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PROSPECTS OF SERCA2A-BASED GENE THERAPY FOR HEART FAILURE

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ABSTRACT
Heart failure (HF) remains one of the leading causes of morbidity and mortality worldwide, and despite substantial progress in pharmacological and device-based management, a considerable proportion of patients continue to progress toward refractory disease. At the cellular level, HF of virtually any etiology converges on a common defect: impaired cardiomyocyte calcium handling, of which downregulation of the sarco(endo)plasmic reticulum Ca2+-ATPase 2a (SERCA2a) is a central feature. This has made SERCA2a one of the most extensively pursued molecular targets for gene-based intervention in cardiology. This review summarizes the biology of SERCA2a — its structural family, cardiac localization, and role in excitation–contraction coupling — and evaluates the genetic and pharmacological evidence linking its loss to contractile failure. The core of the review is a critical, trial-by-trial analysis of preclinical and clinical gene therapy programs built around SERCA2a restoration or activation, from the original AAV1.SERCA2a (CUPID) program through its clinical failure in CUPID-2, to second-generation strategies employing AAV9 vectors, indirect SERCA2a activation via phosphatase-1 inhibitor 1c (AB-1002/GenePHIT), and small-molecule, RNA-interference-, and antisense-based modulators of the SERCA2a–phospholamban axis. The advantages and unresolved limitations of this therapeutic direction — vector tropism, transduction efficiency, immunogenicity, and patient selection — are discussed together with emerging strategies intended to overcome them. The overall picture is one of a biologically well-validated target whose clinical translation has been constrained chiefly by delivery technology and trial design rather than by the underlying pathophysiological rationale.

KEYWORDS: SERCA2a; heart failure; gene therapy; calcium handling; adeno-associated virus; phospholamban

1.INTRODUCTION

1.1 Epidemiology and Global Burden of Heart Failure

Heart failure is not a single disease but a clinical syndrome that represents the common endpoint of nearly every form of chronic cardiac injury — ischemic, hypertensive, valvular, genetic, or toxic. According to the Global Burden of Disease framework and the most recent Heart Failure Society of America statistical updates, global HF prevalence is estimated at 1–3% of the adult population and has more than doubled since 1990 [1–4]. In the United States alone, approximately 6.7 million adults live with HF, a number projected to rise to 8.7 million by 2030 and to exceed 11 million by 2050; the lifetime risk of developing HF has climbed to roughly one in four adults [1,2]. Regional distribution is markedly uneven, with Asia accounting for approximately half of global cases, followed by Europe, Africa, and the Americas [3,4]. Notably, the proportion of patients presenting at a younger age is increasing, a trend more plausibly attributable to the rising prevalence of obesity, diabetes, and hypertension in midlife than to demographic aging alone [1].

1.2 Diagnosis and Guideline-Directed Therapy

HF is defined by a constellation of clinical signs and symptoms supported by objective evidence of cardiac dysfunction — echocardiographic assessment of ejection fraction and filling pressures, natriuretic peptide measurement, and, increasingly, cardiac magnetic resonance and biomarker panels that discriminate HF with reduced, mildly reduced, or preserved ejection fraction (HFrEF, HFmrEF, HFpEF) [5,6]. This classification carries direct therapeutic consequences: the evidence base for guideline-directed medical therapy — mineralocorticoid receptor antagonists, beta-blockers, angiotensin receptor–neprilysin inhibitors, and sodium–glucose cotransporter-2 inhibitors — is strongest for HFrEF and considerably thinner for HFpEF, which now accounts for roughly half of all incident HF [5–11]. Landmark trials such as RALES, MERIT-HF, PARADIGM-HF, DAPA-HF, and EMPEROR-Reduced established the current four-pillar pharmacological framework for HFrEF and meaningfully reduced hospitalization and mortality [7–11]. Device-based therapies — cardiac resynchronization, implantable defibrillators, and mechanical circulatory support — extend survival in selected subgroups but do not correct the underlying molecular deficit, and cardiac transplantation remains constrained by donor organ scarcity.

1.3 Rationale for Gene-Based Therapy

Even with optimal guideline-directed therapy, five-year mortality after an HF diagnosis remains close to 50%, and each recurrent hospitalization for decompensated HF accelerates myocardial deterioration through a cycle that current drugs slow but rarely interrupt [1,2]. Conventional HF pharmacotherapy acts largely on neurohormonal signaling — unloading the heart or blunting maladaptive receptor activation — rather than correcting the primary molecular lesion that underlies contractile failure at the level of the cardiomyocyte [12,13]. Gene therapy offers a fundamentally different logic: rather than modulating the neurohormonal milieu around a failing myocyte, it aims to restore a specific molecular deficiency inside the cell itself. Among the many candidate targets explored — beta-adrenergic signaling components, S100A1, adenylyl cyclase type 6, phospholamban, and regulatory microRNAs — SERCA2a has accumulated by far the largest body of mechanistic, preclinical, and clinical data, and it is on this trajectory, its successes as well as its instructive failures, that the present review is focused [12,13].

2. PATHOGENESIS OF HEART FAILURE

2.1 Neurohormonal Activation and Ventricular Remodeling

Regardless of the initiating insult, HF develops through a sequence of compensatory responses that, sustained over time, become themselves pathogenic. An initial reduction in cardiac output triggers activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system, which acutely support arterial pressure and organ perfusion but, when chronically engaged, produce cardiomyocyte hypertrophy, beta-1 adrenergic receptor desensitization, interstitial and perivascular fibrosis, and endothelial dysfunction [50,51]. Natriuretic peptides (ANP, BNP) are released as a counter-regulatory mechanism, but their vasodilatory and natriuretic effects are progressively overwhelmed by the dominant vasoconstrictor and sodium-retaining systems — a mismatch that is directly relevant to SERCA2a biology, since BNP itself has been shown to suppress SERCA2a expression, a mechanism later implicated in interpreting the negative outcome of the CUPID 2 trial (Section 4) [17]. At the tissue level, these stresses drive ventricular remodeling: myocyte hypertrophy, progressive cardiomyocyte loss through apoptosis and necrosis, and expansion of the extracellular matrix by activated fibroblasts, producing a stiffer, less compliant, and electrically heterogeneous myocardium that is prone to reentrant arrhythmias [51,52].

2.2 Calcium-Handling Defects in the Failing Cardiomyocyte

The cellular correlate of this remodeling is a defect in calcium handling recognized as a near-universal feature of the failing cardiomyocyte, irrespective of etiology [14,53]. Normal excitation–contraction coupling begins with depolarization-triggered Ca^{2+} influx through L-type calcium channels, which triggers calcium-induced calcium release from the sarcoplasmic reticulum (SR) via ryanodine receptors. This cytosolic Ca^{2+} rise activates the myofilaments and produces contraction; relaxation then depends on the rapid removal of cytosolic Ca^{2+}, achieved predominantly by SERCA2a pumping Ca^{2+} back into the SR, with a smaller contribution from the sarcolemmal Na^{+}/Ca^{2+} exchanger [14,15]. In the failing heart, this cycle is disturbed at multiple points: ryanodine receptors become 'leaky,' producing diastolic Ca^{2+} efflux that predisposes to delayed afterdepolarizations and arrhythmia; the Na^{+}/Ca^{2+} exchanger is often upregulated, raising diastolic Ca^{2+}; and, centrally, SERCA2a expression and activity are reduced, slowing SR Ca^{2+} reuptake and depleting the SR Ca^{2+} store available for the next contraction [16,54,55]. This decline in SERCA2a function results from reduced transcription, accelerated protein turnover, and a growing list of inhibitory post-translational modifications — including oxidation of specific cysteine residues and lysine acetylation — that are increasingly recognized as druggable nodes in their own right [21]. The consequence is a self-reinforcing loop: impaired Ca^{2+} handling reduces contractile performance, which intensifies neurohormonal activation, which in turn further suppresses SERCA2a and promotes remodeling — a loop that current pharmacotherapy interrupts only partially, and that direct molecular correction of SERCA2a activity is designed to address at its origin.

3. SIGNIFICANCE OF SERCA2A FOR CARDIAC FUNCTION

3.1 Molecular Family, Structure, and Cardiac Localization

SERCA2a belongs to the P-type ATPase superfamily, specifically the SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) subfamily encoded in mammals by three genes — ATP2A1, ATP2A2, and ATP2A3 — giving rise to the SERCA1, SERCA2, and SERCA3 protein groups, with further tissue-specific isoforms generated by alternative splicing (SERCA2a and SERCA2b from ATP2A2; SERCA3a–3c from ATP2A3) [20]. SERCA1 is essentially restricted to fast-twitch skeletal muscle; SERCA2b is ubiquitously expressed at low abundance as the housekeeping isoform maintaining endoplasmic reticulum Ca^{2+} stores in virtually all cell types; SERCA3 isoforms are found mainly in non-muscle and secretory cells [20]. SERCA2a, the alternatively spliced cardiac/slow-twitch-muscle variant of ATP2A2, is expressed at extraordinarily high density in cardiomyocytes: absolute quantitative PCR in the murine heart shows SERCA2a constitutes roughly 99% of total cardiac SERCA transcript, with SERCA2b and the SERCA3 variants together contributing well under 1% [19]. At the subcellular level, SERCA2a localizes predominantly to the longitudinal (free) sarcoplasmic reticulum, positioning it to rapidly sequester the bulk cytosolic Ca^{2+} transient during diastole, in contrast to SERCA3b, which concentrates at the junctional SR [19]. This near-exclusive cardiac dominance is why SERCA2a — rather

than ATP2A2 as a whole, or another family member — has been selected as the therapeutic transgene of choice: restoring this single isoform is sufficient to address the great majority of SR Ca2+-ATPase activity lost in the failing cardiomyocyte.

3.2 Regulation by Phospholamban and the PP1/Inhibitor-1 Axis

Structurally, SERCA2a is a ten-transmembrane-domain, single-polypeptide pump that couples ATP hydrolysis to the transport of two Ca2+ ions per cycle from the cytosol into the SR lumen against a steep concentration gradient [70]. Its activity is tightly regulated by phospholamban (PLN), a small SR membrane protein that, in its dephosphorylated state, binds SERCA2a and inhibits its Ca2+ affinity; phosphorylation of PLN by protein kinase A or CaMKII, downstream of beta-adrenergic stimulation, relieves this inhibition and accelerates SR Ca2+ uptake, providing the principal mechanism of lusitropic reserve during exercise or stress [15,16]. This PLN–SERCA2a interaction is itself governed by the balance of protein kinase A and protein phosphatase 1 (PP1) activity, the latter modulated by the endogenous inhibitor protein I-1 — a regulatory node that has itself become a gene therapy target distinct from, but mechanistically convergent with, direct SERCA2a gene transfer (Section 4.6) [16].

3.3 Evidence from Genetic Loss-of-Function Models

Because SR Ca2+ reuptake by SERCA2a governs both the rate of myocardial relaxation and the amount of Ca2+ available for the subsequent beat, its activity is a primary determinant of both diastolic and systolic performance. In the failing human heart, SERCA2a mRNA and protein are consistently reduced, reflecting the combined effects of reduced expression, altered PLN stoichiometry, and inhibitory post-translational modification — notably oxidation at Cys498, recently shown to be normally protected by the NADPH-oxidase subunit p22phox, whose loss accelerates SERCA2a degradation and worsens dysfunction after pressure overload [21]. Whether reduced SERCA2a is a cause of contractile failure or merely an epiphenomenon of the failing phenotype has been addressed directly through genetic loss-of-function studies in animals, summarized in Table 1, which collectively establish causality. Constitutive heterozygous Serca2 knockout mice (Serca2+/–), expressing roughly 55–65% of normal SERCA2a protein, display only mild baseline dysfunction under resting conditions; this reserve is exhausted under stress, since Serca2+/– hearts subjected to ischemia–reperfusion show significantly impaired postischemic relaxation, elevated diastolic Ca2+, and — after a more severe infarction protocol — larger infarct size than wild-type hearts [24]. A more severe genetic model, using an inducible, cardiomyocyte-restricted Serca2 knockout in adult mice, showed a clear dose–response relationship between residual SERCA2a content and organ-level function: at one week post-induction, with SERCA2a protein at roughly 32% of normal, isolated whole-heart function was only modestly impaired, but by four weeks, with SERCA2a protein near-undetectable, ventricular performance was severely compromised and the animals progressed to overt, lethal heart failure within seven to ten weeks [25]. Together, these studies provide a graded causal chain — from partial to near-complete SERCA2a loss, and from subclinical dysfunction to lethal failure — that constitutes the principal mechanistic justification for pursuing SERCA2a restoration as a therapeutic strategy.

Table 1. Genetic and gene-transfer models establishing the causal role of SERCA2a in cardiac contractile function.

Model	Species	Genetic manipulation	Key cardiac phenotype	Ref.
Serca2 heterozygous KO (Serca2+/–)	Mouse	~35–45% reduction in SERCA2a protein/activity	Normal baseline function; impaired postischemic relaxation and larger infarct size after I/R	[24,25]
Inducible cardiomyocyte-specific SERCA2 KO	Mouse (adult)	Cre/loxP-mediated deletion of Atp2a2	32% residual SERCA2a at 1 week (mild dysfunction); near-absent SERCA2a at 4 weeks (severe dysfunction, lethal HF at 7–10 weeks)	[26]
AAV1.SERCA2a overexpression, ex vivo	Isolated human failing cardiomyocytes	AAV1-mediated SERCA2a transgene	Restored Ca2+ transient amplitude and cell shortening	[27,28]
AAV1.SERCA2a overexpression, pig volume-overload model	Pig	Intracoronary AAV1.SERCA2a	Increased cardiac SERCA2a expression; improved hemodynamics	[29]
AAV9-S100A1 (comparator Ca2+-handling target)	Pig, post-MI	Retrograde coronary-venous AAV9-S100A1	Prevented/reversed LV remodeling; restored SR Ca2+ handling	[30,31]

shRNA/miR-PLN AAV9 (indirect SERCA2a activation)	Rat / sheep / dog	AAV-mediated phospholamban knockdown	Restored systolic/diastolic function in rat and sheep; toxicity signal in dogs	[32–35]
AAV9-SERCA2a, diabetic cardiomyopathy model	Mouse (ob/ob)	Cardiac-specific AAV9-SERCA2a	Improved LV function and glucose/lipid metabolism	[36]

Abbreviations: I/R, ischemia–reperfusion; SR, sarcoplasmic reticulum; MI, myocardial infarction; KO, knockout.

4. SERCA2A-BASED GENE THERAPY FOR HEART FAILURE

4.1 Preclinical Foundation and Vector Considerations

Before clinical application, restoration of SERCA2a protein and activity by AAV-mediated gene transfer was tested across small- and large-animal HF models, including rodent pressure- and volume-overload models and pig models of ischemic and pacing-induced cardiomyopathy, with a consistent pattern of recovered LV systolic and diastolic function, increased SR Ca2+ content, and — importantly, given that increasing contractility is often pro-arrhythmic — a reduction rather than an increase in ventricular arrhythmia incidence [27–29]. Isolated failing human cardiomyocytes transduced ex vivo with AAV1.SERCA2a likewise showed restored Ca2+ transient amplitude and contractile shortening [27], providing a direct link between the animal data and human myocardial biology and forming the immediate rationale for first-in-human testing.

4.2 The CUPID Clinical Program

The Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) program was the first, and remains the most extensively studied, clinical test of SERCA2a gene therapy. An initial open-label, non-randomized dose-escalation study in nine patients with stable, symptomatic HFrEF receiving intracoronary AAV1.SERCA2a reported no serious adverse events over twelve months, alongside signals of symptomatic, functional, biomarker, and left-ventricular improvement, providing the basis for the subsequent randomized trials [26]. CUPID 1 (phase 2a) then randomized 39 patients with advanced HFrEF and an implanted defibrillator to placebo or one of three escalating doses of intracoronary AAV1.SERCA2a; the vector was well tolerated, and the high-dose cohort met a pre-specified composite six-month efficacy endpoint spanning symptoms, functional capacity, biomarkers, LV volumes, and clinical events, with benefit apparently sustained on longer-term follow-up out to three years [34,35]. These encouraging phase 2a results directly motivated CUPID 2, a considerably larger phase 2b trial designed as an event-driven, randomized, double-blind, placebo-controlled study in patients at high risk of recurrent HF hospitalization, with a primary endpoint of time to recurrent HF-related hospitalization. CUPID 2 randomized 250 patients (123 to AAV1.SERCA2a, 127 to placebo; 243 in the modified intention-to-treat population) and followed them for a median of 17.5 months. The result was unambiguously negative: 104 recurrent events occurred in the treatment arm versus 128 in placebo (hazard ratio 0.93, 95% CI 0.53–1.65, p=0.81), with no difference in mortality (21% versus 16%) and no safety signal attributable to the vector [36]. As the largest cardiac gene transfer study conducted to date, CUPID 2's failure to replicate the phase 2a signal — despite an unchanged mechanistic rationale and a therapy that remained demonstrably safe — reset expectations for the field [37].

4.3 AGENT-HF and SERCA-LVAD

AGENT-HF was designed as a companion mechanistic trial to CUPID 2, using an identical dose and delivery route of intracoronary AAV1.SERCA2a to assess effects on cardiac remodeling by serial cardiac magnetic resonance in nine patients with symptomatic HFrEF [38]. The trial was terminated prematurely when CUPID 2 reported its neutral primary result, and no benefit on ventricular remodeling was detected in the small cohort studied [38]. SERCA-LVAD extended this line of investigation into a mechanistically informative but similarly underpowered study. Designed as a phase 2a, randomized, double-blind, placebo-controlled trial of intracoronary AAV1/SERCA2a specifically in chronic HF patients supported by a left ventricular assist device — a population offering the unique opportunity for elective endomyocardial biopsy and, in some cases, explanted-heart tissue for direct assessment of vector delivery — the trial was intended to enroll 24 subjects but was halted after only five had been treated, a direct consequence of the neutral CUPID 2 result undermining clinical equipoise [39]. In the five treated patients followed for three years, exogenous viral DNA was confirmed in cardiac tissue in two patients, formally demonstrating that transgene delivery to the human heart is achievable, but absolute copy numbers remained low and no functional benefit was observed; no vector-related safety concerns emerged [39].

4.4 Reasons for the Clinical Failure of First-Generation SERCA2a Gene Therapy

Myocardial biopsy and autopsy analyses from CUPID 2 and SERCA-LVAD revealed strikingly low or

undetectable levels of vector-derived transgene DNA in human myocardium — orders of magnitude below the copy numbers achieved in the preclinical large-animal models on which the therapy's rationale rested — indicating that inadequate gene delivery, rather than a flawed biological hypothesis, was the most probable cause of clinical failure [39]. Contributing factors proposed include a dose that, while at the upper bound judged tolerable at the time, may have been too low relative to doses since shown to be necessary and tolerable in other monogenic-disease gene therapies; substantial first-pass vector washout in the coronary circulation during intracoronary delivery; and the relatively poor cardiotropism of the AAV1 serotype used, compared with serotypes such as AAV9 subsequently shown to achieve markedly higher cardiac transduction efficiency [39,47]. A separate, biologically distinct explanation focuses on the CUPID 2 enrollment criteria themselves: because patients were required to have markedly elevated BNP to enrich for high hospitalization risk, and because BNP suppresses SERCA2a expression, the trial may have selectively enrolled patients whose endogenous SERCA2a-suppressive signaling was maximal, potentially blunting the biological effect of exogenous SERCA2a delivery and confounding interpretation of the null result [17] — a hypothesis that, if correct, argues for biomarker-based patient stratification in future SERCA2a-directed trials rather than abandonment of the target.

4.5 Next-Generation Direct SERCA2a Gene Transfer

Rather than abandoning the SERCA2a hypothesis, the post-CUPID era has pursued improved delivery technology alongside indirect strategies. A 2025 preclinical study directly addressed the vector-tropism hypothesis by comparing cardiac-specific AAV9-mediated SERCA2a gene transfer with pharmacological SERCA2a activation (the allosteric small-molecule agonist CDN1163) in leptin-deficient (ob/ob) diabetic mice, a model of metabolic heart failure with reduced SERCA2a expression and combined systolic/diastolic dysfunction [22,33]. Both interventions improved ventricular function and, notably, produced favorable downstream effects on glucose and lipid metabolism, mitochondrial function, and oxidative stress, extending the therapeutic rationale for SERCA2a beyond pure contractile mechanics into cardiometabolic disease [22]. The authors explicitly framed their vector choice as a rationally designed departure from CUPID 2: AAV9 possesses substantially superior native cardiac tropism compared with AAV1, offering a plausible route to the higher myocardial transduction efficiency that biopsy data from the CUPID and SERCA-LVAD trials indicated was lacking in the earlier program [22,39]. A parallel, ongoing clinical program — MUSIC-HFpEF — is testing AAV1.SERCA2A specifically in patients with heart failure with preserved ejection fraction, discussed in Section 4.8 [40].

4.6 Indirect SERCA2a Activation: AB-1002 and the GenePHIT Trial

The most clinically advanced indirect strategy is AB-1002, a chimeric cardiotropic AAV2/AAV8 (AAV2i8) vector delivering a constitutively active form of protein phosphatase-1 inhibitor 1 (I-1c) [41,43]. Rather than supplying SERCA2a directly, AB-1002 works one regulatory step upstream: by chronically inhibiting PP1, it shifts phospholamban toward its phosphorylated, non-inhibitory state, thereby de-repressing the patient's own endogenous SERCA2a and restoring SR Ca2+ uptake without overexpressing the pump protein itself — an approach with the theoretical advantage of preserving physiological stoichiometry between the SERCA2a–phospholamban complex and its regulatory partners [41]. Having shown improved hemodynamics and contractile function in porcine HF models, AB-1002 progressed to a first-in-human, open-label, sequential dose-escalation phase 1 trial in patients with non-ischemic cardiomyopathy, NYHA class III symptoms, and LVEF 15–35% [43]. Across two dose cohorts (11 patients total), a single antegrade intracoronary infusion was well tolerated through 12 months, with no serious treatment-related adverse events, only transient and mild hepatic enzyme elevation, and a transient T-cell response; myocardial transduction efficiency, measured directly, was roughly thirty-fold higher than in the corresponding porcine model, and tissue from one explanted heart confirmed both transgene expression and the expected increase in phospholamban phosphorylation [43]. Full 12-month efficacy data, presented in 2025, showed clinically meaningful LVEF improvement in five of six patients in the lower-dose cohort and improvement or stabilization of NYHA functional class in five of six patients, a considerably more encouraging signal than CUPID 2 produced at a comparable stage, albeit in an open-label, non-randomized, small cohort that precludes firm efficacy conclusions [44]. On the strength of this dataset, AB-1002 has advanced into GenePHIT, a placebo-controlled, multicenter phase 2 trial testing two dose levels in roughly 150 patients with non-ischemic HFrEF, explicitly including patients with pre-existing AAV seropositivity — a population historically excluded from cardiac gene therapy trials [42].

4.7 Complementary Calcium-Cycling Gene and RNA-Based Therapies

A further set of approaches targets the same functional axis from complementary angles. Phospholamban is the natural counterpart of the AB-1002 strategy: early large-animal work using AAV- and adenoviral-mediated PLN silencing or dominant-negative PLN peptides restored SR Ca2+ uptake and improved contractile function in sheep and rat HF models [31,32], and an AAV-delivered artificial microRNA against PLN improved SR Ca2+ uptake in cardiomyocytes [33]; however, a shRNA-based approach tested in healthy canine hearts produced troponin elevation and cardiac dysfunction, illustrating that potent PLN suppression carries a toxicity risk that must be carefully titrated [34]. S100A1, a calcium-binding positive inotropic regulator that becomes depleted in

failing cardiomyocytes in parallel with SERCA2a, has shown durable functional rescue with AAV9-mediated gene transfer in a post-infarction porcine model, together with an acceptable safety profile at high expression levels in subsequent dose-escalation studies, positioning it as a calcium-handling target that could plausibly be combined with SERCA2a-directed approaches [30,31]. Adenylyl cyclase type 6 (AC6) gene transfer, which augments cAMP-generating capacity upstream of PLN phosphorylation, was tested in a 56-patient randomized phase 2 trial and found to be safe with a dose-range signal for improved ejection fraction and exercise duration [46]. At the RNA level, the miR-132 antisense oligonucleotide CDR132L — which targets a microRNA that suppresses SERCA2a expression among other Ca2+-handling and remodeling genes — was tested in a first-in-human, placebo-controlled phase 1b trial in 28 patients with chronic ischemic HF, showing a clean safety profile, dose-dependent target engagement, and a 23.3% median reduction in NT-proBNP at the effective dose, becoming the first antisense drug tested in an HF population [47,48]. Collectively, these programs illustrate that 'SERCA2a-based gene therapy' has evolved from a single-transgene concept into a family of convergent strategies — direct overexpression with improved vectors, disinhibition via the PP1–I-1–phospholamban axis, and RNA-based modulation of upstream regulators — unified by the same underlying molecular target.

4.8 SERCA2a Gene Therapy in HFpEF

HFpEF, which now accounts for roughly half of incident HF and for which pharmacological options remain comparatively limited, presents a distinct rationale for SERCA2a-directed therapy: abnormal myocardial relaxation in HFpEF is mechanistically tied to impaired SERCA2a-mediated Ca2+ reuptake, and, critically, HFpEF patients typically have lower circulating BNP than HFrEF patients with comparable symptom burden — potentially avoiding the BNP-mediated SERCA2a suppression implicated in the CUPID 2 failure [17,40]. This rationale has motivated MUSIC-HFpEF, an ongoing phase 1b, open-label, multicenter pilot trial of a single intracoronary infusion of AAV1.SERCA2A in ten patients with hemodynamically confirmed HFpEF, assessing safety and tolerability alongside resting and exercise hemodynamics, echocardiographic relaxation parameters, NT-proBNP, and functional status [40].

Table 2. Clinical trials of SERCA2a-directed and mechanistically related gene and RNA-based therapies for heart failure.

Trial	Phase	Agent / vector	Population (n)	Route	Principal outcome
Pilot open-label	1/2 (open-label)	AAV1.SERCA2a, dose escalation	HFrEF (n=9)	IC	Safe; signals of symptomatic/functional benefit at 12 months
CUPID 1	2a	AAV1.SERCA2a, 3 doses vs. placebo	Advanced HFrEF (n=39)	IC	Well tolerated; high-dose group met 6-month composite endpoint; benefit reported to 3 years
CUPID 2	2b	AAV1.SERCA2a (high dose) vs. placebo	HFrEF (n=243 mITT)	IC	Negative: HR 0.93 (95% CI 0.53–1.65) for recurrent HF events; no safety signal
AGENT-HF	2	AAV1.SERCA2a vs. placebo	Advanced HFrEF (n=9)	IC	Terminated early after CUPID 2; no remodeling benefit detected
SERCA-LVAD	2a	AAV1.SERCA2a vs. placebo	LVAD-supported HF (n=5, of planned 24)	IC	Terminated early; vector DNA confirmed in myocardium in 2/5 patients; no functional benefit
MUSIC-HFpEF	1b (ongoing)	AAV1.SERCA2a	HFpEF (n=10, planned)	IC	Safety/tolerability and hemodynamic pharmacodynamics; recruiting
AB-1002 first-in-human	1	AAV2i8–I-1c (indirect SERCA2a activation)	Non-ischemic HFrEF, NYHA III (n=11)	IC	Well tolerated; LVEF improvement in 5/6 lower-dose patients; PLN phosphorylation confirmed on biopsy
GenePHIT	2 (ongoing)	AAV2i8–I-1c, 2 dose levels vs. placebo	Non-ischemic HFrEF (n≈150, planned)	IC	Recruiting, including AAV-seropositive patients

AC6 gene transfer	2	Ad5.hAC6 (calcium-independent inotropic target)	HFrEF (n=56)	IC	Safe; dose-range signal for EF and exercise duration improvement
CDR132L phase 1b	1b	Antisense oligonucleotide vs. miR-132 (upstream of SERCA2a)	Chronic ischemic HF (n=28)	IV	Safe; dose-dependent miR-132 reduction; NT-proBNP fell 23.3% at ≥1 mg/kg

Abbreviations: IC, intracoronary; IV, intravenous; mITT, modified intention-to-treat; LVAD, left ventricular assist device; HR, hazard ratio; CI, confidence interval; NYHA, New York Heart Association; PLN, phospholamban; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

5. DISCUSSION

5.1 Advantages of SERCA2a-Directed Gene Therapy

The pathophysiological rationale for SERCA2a-directed therapy is arguably the most rigorously established of any cardiac gene therapy target, resting on concordant human tissue data, graded genetic loss-of-function studies across rodent models, and consistent efficacy across small- and large-animal HF models of diverse etiology [24–29]. Mechanistically, SERCA2a restoration offers something few pharmacological inotropes achieve: it increases contractility while reducing, rather than increasing, arrhythmic risk, because it corrects rather than bypasses the calcium-handling defect that itself predisposes to arrhythmia [27,29]. As a one-time intervention, gene therapy also carries the theoretical appeal of durable effect without the adherence burden that limits real-world effectiveness of chronic oral HF regimens.

5.2 Limitations and Unresolved Barriers

Against these strengths stand limitations that CUPID 2 exposed with particular clarity and that subsequent trials have only partially addressed [36,39]. Myocardial transduction efficiency achieved with first-generation AAV1 vectors delivered by intracoronary infusion has proven insufficient in humans despite adequacy in animal models, indicating that delivery technology, dosing, and administration route — not the underlying biology — have been the principal bottleneck [39]. Pre-existing humoral immunity to AAV serotypes excludes a meaningful fraction of potential patients from vector administration and complicates trial enrollment, though newer trials such as GenePHIT are beginning to include seropositive patients as tolerability data accumulate [42]. Patient heterogeneity and enrollment criteria matter more than initially appreciated, as the BNP-related hypothesis for CUPID 2's failure suggests [17]. Manufacturing scale, cost, and the practical complexity of intracoronary vector delivery in an interventional cardiology setting also remain nontrivial barriers to eventual clinical adoption even if efficacy is demonstrated.

5.3 Future Directions

The field's near-term trajectory is visible in the programs reviewed above: vectors with genuinely superior cardiac tropism (AAV9 and engineered capsids) aimed directly at correcting the transduction shortfall identified in CUPID biopsy data [22]; indirect disinhibition strategies (AB-1002/GenePHIT, PLN-directed RNA interference and antisense approaches) that may offer a more physiologically balanced restoration of Ca2+ handling than transgene overexpression [41–44,47]; and combination or precision approaches — pairing gene therapy with biomarker-defined patient selection, or coupling SERCA2a restoration with correction of specific inhibitory post-translational modifications such as Cys498 oxidation — that were not feasible when the CUPID program was designed [21]. Extension into HFpEF, a population with a distinct calcium-handling and neurohormonal profile that may be more receptive to SERCA2a restoration, represents a further rational direction now being tested prospectively [40]. Whether any of these routes converts a sound biological hypothesis into a clinically validated therapy will depend on the outcomes of GenePHIT, MUSIC-HFpEF, and the next generation of AAV9-based programs now entering translational development.

6. CONCLUSIONS

SERCA2a occupies a distinctive position among cardiac gene therapy targets: it is supported by an unusually coherent chain of evidence — from its near-exclusive cardiac localization and central role in excitation–contraction coupling, through graded genetic loss-of-function studies in animals, to consistent benefit across preclinical HF models — yet its clinical translation, embodied in the CUPID program, produced a stark contrast between compelling phase 2a signals and a definitively negative phase 2b outcome. Retrospective analysis points overwhelmingly to inadequate myocardial transduction and possibly unfavorable patient selection, rather than a flawed biological premise, as the explanation for this failure. The current generation of SERCA2a-directed strategies — improved AAV9-based vectors, indirect activation through the phosphatase-1/I-1/phospholamban axis exemplified by AB-1002, and RNA- or small-molecule-based modulators of the same regulatory network — represents a rational, evidence-driven response to these specific shortcomings rather than a departure from the underlying hypothesis. Given the magnitude and continued growth of the global HF burden and the persistent ceiling on benefit from neurohormonal pharmacotherapy alone, SERCA2a remains one of the most scientifically justified targets for a genuinely disease-modifying, mechanism-based intervention in heart

failure, and the results of the ongoing GenePHIT and MUSIC-HFpEF trials, together with next-generation vector programs, will be decisive in determining whether this two-decade research effort finally reaches clinical fruition.

Author Contributions
AVB and ANO conceived and designed the review. AVB, AAV, DDB and AOM performed the literature search and analysis. AVB wrote the original draft. AAV, DDB, AOM and ANO reviewed and edited the manuscript. ANO supervised the work. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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Not applicable.

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Conflict of Interest
The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies
During manuscript preparation, the corresponding author used an AI-based assistant (Claude, Anthropic) to perform literature retrieval and to draft and format sections of the text. All authors reviewed and edited the resulting content and take full responsibility for the content of the manuscript.

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