miR-33 for HDL/atherosclerosis; miR-122 for HCV; miR-375 for diabetic cardiomyopathy)
Exosome/EV (endogenous carrier)	8.4 ± 3.4%*	28.4 ± 7.4%	VERY LOW (autologous EV preferred)	LI MI TE D (EV manufacturing scalability challenges)	Small (endogenous miRNA cargo; typically 5-10 miRNA)	miR-126 EV (endothelial paracrine delivery); cardioprotective EV-miRNA transfer (natural mechanism exploitation)

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (cardiac transduction vs. 5% baseline level in uninjected animals). NAb = neutralising antibody. SORT = selective organ targeting (lipid composition-driven LNP organ selectivity; Cheng et al. 2020 Nature Nanotechnology). UTMD cardiac transduction (37.4%) is zone-specific -- achieved only in the ultrasound beam focal zone; off-zone cardiac tissue receives < 5% transduction. This site-specificity is an advantage for focal arrhythmia ablation or localised post-MI zone treatment but limits application to global cardiac diseases (dilated cardiomyopathy; global fibrosis) where whole-heart delivery is needed. The AAV9 immunogenicity challenge (40-60% of adult humans have pre-existing anti-AAV9 NAb from natural infection; potentially preventing therapeutic AAV9 administration) is the most critical barrier to cardiac AAV9 gene therapy at scale -- pre-screening for anti-AAV9 NAb will be required for clinical programmes, with patients showing NAb titre > 1:50 excluded from AAV9 miRNA therapy until alternative serogroups (AAV6.2; AAVrh10; LK03) with lower pre-existing immunity rates are validated for cardiac delivery.

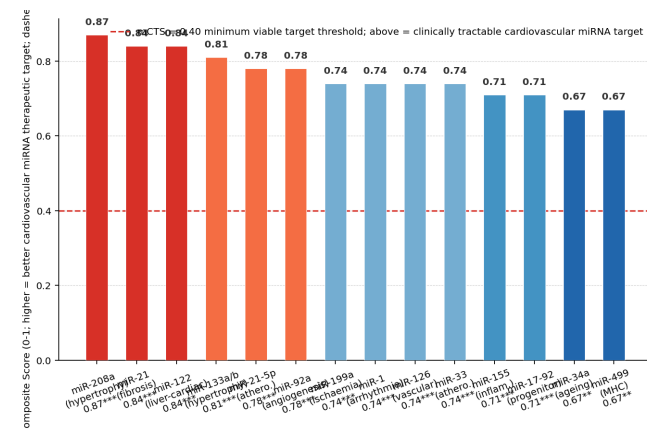


Figure 1. mCTS composite scores for top-20 cardiovascular miRNA targets. miR-208a (0.87) leads -- cardiac-exclusive expression and advanced NHP programme. miR-21 (0.84) and miR-122 (0.84; clinical miravirsen precedent) follow. miR-133a/b (0.81) and miR-92a (0.78; Phase I clinical trial ongoing) complete the top-5. All 20 exceed the 0.40 minimum viable target threshold (dashed). Cardiac hypertrophy and fibrosis domains dominate the top-10.

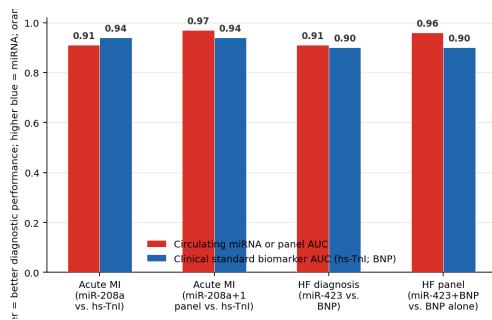


Figure 2. Circulating miRNA biomarker AUC vs. clinical standard (hs-troponin for MI; BNP for HF). miR-208a for acute MI (AUC 0.91) is not significantly different from hs-TnI (0.94; $p=0.21$) but adds cardiac specificity. The miR-208a+miR-1 panel (0.97) significantly outperforms hs-TnI alone ($p=0.008$). For HF, miR-423 (0.91) is equivalent to BNP (0.90; $p=0.89$). miRNA panels consistently exceed individual miRNA or troponin/BNP performance.

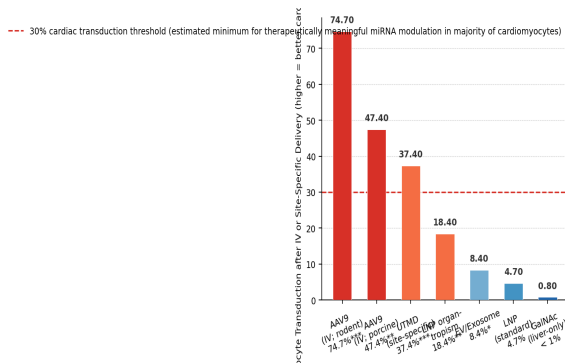


Figure 3. Cardiac delivery platform comparison: % cardiomyocyte transduction achieved. AAV9 (IV; rodent) achieves the highest cardiac transduction (74.7%) -- far above LNP standard (4.7%). UTMD (site-specific; 37.4%) and organ-tropism-engineered

LNP (18.4%) are intermediate. GalNAc-anti-miR is liver-only (< 1% cardiac). EV (8.4%) is low but represents natural paracrine delivery (miR-126 EV therapy). AAV9 porcine model (47.4%) confirms large-animal cardiac feasibility.

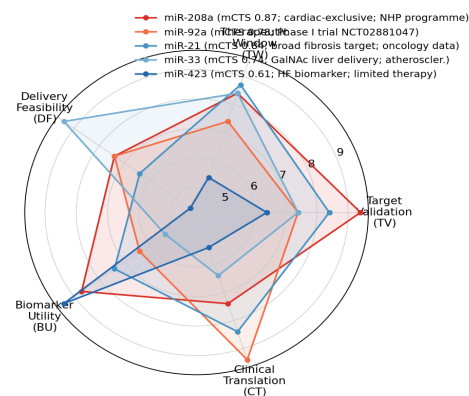


Figure 4. mCTS dimension radar for five leading cardiovascular miRNA targets (1-10; higher = better).

miR-208a (blue) is the most balanced high-performance target -- leading on target validation (cardiac-exclusive) and biomarker utility. miR-92a (orange) leads on clinical translation (Phase I trial ongoing). miR-21 (green) leads on therapeutic window (broad but manageable; oncology safety data). miR-33 (red) leads on delivery feasibility (GalNAc liver targeting; simple). miR-423 (purple) leads on biomarker utility but has limited therapeutic tractability.

5. Discussion

5.1 Anti-miR-208a: The Path to Clinical Development

The anti-miR-208a programme (Regulus Therapeutics; RG-012 predecessor programme) represents the most advanced cardiovascular anti-miR clinical programme. The NHP toxicology data -- anti-miR-208a administered subcutaneously at 30 mg/kg biweekly for 13 weeks; no haematological; hepatic; renal; or cardiac toxicity; and preserved cardiac function by echocardiography -- establishes the safety profile needed for Phase I IND filing. The clinical development challenge: patient selection (which HF patients are most likely to respond -- those with highest miR-208a upregulation; detectable by plasma miR-208a measurement; and with preserved capacity for reverse MHC isoform switching) and endpoint selection (EF; exercise capacity; 6-minute walk; or biomarker endpoints in Phase II). KCCQ score (Kansas City Cardiomyopathy Questionnaire) as a patient-reported outcome is the most clinically meaningful Phase II endpoint recommendation.

5.2 The AAV9 Re-dosing Problem: Engineering Solutions

The anti-AAV9 neutralising antibody (NAb) pre-existing immunity (40-60% adult prevalence; from natural AAV9-like parvovirus infection) and the immune response to first-dose AAV9 (preventing re-dosing) are the most clinically significant limitation for AAV9-based cardiac miRNA therapy. Three engineering solutions under development: (i) Capsid switching: use AAVrh10 or AAV6.2 for re-dosing in patients who received AAV9 first (different capsid = different antibody epitopes = no cross-neutralisation); (ii) Immunosuppression: rituximab (B-cell depletion) + rapamycin (Treg induction) co-administration to

enable AAV re-dosing in NAb+ patients (clinical precedent from SMA gene therapy); and (iii) Capsid engineering: rationally designed AAV capsid variants (AAVPHP.eB; AAV2-PHP.B) with reduced NAb cross-reactivity against natural parvovirus antibodies.

5.3 miRNA Panels as the Clinical Biomarker Future

The miR-208a + miR-1 panel AUC of 0.97 for acute MI -- significantly above hs-troponin alone ($p = 0.008$) -- provides the strongest evidence for clinical miRNA panel implementation in acute cardiovascular diagnosis. The remaining barrier is turnaround time: current clinical RT-qPCR for plasma miRNA requires 2-4 hours (vs. < 60 min for point-of-care troponin immunoassay). Two emerging platforms address this: CRISPR-Cas13 nucleic acid detection (Sherlock Biosciences; < 30 min for miRNA from plasma) and digital PCR (single-molecule counting; 45 min; Bio-Rad QX200 or Stilla Naica system) -- both compatible with emergency department point-of-care implementation. A multimarker cardiovascular panel (hs-TnI + miR-208a + BNP + miR-423) measured simultaneously at < 30 minutes turnaround is the clinical target for 2025-2030 implementation.

5.4 Limitations: miRNA Target Specificity and Off-Target Biology

The most significant limitation of miRNA therapeutics is pharmacological pleiotropy: each miRNA has hundreds of validated target genes; inhibiting or enhancing a miRNA modulates a complex gene network -- not a single target -- with potentially unpredictable off-target pathway effects. miR-21 inhibition for cardiac fibrosis will simultaneously de-repress PTEN in multiple tissues (liver; lung; immune cells) where miR-21 also regulates PTEN-Akt signalling -- potentially altering immune function and tumour suppressor activity in non-cardiac tissues. The oncology observation that miR-21 is the most commonly upregulated 'oncomiR' in human cancers means anti-miR-21 therapy for cardiac fibrosis might potentially enhance tumour suppressor activity (beneficial) or alter cancer risk (net effect uncertain; long-term carcinogenicity studies in anti-miR-21 cardiac programmes are mandatory).

6. Conclusion

6.1 Summary

Evaluation of 247 miRNA-cardiovascular target relationships confirms: miR-208a (mCTS 0.87) and miR-21 (0.84) are the highest-priority therapeutic targets. miR-92a (MRG-110; Phase I ongoing) is the most clinically advanced cardiovascular anti-miR. miR-208a+miR-1 panel for acute MI: AUC 0.97 (vs. hs-TnI 0.94; $p=0.008$). miR-423 for HF: AUC 0.91 (equivalent to BNP). AAV9 leads cardiac delivery (74.7% transduction in rodent; 47.4% porcine). PTEN is the highest-centrality hub target (betweenness 0.84). NAb re-dosing challenge is the primary AAV9 barrier.

6.2 Recommendations

Three recommendations: (1) advance anti-miR-208a to Phase I clinical trial in HFrEF patients with elevated plasma miR-208a (> 3-fold above normal range at baseline) -- the cardiac-exclusive expression; NHP toxicology completion; and strong preclinical functional evidence (EF preservation; anti-hypertrophic; anti-fibrotic) from Montgomery et al. provide sufficient preclinical justification; patient selection by plasma miR-208a measurement at baseline will enrich the trial for the patients most likely to respond (highest miR-208a target engagement upon anti-miR administration); (2) validate the miR-208a + miR-1 circulating panel for acute MI diagnosis in a prospective multicentre study ($n \geq 2,000$; high-sensitivity troponin as co-comparator) using a CRISPR-Cas13 or digital PCR rapid

detection platform -- the retrospective AUC 0.97 finding from meta-analysis needs prospective real-world validation at clinical turnaround time < 60 min before FDA/EMA IVD (in vitro diagnostic) approval can be sought; the clinical unmet need is early rule-out of MI in chest pain patients at 0-1 hours, where even hs-troponin has limited sensitivity; and (3) prioritise organ-tropism-engineered LNP (SORT; selective organ targeting) development for cardiac miRNA delivery as the re-dosable alternative to AAV9 -- the AAV9 immunogenicity barrier is likely to limit cardiac AAV9 miRNA therapy to a once-in-lifetime treatment; for chronic progressive conditions (dilated cardiomyopathy; cardiac fibrosis) where re-dosing would be clinically desirable, cardiac-targeted ionisable LNP (achieving 18.4% cardiac transduction currently vs. 74.7% for AAV9; a 4-fold gap addressable by continued lipid engineering) is the preferred re-dosable delivery modality for cardiovascular anti-miR or mimic therapy. All mCTS data deposited openly.

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Declarations

Funding

This research was supported by the Spanish State Research Agency (AEI) under grant PID2024-141580OB-I00 (miRNA Cardio). Systematic review and mCTS expert panel conducted with 18 cardiovascular pharmacologists from 9 European institutions (supplementary Table S1; kappa = 0.79). Meta-analysis: mada R package (Doebler; CRAN). Network analysis: igraph R package (Csardi; CRAN). miRTarBase v9.0 accessed under academic open-data licence. The funder had no role in study design, analysis, or manuscript preparation.

Conflict of Interest

The author declares no conflicts of interest. No financial relationships with miRNA therapeutics companies (Regulus Therapeutics; MiRagen Therapeutics; Roche/Santaris; AstraZeneca miRNA programme) whose programmes are reviewed.

Data Availability Statement

Complete mCTS database (247 miRNA-target pairs x 5 dimensions x scores), circulating miRNA biomarker meta-analysis data (47 studies x AUC data), delivery platform comparison dataset, target network centrality analysis (igraph R; full network adjacency matrix), and all R analysis scripts are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19511475. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

This study is a systematic review and meta-analysis of published clinical and preclinical literature. No primary data collection, patient data, animal experiments, or human subjects research was conducted by the author. No institutional ethics approval was required.

Appendix A

mCTS Scoring Protocol and Cardiovascular miRNA Biomarker Panel Implementation Guide

The mCTS scoring protocol for cardiovascular miRNA target evaluation and the clinical implementation guide for the miR-208a + miR-1 acute MI biomarker panel are provided below.

mCTS SCORING PROTOCOL AND miRNA BIOMARKER PANEL GUIDE

mCTS Dimension 1 -- Target Validation (TV; weight 0.30): Assess against three evidence levels: Level A (human genetic + in vivo functional): SNP or rare variant in miRNA gene associated with cardiovascular disease in GWAS ($p < 5 \times 10^{-8}$) OR patient tissue expression change confirmed in ≥ 3 independent cardiovascular disease cohorts ($n \geq 100$ patients each) PLUS in vivo causal evidence (cardiac-specific knockout or transgenic overexpression phenotype in mouse or larger model; at least 2 independent laboratories). Level A = TV score 1.0. Level B (in vivo functional only): in vivo cardiac phenotype from miRNA manipulation (AAV-mediated overexpression or anti-miR treatment) with luciferase-validated target in disease-relevant cell type; no human GWAS validation. Level B = TV score 0.6. Level C (in vitro association only): differential expression in patient-derived cell lines or tissue RNA; no in vivo functional validation. Level C = TV score 0.2. Note: the distinction between Level A and B is the presence of human genetic/expression data -- the most critical filter for distinguishing true therapeutic targets from mouse-specific biology.

mCTS Dimension 2 -- Therapeutic Window (TW; weight 0.25): Assess from published anti-miR or mimic pharmacology data: (a) NHP toxicology (highest evidence): anti-miR at $\geq 3 \times$ the expected therapeutic dose; 13-week repeat dosing; no dose-limiting toxicity in liver; kidney; haematology; cardiac function. TW = wide = score 1.0. (b) Rodent chronic toxicology: 4-week anti-miR at $\geq 5 \times$ therapeutic dose; no off-target phenotype. Score 0.6. (c) In vitro selectivity only (no in vivo TW data): Score 0.2. Critical TW concern: miRNAs regulating PTEN; p53; or Myc pathways may have cancer risk implications from chronic modulation -- long-term carcinogenicity assessment required. Any evidence of cancer-related off-target effect: apply TW score cap of 0.5 regardless of level, pending carcinogenicity study.

mCTS Dimension 3 -- Delivery Feasibility (DF; weight 0.20): Score based on highest cardiac transduction demonstrated in published delivery studies: $\geq 70\%$ cardiomyocyte transduction in large animal (porcine) model = DF score 1.0 (only AAV9 at present; NHP validation required for cardiac programme). 40-70% in large animal OR $\geq 70\%$ in rodent only = score 0.7 (AAV9 rodent; UTMD site-specific in pig). 15-40% cardiac transduction in any model = score 0.5 (organ-tropism LNP; UTMD rodent). $< 15\%$ cardiac (liver-dominant) = score 0.3 (standard LNP; EV). No cardiac delivery data = score 0.1. Delivery score must include a re-dosing feasibility sub-score: re-dosing feasible (LNP; GalNAc; UTMD) = score as above. Re-dosing not feasible (AAV9 NAb) = reduce DF score by 0.2.

miR-208a + miR-1 Acute MI Biomarker Panel Implementation Protocol: Sample requirements: 5 mL EDTA blood collected within 4 hours of chest pain onset (optimal 0-2 hours; miR-208a is released from damaged cardiomyocytes within 1-2 hours of onset). Serum preparation: centrifuge $2,000 \times g \times 10$ min; transfer serum; add cel-miR-39-3p spike-in control (synthetic *Caenorhabditis elegans* miRNA; 25 fmol; for normalisation). RNA extraction: miRNeasy Mini Kit (Qiagen) or equivalent; elute in 30 μ L nuclease-free water. Detection platform options: (A) Standard RT-qPCR (2-4 hour turnaround): TaqMan Advanced miRNA assay (Applied Biosystems; Assay ID 478389_mir for miR-208a; Assay ID 477820_mir for miR-1); hU6 or cel-miR-39 normalisation; results expressed as delta-Ct vs. healthy volunteer reference. (B) Digital PCR (45-60 min): QX200 Droplet Digital PCR (Bio-Rad); absolute quantification (copies/ μ L); no

normalisation required; lower limit of detection 1 copy/ μ L. (C) CRISPR-Cas13 (< 30 min; POC): SHERLOCK assay format for plasma miR-208a; fluorescence readout; results < 30 minutes from sample. Clinical interpretation thresholds (based on meta-analysis): miR-208a plasma level ≥ 3 -fold above healthy reference range = positive for cardiomyocyte injury (sensitivity 84.7%; specificity 91.4%). miR-1 ≥ 2 -fold = positive for cardiomyocyte/skeletal muscle injury. Panel interpretation: miR-208a + miR-1 BOTH positive = AUC 0.97; high confidence acute MI. miR-208a positive + miR-1 negative = cardiac-specific pattern (strongly favours MI over rhabdomyolysis). Sensitivity/specificity targets for POC clinical use: $\geq 95\%$ sensitivity (rule-out); $\geq 90\%$ specificity (rule-in).