Received: 27 APR 2026 | Accepted: 12 MAI 2026 | **DOI:** https://doi.org/10.57849/ulisboa.fm.jscml.0000053.2026

JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Targeted Therapeutic Approaches in Hypertrophic Cardiomyopathy

This article was prepared as the final work for the Master's Degree in Medicine at the Faculty of Medicine, University of Lisbon.

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ABSTRACT: Hypertrophic Cardiomyopathy (HCM) is a common autosomal dominant inherited myocardial disease characterised by left ventricular hypertrophy, hypercontractility and diastolic dysfunction. Most cases are caused by mutations in sarcomeric protein genes, such as *MYH7* or *MYBPC3*. Clinical manifestations are heterogeneous, varying from none or mild exercise intolerance to severe lifestyle-limiting symptoms, heart failure, ventricular arrhythmias or sudden cardiac death. Diagnosis relies on multimodal imaging techniques and genetic testing. Current management includes symptom control with β-blockers, calcium channel blockers, and antiarrhythmics, as well as invasive strategies like septal myectomy in obstructive forms. Recently, myosin inhibitors (e.g., Mavacamten, Aficamten) have demonstrated significant improvements in functional status and left ventricular outflow tract (LVOT) obstruction gradients in obstructive HCM, although their benefits in non-obstructive HCM remain limited.

Target therapies are emerging as a potentially disease-modifying approach that targets the underlying genetic defects, even before the onset of phenotype. *In vitro* and pre-clinical studies using animal models and human induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) have demonstrated that techniques such as gene replacement, gene editing, allelic silencing, and other RNA based therapies can restore normal cardiomyocyte function.

These approaches are designed to target cardiomyocytes, employing viral vectors or nanoparticle-based delivery systems to reduce off-target effects. Despite significant progress, many challenges remained unsolved, including efficient delivery, immune system responses, long-term safety, and determining the optimal timing for intervention. Even so, gene and RNA based approaches may represent a transformative shift in HCM treatment, moving from symptom management to directly targeting the underlying cause of the disease.

KEYWORDS: Hypertrophic cardiomyopathy, Sarcomeric mutations, Gene therapy, RNA-based therapies, Cardiac myosin inhibitors.

1. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease, with an estimated prevalence ranging from 1:200 to 1:500 individuals in the general population. It is defined by the presence of left ventricular hypertrophy in the absence of abnormal loading conditions sufficient to explain it, such as long-standing hypertension or aortic stenosis[1]. HCM is usually inherited as an autosomal dominant disorder and is characterised by marked phenotypic heterogeneity, ranging from asymptomatic individuals to patients with heart failure, atrial or ventricular arrhythmias, and sudden cardiac death [2].

In most cases, HCM is caused by pathogenic variants in genes encoding sarcomeric proteins that integrate the contractile unit of heart muscle, the sarcomere (Fig. 1). Rather than causing primary structural instability, these variants predominantly alter sarcomere function. Although the underlying mechanisms are not yet fully elucidated, two major pathophysiological hallmarks have consistently emerged: hypercontractility with impaired relaxation, and increased myofilament calcium sensitivity, both of which contribute to excessive contractile activity and diastolic dysfunction [3].

At the molecular level, three-dimensional structural studies have shown that the motor domain of β-myosin heavy chain (β-MHC) can adopt distinct conformational states that regulate the availability of myosin heads for actin binding. In the super-relaxed state (SRX), the two myosin heads fold back against the thick filament backbone, forming the interacting-heads motif (IHM), an energy-conserving "OFF state". In the disordered-relaxed state (DRX), one head remains associated with the thick filament backbone, whereas the other becomes more accessible for actin interaction. In the active "ON state", both heads are available to bind actin and generate force [4]. Cardiac myosin-binding protein C (cMyBP-C) contributes to stabilisation of the IHM, thereby limiting myosin head mobility and reducing the number of heads available for contraction [5].

Cardiac contraction depends on ATP binding and hydrolysis, followed by phosphate release, which induces conformational changes in the myosin head and powers actin filament sliding. Phosphorylation of the regulatory light chain weakens head–tail interactions, destabilises the IHM, and shifts myosin heads towards the "ON state" [6]. In HCM, pathogenic variants in thick-filament genes, particularly *MYH7* and *MYBPC3*, disrupt this regulatory equilibrium and increase the proportion of myosin heads in the "ON state", thereby enhancing actin–myosin interaction [5,7,8]. By contrast, mutations affecting thin-filament proteins, such as troponin T and troponin I, generally increase myofilament calcium sensitivity and prolong thin- filament activation. Together, these abnormalities promote hypercontractility and impair myocardial relaxation, contributing to diastolic dysfunction [6,9].

Both preclinical and clinical studies indicate that the proportion of myosin heads in the energy-conserving "OFF state" is markedly reduced in HCM, falling to approximately 15–20%, compared with 40–50% in healthy

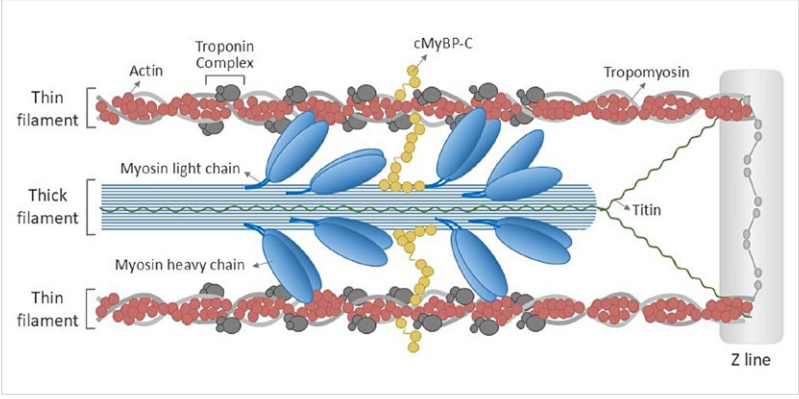

FIGURE 1. The cardiac sarcomere and its components.
Adapted from Lehman, 2022 [4]

myocardium (Fig. 2). This shift leads to increased ATP consumption, excessive cross-bridge formation during both systole and diastole, and energetically inefficient contraction [10].

The resulting hypercontractility, together with altered calcium handling and metabolic stress, promotes mitochondrial dysfunction and activates stress-responsive and pro- hypertrophic signalling pathways, including calcineurin–NFAT, MAPK, PI3K–mTOR, and TGF-β. These cascades drive pathological remodelling and contribute to the classical histopathological hallmarks of HCM, namely cardiomyocyte hypertrophy, myofibrillar disarray, interstitial fibrosis, increased myocardial stiffness, and small-vessel disease [11–13].

These molecular and cellular abnormalities arise from pathogenic variants in genes encoding sarcomeric proteins. Among these, the two most frequently implicated genes are *MYH7*, which encodes β-myosin heavy chain, and *MYBPC3*, which encodes cMyBP-C [14].

Together, pathogenic variants in these genes account for approximately 55–70% of genetically confirmed cases [15,16]. Other well-established sarcomeric genes include *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL2*, and *MYL3*, although each account for a smaller proportion of cases.

As an autosomal dominant disease, HCM is characterised by incomplete penetrance and variable expressivity, such that individuals carrying the same pathogenic variant may exhibit markedly different phenotypes[17]. Importantly, genetic abnormalities are thought to initiate myocardial dysfunction long before overt structural changes become evident. This concept is particularly relevant to the development of targeted therapies, since it supports the possibility of intervening early in the disease course, before irreversible remodelling has occurred [17–19].

Backwell and Marsh classified pathogenic variants in autosomal dominant diseases according to three principal molecular mechanisms: haploinsufficiency, gain-of-function, and dominant-negative effects [20]. Haploinsufficiency usually results from nonsense, frameshift or splicing variants that introduce premature stop codons and activate nonsense-mediated decay, thereby reducing the amount of functional protein. Gain-of-function mutations, often missense, produce a protein with increased or novel activity. On the other hand, dominant-negative mutations generate a mutant protein that interferes, directly or indirectly, with the normal function of the wild-type protein [20].

These concepts are highly relevant in HCM. Most *MYBPC3* variants are nonsense, frameshift, or splice-altering mutations that lead to haploinsufficiency, reduced levels of functional cMyBP-C, and impaired sarcomeric regulation; clinically, they are often associated with later disease onset [16,17,21]. By contrast, many pathogenic *MYH7* variants are missense mutations that exert dominant-negative effects, directly altering myosin head function and frequently producing a more severe and earlier phenotype [22,23]. The principal sarcomeric genes involved in HCM, together with their predominant mutation types and pathogenic mechanisms, are summarised in Table I [3,16].

Hypertrophic cardiomyopathy, however, is not exclusive to sarcomeric HCM. It may also occur in a range of genetic phenocopies, including Fabry disease caused by mutations in *GLA*, PRKAG2 syndrome associated with *PRKAG2* mutations, Danon disease caused by *LAMP2* mutations, and transthyretin cardiac amyloido-

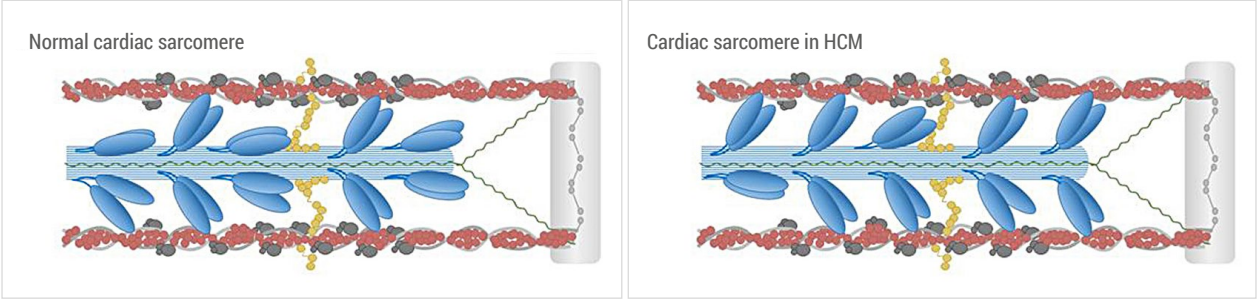

FIGURE 2. Comparison of sarcomeric structure under normal conditions and HCM. Adapted from Lehman, 2022 [4]

sis associated with *TTR* mutations [19,24–26]. These conditions may resemble HCM morphologically, but differ significantly in pathogenesis, extracardiac manifestations, prognosis, and treatment. Their main distinguishing features are summarised in Table II.

From a clinical perspective, HCM is commonly classified according to the presence or absence of left ventricular outflow tract (LVOT) obstruction, which results from the interaction between septal hypertrophy and systolic anterior motion (SAM) of the mitral valve [2]. In obstructive HCM, LVOT obstruction is dynamic and may be present at rest or provoked by physiological manoeuvres such as Valsalva or exercise. In some patients, the Valsalva manoeuvre may accentuate a systolic crescendo–decrescendo murmur. Non-obstructive HCM is diagnosed when characteristic hypertrophy is present

TABLE I. Major sarcomeric genes implicated in HCM and their pathogenic mechanisms

Gene	Encoded protein	Variant type	Main pathogenic mechanism	Typical phenotypic features
MYH7	β-myosin heavy chain	Missense	Dominant-negative effect; increased actin–myosin cross-bridge cycling and hypercontractility	Early onset, marked hypertrophy, variable arrhythmic risk
MYBPC3	Cardiac myosin-binding protein C	Frameshift, Nonsense, splicing	Haploinsufficiency leading to reduced cMyBP-C levels and impaired sarcomeric regulation	Later onset, milder hypertrophy, progressive diastolic dysfunction
TNNT2	Cardiac troponin T	Missense	Increased myofilament Ca^{2+} sensitivity with minimal hypertrophy	Mild hypertrophy, disproportionate arrhythmic risk
TNNI3	Cardiac troponin I	Missense	Impaired inhibitory regulation of actin–myosin interaction, delayed relaxation	Diastolic dysfunction, variable hypertrophy
TPM1	α-tropomyosin	Missense	Altered thin-filament regulation and Ca^{2+} sensitivity	Variable hypertrophy, familial clustering
ACTC1	Cardiac actin	Missense	Disrupted actin–myosin interaction and force transmission	Early-onset hypertrophy, variable severity
MYL2 / MYL3	Myosin regulatory and essential light chains	Missense	Altered modulation of myosin head activity	Hypertrophy with variable systolic and diastolic involvement

Adapted from Argirò, 2025 and Nogueira-Garcia, 2025.[3,16]

TABLE II. Major genetic phenocopies in the differential diagnosis of HCM

Condition	Key features	Distinguishing elements
Sarcomeric HCM	Asymmetric septal hypertrophy; SAM; dynamic LVOT obstruction	Pathogenic variants in sarcomeric genes; patchy mid-wall LGE
Fabry disease (GLA	Concentric LVH; inferolateral fibrosis	Low native T1; angiokeratomas; α- galactosidase A deficiency
Cardiac Amyloidosis (TTR)	Increased wall thickness; HFpEF	Apical sparing strain; global subendocardial LGE; extracardiac involvement
PRKAG2 syndrome	LVH with pre-excitation	WPW pattern; glycogen storage; PRKAG2 mutation
Danon disease (LAMP2)	Severe early LVH; systolic dysfunction	Skeletal myopathy; intellectual disability; X-linked inheritance

Abbreviations: HCM, hypertrophic cardiomyopathy; **LVH,** left ventricular hypertrophy; **SAM,** systolic anterior motion; **LVOT,** left ventricular outflow tract; **LGE,** late gadolinium enhancement; **HFpEF,** heart failure with preserved ejection fraction. *Adapted from Azevedo 2021; Bennett, 2023; Felix, 2025; Lopes, 2024; Teresi, 2025.[19,24–27]*

but no clinically significant resting or provocable LVOT gradient can be demonstrated[1,3].

Transthoracic echocardiography remains the primary imaging modality for the diagnosis of HCM. Current American Heart Association/American College of Cardiology (AHA/ACC) and European Society of Cardiology (ESC) guidelines define HCM as unexplained left ventricular wall thickness ≥15 mm in one or more myocardial segments, with or without right ventricular hypertrophy, that cannot be accounted for by abnormal loading conditions such as hypertension or aortic stenosis [2,26,28–30].

Cardiac magnetic resonance (CMR) imaging is recommended as part of the baseline assessment because of its superior spatial resolution and reproducibility, which enable accurate identification of apical or anterolateral hypertrophy, apical aneurysm, and myocardial fibrosis. CMR also plays an important role in distinguishing sarcomeric HCM from phenocopy conditions [27,28,31].

Genetic testing has become a central component of HCM evaluation, particularly in borderline cases and for family screening. Current guidelines recommend comprehensive or targeted next-generation sequencing panels in individuals with a definite or probable clinical diagnosis of HCM [28]. Identification of a pathogenic variant supports screening of first-degree relatives. Family members who test negative for the familial variant may be discharged from serial cardiac surveillance, whereas genotype-positive individuals require longitudinal follow-up to monitor for phenotypic conversion [28,32]. Despite the identification of more than 1,500 pathogenic variants in different genes, an estimated 40–60% of clinically diagnosed patients still lack a genetic diagnosis [32,33].

A multimodal diagnostic approach also facilitates recognition of disease stage. A commonly used clinical staging model divides HCM into four broad phases [10,19,27]: Stage I, genotype-positive/phenotype-negative disease, also referred to as the pre-phenotypic or non-hypertrophic phase; Stage II, genotype-positive/phenotype-positive disease, corresponding to the classic HCM phenotype and representing the most common clinical presentation; Stage III, adverse remodelling, characterised by myocardial fibrosis, worsening diastolic dysfunction, progressive atrial and ventricular dilatation, and a decline in ejection fraction to approximately

50–65%, with increased risk of heart failure and arrhythmias; and Stage IV, end-stage HCM, which occurs in a minority of patients, approximately 5–10%.

The epidemiology of HCM varies across countries and populations, reflecting disease heterogeneity, differences in screening practices, and under-recognition of subclinical disease. As a result, the true prevalence is likely underestimated. According to the ESC, prevalence ranges from approximately 1 in 500 to 1 in 200 individuals when current imaging and genetic criteria are applied [28]. Large registries such as the Sarcomeric Human Cardiomyopathy Registry (SHaRe) have substantially improved understanding of disease burden and natural history by providing data from specialised centres worldwide [34].

In Portugal, the Portuguese Registry of Hypertrophic Cardiomyopathy (PRo-HCM) remains the main national source of epidemiological data. In 2018, this registry included 1,042 patients diagnosed across several centres. The mean age at diagnosis was 53 years, approximately one- third of patients had a family history of disease, and LVOT obstruction was present in about 35% of cases [35].

Over recent years, HCM treatment has evolved from symptom control alone towards a more mechanistically oriented approach. Current guidelines emphasise treatment individualisation, guided by symptom burden, haemodynamic profile, complications, and 5-year sudden cardiac death risk estimation, while also increasingly incorporating strategies that may modify disease progression [2,28,34].

Conventional pharmacological therapy remains the cornerstone of symptomatic management. β-blockers are considered first-line treatment in symptomatic obstructive HCM because they reduce LVOT gradients and improve exercise tolerance. When β-blockers are ineffective or poorly tolerated, non-dihydropyridine calcium channel blockers such as verapamil may be used. Disopyramide may be added in selected patients with persistent obstruction because of its potent negative inotropic effect. Associated arrhythmias, especially atrial fibrillation, often require dedicated rhythm-control strategies, including amiodarone in selected cases [13,28,36].

For patients who remain severely symptomatic despite optimal medical therapy, septal reduction therapies are an important option. When performed at experienced centres, surgical septal myectomy achieves excellent symptomatic and haemodynamic outcomes, with low procedural mortality and morbidity. Alcohol septal ablation offers a less invasive alternative for selected patients with suitable coronary anatomy, particularly older individuals or those at higher surgical risk, although procedural success is somewhat lower [37].

More recently, cardiac myosin inhibitors have introduced a major shift in the therapeutic landscape of HCM. Mavacamten and aficamten directly target the hypercontractile state by reducing the number of functionally available myosin heads, attenuating excessive cross-bridge formation, and stabilising the myosin "OFF state". By acting at the level of sarcomeric mechanics, these agents reduce LVOT gradients, improve diastolic function, and alleviate symptoms, representing the first pharmacological therapies to directly target a core disease mechanism rather than its downstream consequences [38].

Nevertheless, current therapies still primarily act on the established phenotype. By contrast, gene-based therapies will act directly on the molecular substrate of disease. Although these strategies remain largely in preclinical or early clinical stages, they seek to correct or silence the primary molecular defect rather than simply manage its consequences [1,18,29,39].

2. TARGETED THERAPEUTIC STRATEGIES

Targeted therapeutic strategies for HCM can be broadly divided into three mechanistic levels: protein-level modulation, downstream pathway modulation, and gene- or RNA-targeted therapies. Together, these approaches move treatment beyond conventional symptomatic control towards therapies that directly address the molecular basis of disease.

The most clinically advanced mechanism-targeted therapies currently available for HCM are the cardiac myosin inhibitors mavacamten and aficamten, which act at the sarcomere level by attenuating hypercontractility and restoring a more physiologic balance of myosin head availability. Both agents reduce the number of actin–myosin cross-bridges by stabilising the energy-efficient super-relaxed (SRX) "OFF state" of myosin (Fig. 2) [36,38]. Mavacamten binds to the catalytic domain of the myosin head, inhibits ATPase activity, and promotes formation of the interacting-heads motif (IHM), thereby reducing myosin head availability and ATP consumption [40]. Aficamten acts through a similar principle, reducing contractility by slowing phosphate release, decreasing ATP turnover, and altering myosin head conformation, ultimately resulting in fewer myosin heads entering the active contractile cycle [38]. By counteracting sarcomeric hypercontractility, these agents directly target one of the central pathophysiological mechanisms of HCM.

A second therapeutic layer targets downstream signalling pathways activated by sarcomeric dysfunction, including pro-hypertrophic pathways such as MAPK, TGF-β, and Ca^{2+}/calmodulin-dependent calcineurin signalling, as well as the metabolic stress responses that contribute to the HCM phenotype [41]. Although no approved therapies currently act directly on these pathways in HCM, experimental approaches such as RNA interference (RNAi), antisense oligonucleotides (ASOs), and small-molecule pathway modulators aim to attenuate or prevent maladaptive hypertrophic remodelling [42].

The most upstream strategies are gene- and RNA-targeted therapies, which seek to correct, suppress, or bypass the causal molecular defect itself. These include gene replacement, gene editing, allelic silencing, splicing modulation, RNA editing, and non-coding RNA based therapies. Collectively, these approaches represent a spectrum of molecular interventions ranging from permanent genomic correction to reversible transcript-level modulation, each with distinct mechanistic advantages and translational challenges [23,43,44]. As illustrated in (Fig. 3), these strategies aim to intervene early in the pathogenic cascade by acting at the DNA, RNA, or protein level.

A) GENE REPLACEMENT

Gene replacement therapy is particularly relevant for *MYBPC3* related HCM, in which pathogenic variants commonly result in haploinsufficiency. In this setting, delivery of a functional wild-type copy of the gene can restore cMyBP-C expression and directly address the underlying molecular defect [45,46]. In this approach, full-length wild-type *MYBPC3* complementary DNA (cDNA) is packaged into a viral vector and delivered to cardiomyocytes, where it is transcribed and translated into functional protein that compensates for reduced endogenous expression [42].

Adeno-associated viral vectors (AAVs) are especially attractive for this purpose because of their strong cardiotropism and potential for sustained transgene expression. In recombinant AAV vectors, viral genes are replaced by the therapeutic transgene. Following delivery, the AAV genome generally persists episomally in the nucleus rather than integrating into the host genome, allowing long-term expression while reducing the risk of insertional mutagenesis [47,48].

Preclinical studies have shown that AAV-mediated *Mybpc3* delivery can rescue sarcomeric function and prevent development of the HCM phenotype in mouse models carrying frameshift *Mybpc3* mutations [42]. Mearini and colleagues demonstrated in a knock-in mice

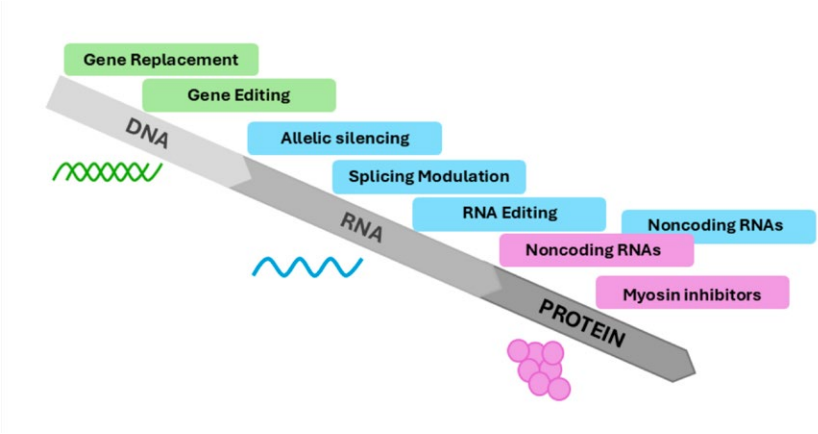

FIGURE 3. Targeted therapies for HCM target the molecular mechanisms underlying the disease at gene level (green boxes), RNA level (blue boxes) or protein level (pink boxes).

that a single systemic injection of AAV9-*Mybpc3*, driven by the cardiac troponin T promoter, increased *Mybpc3* mRNA and wild-type cMyBP-C protein levels, with effects persisting for more than 34 weeks [49]. In human iPSC-derived cardiomyocytes, the same strategy produced a twofold increase in *MYBPC3* mRNA and cMyBP-C protein expression, which was sufficient to suppress the hypertrophic phenotype at the cellular level [50].

This strategy has now entered clinical translation. In the ongoing MyPEAK-1 phase 1b/2 trial, patients with symptomatic HCM carrying pathogenic or likely pathogenic *MYBPC3* variants received a single intravenous infusion of TN-201, an AAV9 vector encoding *MYBPC3* cDNA. Early results indicate that TN-201 is generally well tolerated, with evidence of myocardial transgene expression, increased cMyBP-C protein levels, and favourable biomarker trends. However, patient numbers remain small and follow-up are still limited [51].

B) GENE EDITING, BASE EDITING, AND PRIME EDITING

Gene editing aims to correct the pathogenic variant directly within the genome. The best- studied platform is CRISPR/Cas9, together with more recent high-precision derivatives such as base editing and prime editing [12].

The CRISPR/Cas system was originally identified in bacteria as an adaptive immune mechanism against viruses. In genome editing, CRISPR/Cas9 introduces a double-strand DNA break at a genomic locus specified by a guide RNA (gRNA), enabling highly specific targeting [52]. Following DNA cleavage, endogenous repair pathways are activated, primarily non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is active throughout most of the cell cycle and generally introduces small insertions or deletions (indels), often resulting in gene disruption. This feature can be exploited therapeutically to silence dominant-negative alleles [53,54]. By contrast, HDR enables precise sequence correction when an exogenous therapeutic donor DNA is provided but is less efficient in post-mitotic cardiomyocytes [44].

A landmark proof-of-concept study by Ma et al. reported correction of a pathogenic MYBPC3 variant in human heterozygous embryos, suggesting that early embryonic cells may preferentially exploit HDR, with reduced mosaicism. However, the mechanisms underlying these observations remain debated, and both the reproducibility of the findings and the associated ethical issues have been questioned [43].

CRISPR/Cas9 has also been applied in vitro to model and correct HCM-associated variants. Pavlova and colleagues created iPSC-CMs carrying the likely pathogenic *MYBPC3* p.N515del variant and successfully corrected this in-frame deletion using CRISPR/Cas9. Comparison of the mutant and corrected isogenic lines showed that the variant was associated with increased cell size, whereas correction restored a normal phenotype without detectable off-target effects or karyotypic abnormalities [55]. In vivo, CRISPR/Cas9-mediated HDR targeting of the murine *Mybpc3* p.W1098X mutation produced low correction efficiency, but still yielded

modest functional benefit, suggesting that even partial editing may be biologically meaningful [56].

To overcome the limitations associated with double-strand DNA breaks and HDR dependence, newer platforms have been developed. Base editing uses a catalytically impaired Cas9 or Cas9 nickase fused to a deaminase enzyme, enabling targeted base transitions without inducing double-strand DNA breaks [57]. Chai et al. showed that adenine base editing could correct the pathogenic *MYH7* p.R403Q variant in iPSC-CMs and in a humanised mouse model, rescuing disease-associated phenotypes with minimal off-target activity [57].

Prime editing further expands the scope of precise editing by enabling base substitutions, as well as small insertions and deletions, without double-strand breaks or donor DNA templates. This system uses a Cas9 nickase fused to a reverse transcriptase and guided by a prime editing guide RNA (pegRNA), which both targets the site and encodes the desired sequence with the genetic modification. Because prime editing does not rely on HDR, it may be particularly advantageous in cardiomyocytes [58,59]. Although not yet directly applied to HCM, successful prime editing-mediated correction of pathogenic variants in dilated cardiomyopathy iPSC-CMs has already been reported, supporting its future potential in inherited cardiomyopathies [58].

C) ALLELIC SILENCING

Allelic silencing is particularly attractive for HCM caused by dominant-negative mutations, because it aims to selectively suppress the mutant allele while preserving the wild-type allele. By reducing production of the pathogenic protein and maintaining wild-type one, this strategy seeks to restore sarcomeric function. Two main molecular approaches have been explored: RNA interference (RNAi) and antisense oligonucleotides (ASOs) [60].

RNAi is a natural mechanism of post-transcriptional gene silencing in which small RNA molecules guide the RNA-induced silencing complex (RISC) to specific transcripts, resulting in mRNA degradation or translational repression. Small interfering RNAs (siRNAs) generally act through near-perfect complementarity, leading to highly specific cleavage of target mRNA, whereas endogenous microRNAs (miRNAs) typically bind with partial complementarity and regulate broader gene networks [60–62].

Using this principle, Migliore et al. designed allele-specific siRNAs targeting two pathogenic *TNNT2* missense mutations and demonstrated selective knockdown of mutant transcripts in reporter-based assays. Although these experiments were performed in HEK293 cells rather than cardiomyocytes, they provided proof of principle that single-nucleotide discrimination is feasible, although highly dependent on precise sequence optimisation [63].

ASOs provide an alternative allele-selective strategy. These short, chemically modified single-stranded oligonucleotides bind complementary RNA sequences and recruit RNase H, which cleaves the RNA strand of the RNA–DNA duplex and thereby degrades the target transcript. Their chemical modifications enhance stability, nuclease resistance, and specificity [64]. Anderson et al. demonstrated the feasibility of SNP-guided ASO-mediated silencing in *MYH7*, using common linked single-nucleotide polymorphisms as allele-specific markers to selectively suppress mutant transcripts in both human iPSC-derived cardiomyocytes and a humanised mouse model [65].

Direct comparison of siRNA- and ASO-based silencing in *MYH7*-R403Q iPSC-CMs showed that siRNAs achieved greater reduction of mutant transcript levels and more pronounced improvement in hypertrophic and contractile phenotypes, whereas ASOs showed higher allele specificity with more modest phenotypic rescue. These findings emphasise the balance between silencing potency and selectivity [64].

Importantly, both siRNA- and ASO-based therapies have already reached clinical application in transthyretin amyloid cardiomyopathy, where they reduce hepatic transthyretin production. Patisiran and vutrisiran act through RNAi-mediated degradation of *TTR* mRNA, whereas eplontersen is an ASO currently under evaluation in the phase 3 CARDIO-TTRansform trial[66]. Although cardiac amyloidosis differs mechanistically from HCM, these studies support the broader clinical feasibility of transcript-directed cardiovascular therapies.

D) SPLICING MODULATION

Splicing modulation targets pre-mRNA processing rather than the DNA sequence itself. Typically achieved using ASOs, this approach redirects the endogenous splicing machinery to promote exon inclusion or exon skipping, restore reading frames, reduce aberrant transcript formation, or prevent nonsense-mediated decay [67–70].

A well-established clinical precedent is nusinersen for spinal muscular atrophy, which promotes exon 7 inclusion in the *SMN2* transcript and restores production of functional SMN protein [67,71]. Applying the same principle to HCM, Gedicke-Hornung et al. used a knock-in *Mybpc3* mouse model carrying a splice-disrupting exon 6 mutation and demonstrated that ASO-mediated exon skipping reduced aberrant transcripts and increased production of a partially functional isoform. These findings provided proof of concept that splicing modulation may represent a causal therapeutic strategy for selected MYBPC3 mutations [72].

E) RNA EDITING

RNA editing has emerged as a promising strategy that targets pathogenic transcripts without altering the underlying DNA sequence. Because it acts at the RNA level, it offers reversible and potentially safer modulation than permanent genome editing. CRISPR-Cas13 systems, together with guide RNAs, enable highly specific recognition and degradation or modification of mutant transcripts, making this approach especially attractive for dominant- negative HCM variants such as those in *MYH7* [73,74].

Yang et al. developed a high-precision Cas13 variant capable of distinguishing transcripts differing by only a single nucleotide. In a murine model carrying the *Myh6* p.R872H variant, this system selectively reduced the mutant transcript while preserving wild-type expression, thereby overcoming a major limitation of earlier Cas13 systems, namely insufficient allele specificity [62].

F) NON-CODING RNA BASED THERAPIES

Non-coding RNAs, particularly miRNAs, regulate multiple gene networks involved in sarcomeric function, calcium handling, hypertrophic signalling, and fibrosis, and therefore contribute to cardiac remodelling in HCM [75].

Among these, miR-133 has emerged as an important regulator of cardiac hypertrophy and fibrosis in cardiomyocytes and cardiac fibroblasts [75]. Experimental studies have shown that miR-133 expression suppresses fibrotic and hypertrophic remodelling by modulating the TGF-β/Smad and PI3K/Akt pathways, as well as histone deacetylases and β-adrenergic signalling [76]. In particular, miR-133 reduces *TGF-β1* expression and downregulates connective tissue growth factor gene (*CTGF*), thereby limiting fibroblast activation and extracellular matrix deposition. However, this regulation is bidirectional, since TGF-β signalling can itself suppress miR-133 expression, creating a complex feedback loop [76].

Although non-coding RNA based therapies do not directly correct the causal mutation, they target convergent downstream mechanisms and may therefore complement gene replacement, gene editing, or allelic silencing approaches. As such, they may become part of combination strategies aimed both at correcting the primary defect and attenuating the maladaptive remodelling that defines clinical HCM.

3. PRECLINICAL MODELS AND DELIVERY PLATFORMS FOR TARGETED THERAPIES

The development of targeted therapies for HCM depends on experimental models that reproduce key genetic and phenotypic features of the disease, as well as on delivery systems capable of safely transporting therapeutic material to cardiomyocytes. In preclinical research, the two main model systems are animal models and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). These complementary platforms are essential for studying disease mechanisms, testing therapeutic efficacy, and evaluating safety [77].

Animal models, particularly genetically engineered mice and pigs, have been instrumental in investigating how sarcomeric mutations give rise to the HCM phenotype. Knock-in models carrying pathogenic variants in *MYH7* or *MYBPC3* genes allow the study of genotype–phenotype relationships and provide an in vivo platform for testing gene replacement, gene editing, and RNA-targeted therapies [77]. For example, porcine models carrying the *MYH7* R723G variant reproduce key features of HCM, including myocyte disarray and early myocardial abnormalities [22]. Similarly, *Mybpc3* knockout models have shown that loss of cMyBP-C leads to hypertrophy, arrhythmias, and a severe HCM phenotype, supporting the concept of *MYBPC3* haploinsufficiency as a major disease mechanism [78,79]. Despite their value, animal models remain limited by interspecies differences in cardiac physiology, whereas large-animal models, although closer to humans, are more costly and technically demanding [29,77].

iPSC-CMs provide a complementary human cellular platform for modelling HCM and testing targeted therapies in vitro. These cells are generated by reprogramming somatic cells from patients with sarcomeric

mutations into pluripotent stem cells and differentiating them into cardiomyocytes [21]. iPSC-CMs carrying pathogenic *MYH7* or *MYBPC3* variants reproduce major cellular hallmarks of HCM, including hypertrophy, myofibrillar disarray, abnormal calcium handling, and altered contractility [80]. They are particularly useful for mutation-specific studies because CRISPR/Cas9 can be used to generate or correct variants, producing isogenic cell lines that differ only at the disease-causing locus [81,82]. Their major limitation is their relative immaturity, as they more closely resemble fetal than adult cardiomyocytes, although maturation strategies such as three-dimensional culture, micropatterning, and mechanical stimulation have improved their physiological relevance [83–85].

Efficient delivery of therapeutic genetic material to cardiomyocytes is another critical requirement for gene-based therapies in HCM. Currently, the main delivery systems explored are viral vectors and non-viral vectors. Among viral platforms, adeno-associated viruses (AAVs) are the most widely used in cardiac gene therapy because of their strong cardiotropism and ability to support sustained transgene expression, usually as episomal DNA, thereby reducing the risk of insertional mutagenesis [47,86]. AAV9 is particularly effective for myocardial delivery after systemic administration and has been widely used for *MYBPC3* gene replacement, CRISPR/Cas9 delivery, and RNA based approaches [47,87]. A major limitation of AAV vectors is their restricted packaging capacity, although this can sometimes be overcome by using cDNA constructs [42,88].

Other viral vectors have a more limited role. Adenoviral vectors can efficiently transduce cardiomyocytes and remain episomal, but their clinical applicability is restricted by strong immunogenicity and pre-existing neutralising antibodies[89,90]. Lentiviral vectors provide stable genomic integration and are useful in vitro, particularly in iPSC-CM studies, but their use in vivo is limited by insertional mutagenesis risk, lower efficiency in adult cardiomyocytes, and the need for invasive delivery [91–93].

Among non-viral systems, lipid nanoparticles (LNPs) are the most advanced platform. They can encapsulate mRNA, ASOs, siRNAs, and CRISPR/Cas9 ribonucleoprotein complexes, do not integrate into the genome, and are easier to manufacture than viral vectors [94–96]. Their main limitation is preferential hepatic accumulation after systemic administration, which reduces delivery to the myocardium. For this reason, cardiac-targeted LNPs are being developed using ligands that enhance myocardial uptake [97,98]. Compared with AAVs, LNPs generally produce faster but more transient expression, a feature that may be advantageous for applications such as genome editing, where prolonged nuclease exposure could increase off- target effects [98,99].

Overall, animal models and iPSC-CMs provide the essential preclinical framework for evaluating targeted therapies in HCM, whereas vector design remains a central determinant of translational success. Progress in both model systems and delivery technologies will be critical for moving gene and RNA based therapies from experimental proof of concept to clinical application.

4. ADVANTAGES AND LIMITATIONS OF TARGETED GENE THERAPIES

Targeted gene therapies offer important conceptual advantages over conventional pharmacological or invasive treatments because they act directly on the molecular mechanisms that drive disease onset and progression. Unlike standard therapies, which mainly relieve symptoms or reduce left ventricular outflow tract obstruction, gene and RNA based strategies aim to correct the primary cause of sarcomeric dysfunction and may therefore prevent downstream hypertrophy, fibrosis, and diastolic impairment [2]. In principle, these approaches will also offer the possibility of durable or even curative benefit after a single intervention, unlike conventional therapies that require lifelong administration and lose effect when discontinued [36,86].

A major strength of these therapies is their potential for personalised, mutation-specific intervention. Allele-specific silencing can selectively suppress pathogenic MYH7 alleles while preserving wild-type expression, and base editing may allow correction of single-nucleotide variants in an individualised manner [65,81]. In addition, because these strategies can be designed to act preferentially in cardiomyocytes, they may reduce systemic exposure and cumulative toxicity compared with chronic pharmacological therapy [2,100]. They may also be integrated with other treatment modalities, including symptom-directed therapies, as part of combined approaches [73].

Perhaps the most transformative feature of targeted therapies is their potential to alter the natural history of HCM, particularly if used at early stages such as genotype-positive, phenotype-negative disease. Sub-

tle abnormalities in energy use, relaxation, and diastolic function may be present before overt hypertrophy develops, raising the possibility that early intervention could delay or prevent structural remodelling [18,41]. Realising this potential will require sensitive biomarkers and multimodal strategies capable of detecting pre-phenotypic myocardial changes and monitoring therapeutic response; in this context, cardiac magnetic resonance may help identify subclinical abnormalities before hypertrophy is present [75,101].

Despite this promise, major limitations remain. Efficient and selective delivery to cardiomyocytes is still a central challenge. Although AAV9 shows marked cardiac tropism, it does not ensure exclusive myocardial specificity, and additional strategies such as cardiomyocyte- specific promoters and capsid engineering are often needed to reduce off-target expression, particularly in the liver and skeletal muscle [45,87,102,103]. Moreover, the dense extracellular matrix of the heart and the limitations of systemic or invasive delivery approaches continue to restrict homogeneous myocardial distribution, while non-viral systems such as lipid nanoparticles still show limited uptake by adult cardiomyocytes [97,104].

Another major barrier is the marked genetic and phenotypic heterogeneity of HCM. Different variants, even within the same gene, may act through distinct mechanisms such as haploinsufficiency or dominant-negative effects, complicating the development of universal therapies. Variable expressivity, incomplete penetrance, modifier genes, and genotype-negative cases further limit patient stratification and therapeutic timing [17,18,31]. In addition, the adult heart is largely post-mitotic, which constrains repair strategies that rely on cell division- dependent mechanisms [105].

Safety also remains a major concern. Pre-existing immunity to viral vectors may reduce therapeutic efficacy, and the high doses required for cardiac transduction can trigger inflammatory responses and limit redosing [90,106]. Gene-editing platforms also carry risks of off-target effects, unintended genomic alterations, and long-term genotoxicity, even with newer approaches such as base and prime editing [102]. Prolonged or excessive expression of therapeutic proteins or nucleases may further disrupt myocardial stability or increase arrhythmic risk, which has driven the development of self-limiting vectors, inducible systems, and tissue-specific promoters [107,108].

Finally, important ethical, regulatory, and practical challenges remain, especially regarding treatment of genotype-positive, phenotype-negative individuals, many of whom may never develop overt disease. Uncertainty in penetrance complicates risk–benefit assessment and patient selection. At the same time, large-scale manufacturing, mutation-specific development, regulatory approval, and cost remain substantial barriers to broad implementation, raising concerns about future healthcare inequities [30,39].

Overall, targeted gene therapies hold unique potential to shift HCM management from symptomatic treatment towards true disease modification. However, their successful clinical translation will depend on overcoming major challenges in delivery, specificity, safety, patient selection, and scalability.

5. CONCLUSION

Hypertrophic cardiomyopathy is a paradigmatic inherited myocardial disease in which pathogenic sarcomeric variants drive a prolonged pathogenic cascade that precedes structural remodelling by many years. Although current therapies have significantly improved symptom burden and clinical outcomes, they largely target the phenotypic consequences of disease rather than its primary molecular basis. Cardiac myosin inhibitors represent an important mechanistic advance, but their clinical role remains mainly confined to established disease.

Gene and RNA based therapies offer the opportunity of disease modification by targeting the causal substrate of HCM. Preclinical studies support the potential of gene replacement, genome editing, allelic silencing, and RNA-directed approaches to restore sarcomeric function and attenuate or prevent pathological remodelling, particularly when applied early in the disease course. Major translational challenges remain, including delivery, specificity, safety, scalability, and patient selection. Nevertheless, these strategies mark a critical shift from symptomatic treatment towards mechanism-based prevention and may ultimately redefine the management of inherited cardiomyopathies.

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