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# Mechanism-driven therapies for hypertrophic cardiomyopathy

Chrisan J. Ramachandra<sup>1,2,#</sup>, Iswaree Devi Balakrishnan<sup>3,4</sup>, Weng Khong Lim<sup>5-8</sup>, Derek J. Hausenloy<sup>1,2,4,9</sup>

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease that manifests with left ventricular hypertrophy in the absence of an identifiable cause. HCM was initially viewed as a disease of the sarcomere, as several mutations in various contractile proteins have been identified in familial cases. However, this ideology is being contested as many cases do not carry sarcomere mutations, and not all sarcomere-mutation carriers develop HCM. The clinical spectrum is vast, ranging from individuals who are completely asymptomatic to those who manifest chest pain, exercise intolerance, heart failure, and arrhythmia. Current management with  $\beta$ -blockers, calcium channel antagonist, and antiarrhythmics is mainly aimed at relieving symptoms and do not address the underlying pathophysiology. Crucially, while mortality remains low, a significant number of patients experience death or hospitalization due to ventricular tachycardia, heart failure, or stroke. Recently, several targeted approaches have been undertaken to attenuate pathological features, including myocardial fibrosis, cardiomyocyte hypercontractility, perturbed metabolism, and oxidative stress. While most of these targeted therapies have shown promise in preclinical animal models, their translation into clinical cohorts has been disappointing, with the exception of cardiac myosin inhibitor, mavacamten. Considering conventional management strategies do not improve long-term prognosis, there is an unmet need to develop mechanism-driven treatments for HCM. While this would require in-depth understanding of the causal effects of each mutation, models based on human physiology may provide unprecedented opportunity to interrogate the pathogenicity of novel mutations and to develop mechanism-driven therapies for improving health outcomes in HCM patients.

**Keywords:** Hypertrophic cardiomyopathy, Clinical management, Diastolic dysfunction, Heart failure, Mavacamten, Induced pluripotent stem cells

### Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease with a prevalence of 1:200-1:500, that manifests as left ventricular hypertrophy (LVH) in the absence of an identifiable cause (Maron, 2018). Approximately 60% of HCM cases have a clear familial link with missense variants in MYH7 and truncating variants in MYBPC3 accounting for most familial cases (Marian and Braunwald, 2017; Sabater-Molina et al., 2018). While this may lend support that HCM is a disease of the sarcomere, the absence of sarcomere mutations in a vast number of cases contests this ideology and may explain the heterogeneous nature of the disease (Maron et al., 2019). Indeed, the clinical spectrum of HCM is vast, ranging from cases who are completely asymptomatic to those who present

with chest pain, shortness of breath, fatigue, and syncope, with a small subset experiencing sudden cardiac death, in particular children and athletes (Ostman-Smith et al., 2008; Maron et al., 2009). HCM patients are grouped into two subsets; those with LV outflow tract (LVOT) obstruction and those without. LVH can precipitate LVOT obstruction at rest or with provocation, reduce LV compliance, and induce diastolic dysfunction. As expected, patients with obstructive HCM (oHCM) are associated with more severe symptoms and adverse outcomes, and though nonobstructive HCM (nHCM) was initially viewed as a benign condition with minimal symptoms, recent findings support high rates of adverse clinical events, comparable to those with oHCM (Lu et al., 2018; Maron et al., 2018a).

The presence or absence of obstruction guides clinical

<sup>1</sup>National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore. <sup>2</sup>Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore. <sup>3</sup>Department of Cardiology, National Heart Centre Singapore, Singapore. <sup>4</sup>Yong Loo Lin School of Medicine, National University Singapore, Singapore. <sup>5</sup>SingHealth Duke-NUS Institute of Precision Medicine, Singapore. <sup>6</sup>SingHealth Duke-NUS Genomic Medicine Centre, Singapore. <sup>7</sup>Cancer & Stem Cell Biology Program, Duke-National University of Singapore Medical School, Singapore. <sup>8</sup>Laboratory of Genome Variation Analytics, Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore. <sup>9</sup>The Hatter Cardiovascular Institute, University College London, London, UK.

Correspondence should be addressed to Dr. Chrisan J. Ramachandra (chrisan.ramachandra@nhcs.com.sg).

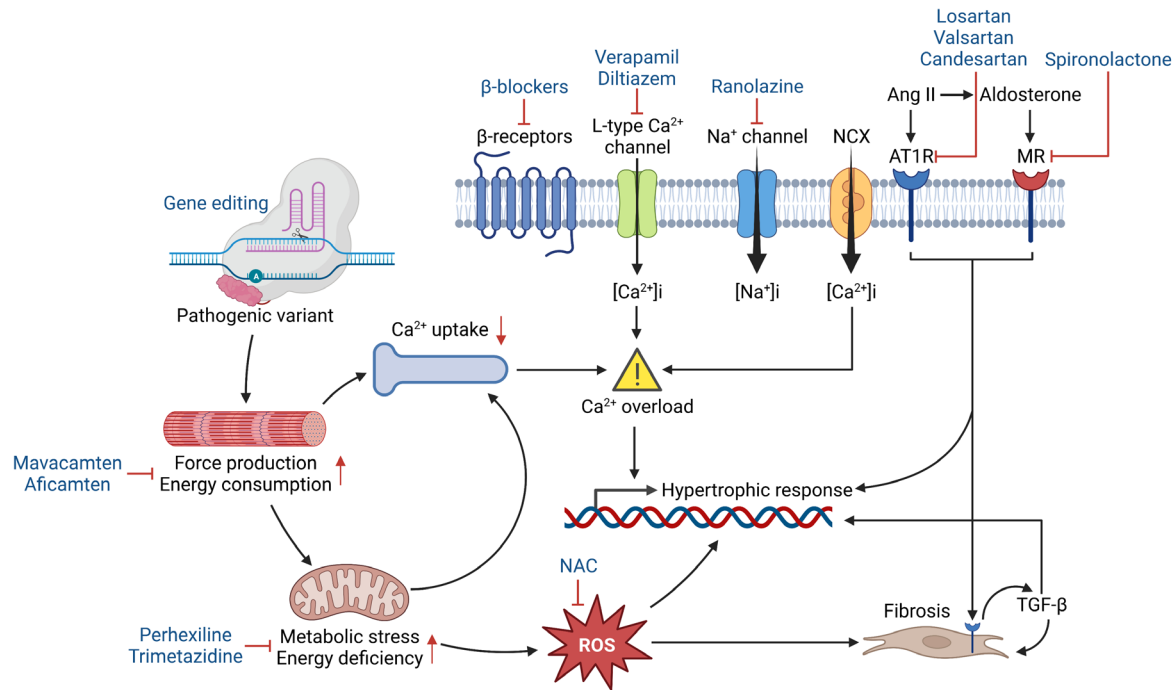


Figure 1. Schematic illustrating the pathophysiology of HCM, whereby pathogenic variants potentiate hypertrophic and fibrotic responses through abnormal sarcomere mechanics, impaired energetics, calcium mishandling, and oxidative stress. Several therapeutic modalities (blue) have been developed to target these maladaptive processes. Abbreviations: NCX- Na<sup>+</sup>/Ca<sup>2+</sup> exchanger; Ang II- angiotensin II; AT1R- angiotensin II receptor type 1; MR- mineralocorticoid receptor; NAC- N-acetylcysteine; ROS- reactive oxygen species; TGF-β- transforming growth factor beta

management. For patients with oHCM, measures that increase cardiac preload and afterload, and those that reduce cardiac contractility are favored. Pharmacotherapies like β-blockers, calcium channel antagonists, and antiarrhythmics represent the latter, where the LVOT obstruction is attenuated by reducing cardiac inotropy and chronotropy, reducing myocardial oxygen demand and enhancing diastolic filling. Invasive septal reduction strategies (either by surgical myectomy or alcohol septal ablation) may be considered for patients whose symptoms are refractory to pharmacotherapy (Rovner et al., 2003), although this can increase the risk of complete heart blockage due to damage of bundle branches (Talreja et al., 2004). Interventions for patients with nHCM mainly attempt to manage arrhythmias and improve diastolic filling; however, these approaches are less effective than for those with oHCM (Matsubara et al., 1995; Olivotto et al., 2018). Ultimately, these conventional management strategies aim to relieve symptoms and are unable to reverse pathological features that accompany LVH, including cardiomyocyte hypertrophy and disarray, or interstitial fibrosis (Cui et al., 2021). Crucially, despite low incidence of mortality, long-term follow-up studies reveal poor prognosis in HCM patients with ~50% experiencing death or hospitalization due to ventricular tachycardia, heart failure, or stroke (Sugiura et al., 2022), which is compounded by the modest efficacy and tolerability of conventional pharmacotherapies (Spoladore et al., 2012). As such, there is an unmet need to develop new treatments to improve health outcomes in HCM patients.

Proteomic analysis of myocardial tissue from HCM patients has revealed alterations in various cellular pathways, including metabolism, muscle contraction, calcium regulation, and oxidative stress (Coats et al., 2018), and recent attempts to target some of these pathways have been promising. In this review, we discuss conventional pharmacotherapies for managing HCM and draw special attention to new treatment strategies that can attenuate pathological features, and highlight the use of human models for interrogating the pathogenicity of

novel mutations with the aim of developing mechanism-driven therapies for improving health outcomes in HCM patients.

### Conventional pharmacotherapies

Since it was first described over 60 years ago, the clinical management of HCM has centered on relieving symptoms and improving quality of life. This section focuses on non-selective pharmacological modalities that are used in line with existing treatment guidelines.

#### β-blockers

The use of non-vasodilating β-adrenergic blockers is one of the oldest strategies for treating symptomatic cases and represents the mainstay of therapy (Figure 1). The beneficial effects of β-blockers are mediated by sympathetic modulation of heart rate, contractility, and stiffness, which in turn improve LV relaxation and increases time for diastolic filling. Several β-blockers have been evaluated over time with some found to be more effective than others (Cohen and Braunwald, 1968; Hubner et al., 1973). For HCM presenting in childhood (which is associated with a higher mortality than when presented in adulthood), high-dose propranolol treatment drastically reduced the risk of death compared to those who were managed conventionally (Ostman-Smith et al., 1999). Due to their negative chronotropic effects, β-blockers are unable to promote improvements in exercise capacity (Ikram and Fitzpatrick, 1981; Gilligan et al., 1993); however, recent studies with metoprolol have shown that this agent can reduce obstruction at rest and during exercise (Dybro et al., 2021) as well as improve exercise hemodynamics (Dybro et al., 2022) and LV global longitudinal strain (Dybro et al., 2023), which imply improvements in LV systolic function. While these studies suggest β-blocker treatment is effective, it is unclear whether these agents can improve long-term prognosis of HCM patients. Moreover, it is unlikely these agents can exert meaningful benefits in patients with more severe gradients and heart failure status (Monda et al., 2022) or attenuate adverse ventricular remodeling (Dybro et

al., 2021).

### **Calcium channel antagonists**

An alternative to  $\beta$ -blocker therapy is the use of the non-dihydropyridine calcium channel antagonist, verapamil, which has been shown to relieve symptoms in patients with different degrees of disease severity (Rosing et al., 1980; Gilligan et al., 1993). Due to its negative inotropic and chronotropic effects, verapamil mediates its action by improving diastolic filling, which has been attributed to reduction in diastolic asynchrony, rather than changes in diastolic velocities of the myocardial fibers (Rosing et al., 1985; Pacileo et al., 2000). Whether verapamil can improve exercise capacity remains controversial (Rosing et al., 1980; Rosing et al., 1985; Gilligan et al., 1993). The rationale for using calcium channel antagonists to treat the disease is supported by several studies that report intracellular calcium handling abnormalities in the setting of HCM (Figure 1). However, despite promoting better therapeutic outcomes than  $\beta$ -blockers (as suggested by comparative studies), verapamil is contraindicated for patients at high risk for adverse electrophysiological events, atrioventricular block, and sinus arrest (Rosing et al., 1985). Diltiazem is another calcium channel antagonist that has been shown to improve LV relaxation and diastolic filling without altering systolic function in HCM patients (Suwa et al., 1984; Iwase et al., 1987). Though it is considered to have a more favorable safety profile than verapamil, diltiazem can increase pulmonary artery wedge pressure, which is likely due to the combined action of a negative inotropic effect with no improvements in LV compliance, and this may increase risk of pulmonary congestion (Betocchi et al., 1996). Another strategy to prevent calcium overload in cardiomyocytes is by inhibiting the late sodium current (INa,late), which is enhanced in the setting of HCM and facilitates the exchange of intracellular sodium for extracellular calcium (Figure 1). However, the INa,late inhibitor, ranolazine, was unable to improve diastolic function, exercise capacity, biomarker levels, or quality of life, despite demonstrating acceptable safety profiles and reducing premature ventricular complex burden (Olivotto et al., 2018).

### **Antiarrhythmics**

Antiarrhythmics, either alone or in combination with other agents, have been shown to decrease pressure gradients through their negative inotropic and lusitropic properties (Pollick, 1988; Cokkinos et al., 1989; Hamada et al., 1997). In one study, the Class Ia antiarrhythmic, disopyramide, relieved symptoms in patients with oHCM by improving diastolic function through afterload reduction but had minimal effect on patients with nHCM (Matsubara et al., 1995). Similarly, cibenzoline was shown to reduce pressure gradients in patients with oHCM (Hamada et al., 1997) by reducing diastolic pressures and attenuating diastolic dysfunction (Hamada et al., 2001; Hamada et al., 2005; Hamada et al., 2007). Although an increase in exercise capacity has been reported following disopyramide treatment (Pollick, 1988), whether improvements in diastolic function truly translate to better exercise tolerance requires validation in larger cohorts. Despite promoting substantial functional improvements in most symptomatic cohorts, antiarrhythmics may promote early incidence of sudden cardiac death by triggering malignant arrhythmias or conduction abnormalities, especially when diastolic filling is reduced post-treatment (Fananapazir et al., 1991).

In summary, these conventional pharmacotherapies have been shown to relieve symptoms in several small cohorts. However, many of these studies were underpowered and employed surrogate endpoints, such as change in LVOT gradients or exercise tolerance; and hence, it has been difficult to assess the true impact of these agents on long-term health outcomes in patients. Strikingly, recent findings imply these

agents may be ineffective in the long-term as a significant number of patients who were on these medications experienced HCM-related adverse events (Sugiura et al., 2022). While this calls for adequately designed clinical trials, the inclusion of functional imaging techniques, genetic information, and hard endpoints should be considered when determining the efficacy of novel treatments in future.

### **Mechanism-driven therapies**

Several approaches have been undertaken to target various pathological features of HCM. This section focuses on therapies designed to attenuate myocardial fibrosis, oxidative stress, impaired myocardial energetics, and hypercontractility.

#### **Angiotensin receptor blockers**

Myocardial fibrosis is an independent predictor of adverse outcomes in HCM patients (O'Hanlon et al., 2010; Raman et al., 2019); and hence, it is not surprising that attempts have been made to prevent the development of fibrosis in the setting of HCM. Elevated levels of transforming growth factor beta-1 (TGF- $\beta$ 1) and its receptor were first identified in ventricular biopsies from HCM patients, suggestive of its involvement in disease pathogenesis (Li et al., 1997; Li et al., 1998). Indeed, the activation of TGF- $\beta$  signaling in non-myocytes mediates an increase in fibrosis, which may contribute to diastolic dysfunction and heart failure in mice with HCM (Teekakirikul et al., 2010) (Figure 1). Interestingly, treatment with the angiotensin II type 1 receptor antagonist, losartan, prevented the development of LVH and fibrosis in these mice, but only if administered prior to the onset of LVH. These findings support the use of angiotensin receptor blockers (ARBs) to treat patients with less established HCM, and consistently, valsartan improved cardiac structure and function in patients with early-stage HCM (Ho et al., 2021). In another study, losartan attenuated progression of LVH and fibrosis in patients who were mostly asymptomatic (Shimada et al., 2013). On the contrary, losartan was unable to attenuate LVH (Axelsson et al., 2015) or improve cardiac function and exercise capacity in patients with overt HCM (Axelsson et al., 2016).

The patient genotype could be a major determinant of the response to ARBs, as though candesartan was able to promote LVH regression, and improve LV function and exercise tolerance in carriers with MYH7 mutations, it had moderate effects in carriers of MYBPC3 mutations and no effect in those with TNNI3 (troponin I) mutations (Penicka et al., 2009). It can be speculated the differential ARB response observed here is governed by TGF- $\beta$  signaling, as mice with different HCM-causing mutations differed in several properties, with only those with troponin T mutations demonstrating a biosignature consistent with elevated TGF- $\beta$  signaling (Vakrou et al., 2018). While this may imply elevated TGF- $\beta$  signaling underpins the pathophysiology in specific genetic subgroups, plasma analysis confirmed this pathway is generally upregulated in HCM patients when compared to those presenting with hypertensive LVH (Shimada et al., 2021) and can also serve as a predictor of major adverse cardiovascular events (Shimada et al., 2022).

#### **Mineralocorticoid receptor antagonist**

In certain cases, myocardial fibrosis has been found to be an early manifestation in patients who are yet to develop LVH, where substantial scar formation precipitates arrhythmia or progressive heart failure in the absence of LVOT obstruction. Aldosterone is thought to be a critical mediator of cardiac hypertrophy and fibrosis as it is elevated in patient myocardium, evokes a hypertrophic response in rat cardiomyocytes, and promotes collagen and TGF- $\beta$  expression in rat fibroblasts (Tsybouleva et al., 2004) (Figure 1). Interestingly, the mineralocorticoid receptor (MR) antagonist, spironolactone, attenuated fibrosis, cardiomyocyte disarray, and diastolic



dysfunction in mice (Tsybouleva et al., 2004), supporting the need for clinical trials to evaluate the potential beneficial effects of MR blockade in the setting of HCM. However, spironolactone was unable to attenuate fibrosis, nor improve LV remodeling and functional capacity in a small cohort of patients with or without LVOT obstruction (Maron et al., 2018b).

#### **Antioxidants**

The occurrence of LVH and fibrosis are considered secondary to the activation of numerous mitotic pathways, including those mediated by oxidative stress (Figure 1). Consistent with being a critical mediator of cardiac hypertrophy and fibrosis (Ramachandra et al., 2021a), oxidative stress is elevated in HCM patient myocardium and correlates with LV dilation and systolic dysfunction (Nakamura et al., 2005), while inhibition of these pathways through direct or indirect measures attenuates the disease phenotype in animal models of HCM (Senthil et al., 2005; Marian et al., 2006; Lombardi et al., 2009; Wilder et al., 2015). N-acetylcysteine (NAC), a precursor of glutathione (the most abundant intracellular defense mechanism against oxidative damage), is one of the most studied compounds for attenuating oxidative stress, and it has been shown to reverse established LVH and fibrosis, and reduce susceptibility to ventricular arrhythmia (Marian et al., 2006; Lombardi et al., 2009). NAC can also attenuate diastolic dysfunction that may arise through oxidative modifications of myofilament proteins (Wilder et al., 2015; Ryba et al., 2019; Ramachandra et al., 2022). Despite these beneficial effects, NAC had no major impact on indices of LVH and fibrosis in HCM patients (Marian et al., 2018). It is important to note most of these patients presented with minimal symptoms and did not have exercise intolerance; and hence, whether NAC can relieve symptoms or prevent disease progression despite the lack of reduction in fibrosis remains to be determined. Interestingly, fingolimod, a sphingosine-1-phosphate receptor modulator used for treating multiple sclerosis, decreased oxidative modifications of myofilament proteins, which in turn attenuated diastolic dysfunction, but with no reduction in fibrosis (Ryba et al., 2019). These findings may suggest that restoring myofilament function is sufficient for improving cardiac function in the setting of HCM.

#### **Metabolic modulators**

Elucidating common pathophysiological mechanisms across diverse HCM genotypes is crucial for developing therapies that can promote maximum response across the entire clinical spectrum. Impaired myocardial energetics is considered an early and common driver of HCM (Ramachandra et al., 2019; Ramachandra et al., 2021b) independent of family history (Jung et al., 2000), clinical status (Jung et al., 1998), and patient genotype (Crilley et al., 2003). In support, reduction in myocardial phosphocreatine/adenosine triphosphate (PCr/ATP) ratios and increases in inorganic phosphate/phosphocreatine (Pi/PCr) ratios are found in asymptomatic patients (Jung et al., 1998), and in carriers with different sarcomere mutations, including those who are yet to develop LVH (Crilley et al., 2003). This energy deficit is exacerbated during exercise and may explain the development of diastolic dysfunction in HCM patients during peak exercise (Dass et al., 2015). Impaired myocardial energetics was initially viewed as a result of ischemia mediated decreases in oxygen supply (Sieverding et al., 1997); however, findings from several animal models suggest the induction of energetic abnormalities and diastolic dysfunction are more likely to be primary effects of sarcomere mutations and are unlikely to be secondary consequences of LVH (Spindler et al., 1998) (Figure 1). Indeed, sarcomere mutations do facilitate chronic mismatch between ATP synthesis and ATP consumption by overall crossbridge activity; and hence, it is not surprising that inefficient use of ATP at

the myofilament level could provoke cardiac dysfunction with the severity dependent on the type of mutation and the amount of mutant protein in the sarcomere (Montgomery et al., 2001; Javadpour et al., 2003). While these studies imply energetic impairment is a direct consequence of alterations of the myocardium, other studies, albeit controversial, suggest the low myocardial PCr/ATP ratios are a result of fibrosis (Esposito et al., 2009; Petersen et al., 2009). However, an argument can be made that some regions of the myocardium are more energy deprived than others, which is supported by findings whereby increased myofilament calcium sensitivity (due to sarcomere mutations) rapidly precipitated energy deprived regions during stress, which in turn decreased intercellular coupling and increased arrhythmia susceptibility (Huke et al., 2013).

Energy and metabolism are tightly coupled cellular processes; and hence, energetic impairment is usually associated with perturbed metabolism. Indeed, comprehensive multiomics profiling of LV septal myectomy samples revealed substantial dysregulation in fatty acid metabolism, reduction of acylcarnitines, and accumulation of free fatty acids that coincided with elevated levels of oxidative stress, mitochondrial damage, and reduced mitochondrial clearance (Ranjbarvaziri et al., 2021). Other studies also report a reduced capacity for fatty acid oxidation, but an increase in ketone bodies and branched chain amino acids may suggest the use of alternate fuels in HCM hearts (Previs et al., 2022). Since these metabolic derangements were observed across diverse genotypes, therapeutic strategies aimed at normalizing myocardial energetics and metabolism could have immediate clinical implications. In support, the carnitine palmitoyl transferase-1 inhibitor, perhexiline, attenuated myocardial energy impairment, improved diastolic function, and increased exercise capacity in patients with symptomatic nHCM (Abozguia et al., 2010), and is being evaluated for its ability to promote LVH regression (Ananthakrishna et al., 2021). Despite these promising outcomes, modalities that suppress fatty acid oxidation could be potentially ineffective or harmful given that fatty acid metabolism is perturbed in HCM hearts (Ranjbarvaziri et al., 2021; Previs et al., 2022). In support, trimetazidine, an inhibitor of fatty acid  $\beta$ -oxidation decreased exercise capacity in patients with nHCM (Coats et al., 2019).

#### **Cardiac myosin inhibitors**

Hypercontractility is another pathological feature of HCM, which is attributed to the destabilization of the myosin super-relaxed (SRX) state (Toepfer et al., 2019; Vander Roest et al., 2021). In healthy hearts, myosin undergoes physiological shifts between the SRX conformation (energy conserving) and the disordered relaxed state (DRX) conformation (energy consuming), while in hypertrophied hearts, pathogenic mutations increase the proportion of myosins in the DRX state evoking hypercontractility, impaired relaxation, and increased energy consumption that precipitates higher rates of heart failure and arrhythmias (Toepfer et al., 2020) (Figure 1). Destabilization of the SRX state is not restricted to carriers with myosin mutations and is found to occur in animal models with mutations in cardiac myosin-binding protein C (McNamara et al., 2016; Toepfer et al., 2019), cardiac myosin essential light chain (Sitbon et al., 2020), and ventricular regulatory light chain (Yadav et al., 2019; Yuan et al., 2022).

Given the DRX state facilitates crossbridge formation with greater ATP consumption, novel cardiac myosin inhibitors have been developed to reduce myosin ATPase activity. Since its discovery, where it attenuated the development of LVH, cardiomyocyte disarray, and fibrosis in mice (Green et al., 2016), MYK-461 (mavacamten) is now a viable therapeutic modality for HCM. Like previous therapies, mavacamten demonstrated a more pronounced therapeutic effect in mice only when administered prior to the onset of overt LVH (pre-

hypertrophic state), which may imply maximum benefit could be achieved in patients with less established HCM. Strikingly, mavacamten has been associated with favorable outcomes in patients with and without obstruction (Heitner et al., 2019; Ho et al., 2020; Olivotto et al., 2020). In the EXPLORER-HCM clinical trial, which evaluated the effect of mavacamten in symptomatic patients with obstruction, this agent was associated with improved New York Heart Association functional class, better exercise capacity, less LVOT obstruction, and improved health status (Olivotto et al., 2020; Spertus et al., 2021). Interestingly, sub-study analysis revealed mavacamten treatment reduced LV mass and LV wall thickness, as well as left atrial volume index (Saberli et al., 2021), with accompanying improvements in diastolic function and systolic anterior motion of the mitral valve (Hegde et al., 2021; Cremer et al., 2022). Consistently, mavacamten treatment in patients without obstruction was associated with improvements in myocardial wall stress as evidenced by reductions in NT-proBNP and troponin I levels (Ho et al., 2020).

Mechanistically, mavacamten is thought to decrease force production, inhibit myosin ATPase activity, and accelerate cross-bridge detachment rate (Anderson et al., 2018; Mamidi et al., 2018; Rohde et al., 2018; Awinda et al., 2021), but as to how this agent attenuates LVH, fibrosis, and diastolic dysfunction warrants further investigation. Mavacamten treatment may also reduce the need for septal reduction therapy in highly symptomatic patients with obstruction (Desai et al., 2023). Finally, when evaluated in patients on  $\beta$ -blockers, mavacamten improved heart-rate independent measures, but not indices that were heart-rate dependent, including peak exercise capacity (Wheeler et al., 2023). This finding may argue for dose reduction or withdrawal of  $\beta$ -blockers to gain maximum effect of this agent, however such decisions should be considered carefully with respect to patient history.

In summary, several mechanism-driven therapeutic modalities have been identified in animal models of HCM. The poor clinical translation of these modalities highlights the large gap between preclinical target discovery and clinical implementation. Mavacamten seems to be an exception, providing symptom relief and reversing established pathological features in clinical cohorts. However, if this agent is to be considered a mainstay therapy, careful dosing will be required to prevent reductions in LV ejection fraction and atrial fibrillation (Heitner et al., 2019).

### Future Perspectives

HCM is a heterogeneous disease with differences in genetic etiology underpinning symptom severity and patient response to pharmacotherapies. Indeed, an association between position of mutation and disease severity has been described in early studies, whereby different mutations in myosin heavy chain beta ( $\beta$ -MHC) revealed variability in the nature and extent of functional impairment in contractile properties (Lankford et al., 1995). Similarly, different mutations in troponin T precipitate different alterations in muscle fibers (Hernandez et al., 2005). Moreover, when the impact of mutations on cardiac structure and function were evaluated in pre-hypertrophic individuals, the R92W troponin T mutation was associated with increased systolic function, the A797T  $\beta$ -MHC mutation with reduced diastolic function, and the R403W  $\beta$ -MHC mutation with both reduced systolic and diastolic function (Revera et al., 2008). These findings support the need for interrogating the causal effect of novel mutations, especially those prevalent in specific ethnic populations (Viswanathan et al., 2018; Pua et al., 2020; Wu et al., 2020), and which are of non-sarcomere origin, as this will facilitate the development of mechanism-driven therapies.

Treatment with mavacamten, or next-in-class, aficamten (Maron et al., 2023) have provided substantial benefits in

symptomatic patients; however, the exclusion of individuals with severe heart failure symptoms and the low representation of non-Caucasian participants and children in these studies argues whether these agents can provide universal benefits across the entire clinical spectrum. Such concerns are derived from past findings whereby several therapeutic compounds that improved cardiac structure and function in animal models of HCM were unable to promote meaningful benefits in humans. For several decades, animal models with various pathogenic mutations (predominantly in contractile proteins) have helped to increase our understanding of HCM, and while these translational disparities could be attributed to shortcomings of the respective clinical trials, it must be acknowledged that animal models may not completely represent human diseases. For instance, most mouse models of HCM fail to develop overt LVH and/or fibrosis amongst other clinical features (Gannon and Link, 2021); and hence, it may be prudent to place greater emphasis on studying human cells or tissue to elucidate the causal effects of novel mutations and to discover new therapeutic targets.

Human induced pluripotent stem cells (iPSCs) offer an unprecedented opportunity to study human physiology and disease at the cellular and organ level. Human iPSCs are pluripotent, which means they can be differentiated into any cell type of the human body, and being stem cells, they can be expanded into millions of cell progeny, circumventing the limitations associated with primary tissues. Being derived from patients, iPSCs allow for the identification and validation of therapeutic targets across diverse genotypes, thereby accounting for clinical heterogeneity and maximizing patient response to novel therapeutics, while mitigating adverse effects. We and others have derived iPSCs from HCM patients with different mutations, and remarkably, cardiomyocytes differentiated from these iPSCs show clinical hallmarks, including cellular hypertrophy, arrhythmia, myofibril disarray, calcium mishandling, and impaired relaxation (Lan et al., 2013; Viswanathan et al., 2018; Prondzynski et al., 2019; Wu et al., 2019; Zhou et al., 2019; Ramachandra et al., 2022). As expected, different mutations evoke different pathological features in these human models, reaffirming the need for mechanism-driven therapies (Prajapati et al., 2018; Smith et al., 2018; Bhagwan et al., 2020). Importantly, these iPSC-derived HCM models have provided new mechanistic insight into disease pathogenesis (Qiu et al., 2021; Vander Roest et al., 2021; Ramachandra et al., 2022), and functionally characterized the pathogenicity of novel mutations, both in sarcomere and non-sarcomere encoding genes (Liu et al., 2014; Pua et al., 2020; Kondo et al., 2022), positioning these human cellular models as effective tools for developing patient-tailored therapies. Establishing patient-specific iPSC models for every uncharacterized HCM genotype will be extremely challenging, and so for now priority could be given for novel mutations with high risk of pathogenicity as determined by combined assessment of *in silico* modelling, population prevalence, and clinical severity.

### Gene therapy as a potential future treatment modality for HCM

Recently, the effect of genomic base editing to correct the pathogenic MYH7 R403Q mutation has been evaluated in two independent studies, where this modality was able to correct and rescue pathological phenotypes in HCM patient-derived cardiomyocytes and in mice with HCM (Chai et al., 2023; Reichart et al., 2023) (Figure 1). These studies mark the first demonstration of efficient single nucleotide gene correction in postnatal mammalian cardiomyocytes *in vivo*, and highlight permanent genomic correction of all cardiomyocytes is not compulsory to prevent the onset of HCM. Gene editing could potentially be a one-time treatment modality for HCM patients



and it would be interesting to see whether this approach can also attenuate pathological features in more established cases, although dosing will require rigorous calculations to minimize bystander editing and prevent adverse effects (Reichart et al., 2023). In summary, gene editing could serve as a very specific therapeutic modality for individuals with known pathogenic mutations, and as we interrogate the causality of other mutations in models relevant to human physiology there is much opportunity to develop mechanism-driven therapies for HCM.

### Conclusions

Recent findings highlight conventional pharmacotherapies as being ineffective in improving long-term prognosis in HCM patients, which can be attributed to their non-selective mode of action and their inability to target the underlying pathophysiology. Much of our mechanistic understanding of HCM is centered on studies interrogating the genotype-phenotype relationship of a few specific pathogenic sarcomere variants. However, it must be acknowledged, we still lack substantial understanding of many other pathogenic sarcomere variants, non-sarcomere variants, and variants prevalent in non-Caucasian populations. To improve health outcomes in HCM patients, we should strongly consider moving away from the one-size fits all approach of using non-selective pharmacotherapies and focus on developing mechanism-driven therapies based on the genetic background of each patient. A workflow combining genetic data, in silico modeling, and patient-specific iPSC models could (1) give impetus for unravelling the complexity of HCM, (2) help to risk stratify genotype-positive/phenotype-negative carriers, and (3) facilitate the development of more effective therapies that can potentially prevent disease progression and improve health outcomes in patients with established HCM.

### Conflicts of interest

The authors declare that they have no conflicts of interest. Chrishan Ramachandra and Derek Hausenloy did not participated at any level in the editorial review of this manuscript.

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### References

Abozguia K, Elliott P, McKenna W, Phan TT, Nallur-Shivu G, Ahmed I, Maher AR, Kaur K, Taylor J, Henning A, Ashrafian H, Watkins H, Frenneaux M (2010) Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy. *Circulation* 122:1562-1569.

Ananthkrishna R, Lee SL, Foote J, Sallustio BC, Binda G, Mangoni AA, Woodman R, Semsarian C, Horowitz JD, Selvanayagam JB (2021) Randomized controlled trial of perhexiline on regression of left ventricular hypertrophy in patients with symptomatic hypertrophic cardiomyopathy

(RESOLVE-HCM trial). *Am Heart J* 240:101-113.

- Anderson RL, Trivedi DV, Sarkar SS, Henze M, Ma W, Gong H, Rogers CS, Gorham JM, Wong FL, Morck MM, Seidman JG, Ruppel KM, Irving TC, Cooke R, Green EM, Spudis JA (2018) Deciphering the super relaxed state of human beta-cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. *Proc Natl Acad Sci U S A* 115:E8143-E8152.
- Awinda PO, Watanabe M, Bishaw Y, Huckabee AM, Agonias KB, Kazmierczak K, Szczesna-Cordary D, Tanner BCW (2021) Mavacamten decreases maximal force and Ca(2+) sensitivity in the N47K-myosin regulatory light chain mouse model of hypertrophic cardiomyopathy. *Am J Physiol Heart Circ Physiol* 320:H881-H890.
- Axelsson A, Iversen K, Vejlstup N, Ho CY, Havndrup O, Kofoed KF, Norsk J, Jensen M, Bundgaard H (2016) Functional effects of losartan in hypertrophic cardiomyopathy-a randomised clinical trial. *Heart* 102:285-291.
- Axelsson A, Iversen K, Vejlstup N, Ho C, Norsk J, Langhoff L, Ahtarovski K, Corell P, Havndrup O, Jensen M, Bundgaard H (2015) Efficacy and safety of the angiotensin II receptor blocker losartan for hypertrophic cardiomyopathy: the INHERIT randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol* 3:123-131.
- Betocchi S, Piscione F, Losi MA, Pace L, Bocalatte M, Perrone-Filardi P, Cappelli B, Briguori C, Manganelli F, Ciampi Q, Salvatore M, Chiariello M (1996) Effects of diltiazem on left ventricular systolic and diastolic function in hypertrophic cardiomyopathy. *Am J Cardiol* 78:451-457.
- Bhagwan JR, Mosqueira D, Chairez-Cantu K, Mannhardt I, Bodbin SE, Bakar M, Smith JGW, Denning C (2020) Isogenic models of hypertrophic cardiomyopathy unveil differential phenotypes and mechanism-driven therapeutics. *J Mol Cell Cardiol* 145:43-53.
- Chai AC, Cui M, Chemello F, Li H, Chen K, Tan W, Atmanli A, McAnally JR, Zhang Y, Xu L, Liu N, Bassel-Duby R, Olson EN (2023) Base editing correction of hypertrophic cardiomyopathy in human cardiomyocytes and humanized mice. *Nat Med* 29:401-411.
- Coats CJ, Pavlou M, Watkinson OT, Protonotarios A, Moss L, Hyland R, Rantell K, Pantazis AA, Tome M, McKenna WJ, Frenneaux MP, Omar R, Elliott PM (2019) Effect of Trimetazidine Dihydrochloride Therapy on Exercise Capacity in Patients With Nonobstructive Hypertrophic Cardiomyopathy: A Randomized Clinical Trial. *JAMA Cardiol* 4:230-235.
- Coats CJ, Heywood WE, Virasami A, Ashrafi N, Syrris P, Dos Remedios C, Treibel TA, Moon JC, Lopes LR, McGregor CGA, Ashworth M, Sebire NJ, McKenna WJ, Mills K, Elliott PM (2018) Proteomic Analysis of the Myocardium in Hypertrophic Obstructive Cardiomyopathy. *Circ Genom Precis Med* 11:e001974.
- Cohen LS, Braunwald E (1968) Chronic beta adrenergic receptor blockade in the treatment of idiopathic hypertrophic subaortic stenosis. *Prog Cardiovasc Dis* 11:211-221.
- Cokkinos DV, Salpeas D, Ioannou NE, Christoulas S (1989) Combination of disopyramide and propranolol in hypertrophic cardiomyopathy. *Can J Cardiol* 5:33-36.
- Cremer PC, Geske JB, Owens A, Jaber WA, Harb SC, Saberi S, Wang A, Sherrid M, Naidu SS, Schaff H, Smedira NG, Wang Q, Wolski K, Lampl KL, Sehnert AJ, Nissen SE, Desai MY (2022) Myosin Inhibition and Left Ventricular Diastolic Function in Patients With Obstructive Hypertrophic Cardiomyopathy Referred for Septal

- Reduction Therapy: Insights From the VALOR-HCM Study. *Circ Cardiovasc Imaging* 15:e014986.
- Crilly JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H (2003) Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol* 41:1776-1782.
- Cui H, Schaff HV, Lentz Carvalho J, Nishimura RA, Geske JB, Dearani JA, Lahr BD, Lee AT, Bos JM, Ackerman MJ, Ommen SR, Maleszewski JJ (2021) Myocardial Histopathology in Patients With Obstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 77:2159-2170.
- Dass S, Cochlin LE, Suttie JJ, Holloway CJ, Rider OJ, Carden L, Tyler DJ, Karamitsos TD, Clarke K, Neubauer S, Watkins H (2015) Exacerbation of cardiac energetic impairment during exercise in hypertrophic cardiomyopathy: a potential mechanism for diastolic dysfunction. *Eur Heart J* 36:1547-1554.
- Desai MY, Owens A, Geske JB, Wolski K, Saberi S, Wang A, Sherrid M, Cremer PC, Naidu SS, Smedira NG, Schaff H, McErlean E, Sewell C, Balasubramanyam A, Lampl K, Sehnert AJ, Nissen SE (2023) Dose-Blinded Myosin Inhibition in Patients With Obstructive Hypertrophic Cardiomyopathy Referred for Septal Reduction Therapy: Outcomes Through 32 Weeks. *Circulation* 147:850-863.
- Dybro AM, Rasmussen TB, Nielsen RR, Andersen MJ, Jensen MK, Poulsen SH (2021) Randomized Trial of Metoprolol in Patients With Obstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 78:2505-2517.
- Dybro AM, Rasmussen TB, Nielsen RR, Ladefoged BT, Andersen MJ, Jensen MK, Poulsen SH (2022) Effects of Metoprolol on Exercise Hemodynamics in Patients With Obstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 79:1565-1575.
- Dybro AM, Rasmussen TB, Nielsen RR, Pedersen ALD, Andersen MJ, Jensen MK, Poulsen SH (2023) Metoprolol Improves Left Ventricular Longitudinal Strain at Rest and during Exercise in Obstructive Hypertrophic Cardiomyopathy. *J Am Soc Echocardiogr* 36:196-204.
- Esposito A, De Cobelli F, Perseghin G, Pieroni M, Belloni E, Mellone R, Canu T, Gentinetta F, Scifo P, Chimenti C, Frustaci A, Luzzi L, Maseri A, Maschio AD (2009) Impaired left ventricular energy metabolism in patients with hypertrophic cardiomyopathy is related to the extension of fibrosis at delayed gadolinium-enhanced magnetic resonance imaging. *Heart* 95:228-233.
- Fananapazir L, Leon MB, Bonow RO, Tracy CM, Cannon RO, 3rd, Epstein SE (1991) Sudden death during empiric amiodarone therapy in symptomatic hypertrophic cardiomyopathy. *Am J Cardiol* 67:169-174.
- Gannon MP, Link MS (2021) Phenotypic variation and targeted therapy of hypertrophic cardiomyopathy using genetic animal models. *Trends Cardiovasc Med* 31:20-31.
- Gilligan DM, Chan WL, Joshi J, Clarke P, Fletcher A, Krikler S, Oakley CM (1993) A double-blind, placebo-controlled crossover trial of nadolol and verapamil in mild and moderately symptomatic hypertrophic cardiomyopathy. *J Am Coll Cardiol* 21:1672-1679.
- Green EM, Wakimoto H, Anderson RL, Evanchik MJ, Gorham JM, Harrison BC, Henze M, Kawas R, Oslob JD, Rodriguez HM, Song Y, Wan W, Leinwand LA, Spudich JA, McDowell RS, Seidman JG, Seidman CE (2016) A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. *Science* 351:617-621.
- Hamada M, Shigematsu Y, Ikeda S, Hara Y, Okayama H, Kodama K, Ochi T, Hiwada K (1997) Class Ia antiarrhythmic drug cibenzoline: a new approach to the medical treatment of hypertrophic obstructive cardiomyopathy. *Circulation* 96:1520-1524.
- Hamada M, Aono J, Ikeda S, Watanabe K, Inaba S, Suzuki J, Ohtsuka T, Shigematsu Y (2007) Effect of intravenous administration of cibenzoline on left ventricular diastolic pressures in patients with hypertrophic cardiomyopathy: its relationship to transmitral Doppler flow profiles. *Circ J* 71:1540-1544.
- Hamada M, Shigematsu Y, Hara Y, Suzuki M, Ohtsuka T, Hiasa G, Ogimoto A, Saeki H, Suzuki J, Hiwada K (2001) Antiarrhythmic drug, cibenzoline, can directly improve the left ventricular diastolic function in patients with hypertrophic cardiomyopathy. *Jpn Circ J* 65:531-538.
- Hamada M, Shigematsu Y, Inaba S, Aono J, Ikeda S, Watanabe K, Ogimoto A, Ohtsuka T, Hara Y, Higaki J (2005) Antiarrhythmic drug cibenzoline attenuates left ventricular pressure gradient and improves transmitral Doppler flow pattern in patients with hypertrophic obstructive cardiomyopathy caused by midventricular obstruction. *Circ J* 69:940-945.
- Hegde SM, Lester SJ, Solomon SD, Michels M, Elliott PM, Nagueh SF, Choudhury L, Zemanek D, Zwas DR, Jacoby D, Wang A, Ho CY, Li W, Sehnert AJ, Olivetto I, Abraham TP (2021) Effect of Mavacamten on Echocardiographic Features in Symptomatic Patients With Obstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 78:2518-2532.
- Heitner SB, Jacoby D, Lester SJ, Owens A, Wang A, Zhang D, Lambing J, Lee J, Semigran M, Sehnert AJ (2019) Mavacamten Treatment for Obstructive Hypertrophic Cardiomyopathy: A Clinical Trial. *Ann Intern Med* 170:741-748.
- Hernandez OM, Szczesna-Cordary D, Knollmann BC, Miller T, Bell M, Zhao J, Sirenko SG, Diaz Z, Guzman G, Xu Y, Wang Y, Kerrick WG, Potter JD (2005) F110I and R278C troponin T mutations that cause familial hypertrophic cardiomyopathy affect muscle contraction in transgenic mice and reconstituted human cardiac fibers. *J Biol Chem* 280:37183-37194.
- Ho CY et al. (2020) Evaluation of Mavacamten in Symptomatic Patients With Nonobstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 75:2649-2660.
- Ho CY et al. (2021) Valsartan in early-stage hypertrophic cardiomyopathy: a randomized phase 2 trial. *Nat Med* 27:1818-1824.
- Hubner PJ, Ziady GM, Lane GK, Hardarson T, Scales B, Oakley CM, Goodwin JF (1973) Double-blind trial of propranolol and practolol in hypertrophic cardiomyopathy. *Br Heart J* 35:1116-1123.
- Huke S, Venkataraman R, Faggioni M, Bennuri S, Hwang HS, Baudenbacher F, Knollmann BC (2013) Focal energy deprivation underlies arrhythmia susceptibility in mice with calcium-sensitized myofilaments. *Circ Res* 112:1334-1344.
- Ikram H, Fitzpatrick D (1981) Double-blind trial of chronic oral beta blockade in congestive cardiomyopathy. *Lancet* 2:490-493.
- Iwase M, Sotobata I, Takagi S, Miyaguchi K, Jing HX, Yokota M (1987) Effects of diltiazem on left ventricular diastolic behavior in patients with hypertrophic cardiomyopathy: evaluation with exercise pulsed Doppler echocardiography. *J Am Coll Cardiol* 9:1099-1105.
- Javadpour MM, Tardiff JC, Pinz I, Ingwall JS (2003) Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. *J Clin Invest* 112:768-775.
- Jung WI, Sieverding L, Breuer J, Hoess T, Widmaier S, Schmidt O, Bunse M, van Erckelens F, Apitz J, Lutz O, Dietze

- GJ (1998) 31P NMR spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy. *Circulation* 97:2536-2542.
- Jung WI, Hoess T, Bunse M, Widmaier S, Sieverding L, Breuer J, Apitz J, Schmidt O, van Erckelens F, Dietze GJ, Lutz O (2000) Differences in cardiac energetics between patients with familial and nonfamilial hypertrophic cardiomyopathy. *Circulation* 101:E121.
- Kondo T et al. (2022) Human-Induced Pluripotent Stem Cell-Derived Cardiomyocyte Model for TNNT2 Delta160E-Induced Cardiomyopathy. *Circ Genom Precis Med* 15:e003522.
- Lan F et al. (2013) Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell* 12:101-113.
- Lankford EB, Epstein ND, Fananapazir L, Sweeney HL (1995) Abnormal contractile properties of muscle fibers expressing beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J Clin Invest* 95:1409-1414.
- Li G, Li RK, Mickle DA, Weisel RD, Merante F, Ball WT, Christakis GT, Cusimano RJ, Williams WG (1998) Elevated insulin-like growth factor-I and transforming growth factor-beta 1 and their receptors in patients with idiopathic hypertrophic obstructive cardiomyopathy. A possible mechanism. *Circulation* 98:II144-149; discussion II149-150.
- Li RK, Li G, Mickle DA, Weisel RD, Merante F, Luss H, Rao V, Christakis GT, Williams WG (1997) Overexpression of transforming growth factor-beta1 and insulin-like growth factor-I in patients with idiopathic hypertrophic cardiomyopathy. *Circulation* 96:874-881.
- Liu Z, Song Y, Li D, He X, Li S, Wu B, Wang W, Gu S, Zhu X, Wang X, Zhou Q, Dai Y, Yan Q (2014) The novel mitochondrial 16S rRNA 2336T>C mutation is associated with hypertrophic cardiomyopathy. *J Med Genet* 51:176-184.
- Lombardi R, Rodriguez G, Chen SN, Ripplinger CM, Li W, Chen J, Willerson JT, Betocchi S, Wickline SA, Efimov IR, Marian AJ (2009) Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. *Circulation* 119:1398-1407.
- Lu DY, Pozios I, Haileselassie B, Ventoulis I, Liu H, Sorensen LL, Canepa M, Phillip S, Abraham MR, Abraham TP (2018) Clinical Outcomes in Patients With Nonobstructive, Labile, and Obstructive Hypertrophic Cardiomyopathy. *J Am Heart Assoc* 7.
- Mamidi R, Li J, Doh CY, Verma S, Stelzer JE (2018) Impact of the Myosin Modulator Mavacamten on Force Generation and Cross-Bridge Behavior in a Murine Model of Hypercontractility. *J Am Heart Assoc* 7:e009627.
- Marian AJ, Braunwald E (2017) Hypertrophic Cardiomyopathy: Genetics, Pathogenesis, Clinical Manifestations, Diagnosis, and Therapy. *Circ Res* 121:749-770.
- Marian AJ, Senthil V, Chen SN, Lombardi R (2006) Antifibrotic effects of antioxidant N-acetylcysteine in a mouse model of human hypertrophic cardiomyopathy mutation. *J Am Coll Cardiol* 47:827-834.
- Marian AJ, Tan Y, Li L, Chang J, Syrris P, Hessabi M, Rahbar MH, Willerson JT, Cheong BY, Liu CY, Kleiman NS, Bluemke DA, Nagueh SF (2018) Hypertrophy Regression With N-Acetylcysteine in Hypertrophic Cardiomyopathy (HALT-HCM): A Randomized, Placebo-Controlled, Double-Blind Pilot Study. *Circ Res* 122:1109-1118.
- Maron BJ (2018) Clinical Course and Management of Hypertrophic Cardiomyopathy. *N Engl J Med* 379:655-668.
- Maron BJ, Rowin EJ, Udelson JE, Maron MS (2018a) Clinical Spectrum and Management of Heart Failure in Hypertrophic Cardiomyopathy. *JACC Heart Fail* 6:353-363.
- Maron BJ, Maron MS, Maron BA, Loscalzo J (2019) Moving Beyond the Sarcomere to Explain Heterogeneity in Hypertrophic Cardiomyopathy: JACC Review Topic of the Week. *J Am Coll Cardiol* 73:1978-1986.
- Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO (2009) Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. *Circulation* 119:1085-1092.
- Maron MS, Chan RH, Kapur NK, Jaffe IZ, McGraw AP, Kerur R, Maron BJ, Udelson JE (2018b) Effect of Spironolactone on Myocardial Fibrosis and Other Clinical Variables in Patients with Hypertrophic Cardiomyopathy. *Am J Med* 131:837-841.
- Maron MS et al. (2023) Phase 2 Study of Aficamten in Patients With Obstructive Hypertrophic Cardiomyopathy. *J Am Coll Cardiol* 81:34-45.
- Matsubara H, Nakatani S, Nagata S, Ishikura F, Katagiri Y, Ohe T, Miyatake K (1995) Salutary effect of disopyramide on left ventricular diastolic function in hypertrophic obstructive cardiomyopathy. *J Am Coll Cardiol* 26:768-775.
- McNamara JW, Li A, Smith NJ, Lal S, Graham RM, Kooiker KB, van Dijk SJ, Remedios CGD, Harris SP, Cooke R (2016) Ablation of cardiac myosin binding protein-C disrupts the super-relaxed state of myosin in murine cardiomyocytes. *J Mol Cell Cardiol* 94:65-71.
- Monda E, Lioncino M, Palmiero G, Franco F, Rubino M, Cirillo A, Verrillo F, Fusco A, Caiazza M, Mazzella M, Moscarella E, Dongiglio F, Sepe J, Pacileo G, Calabro P, Limongelli G (2022) Bisoprolol for treatment of symptomatic patients with obstructive hypertrophic cardiomyopathy. The BASIC (bisoprolol AS therapy in hypertrophic cardiomyopathy) study. *Int J Cardiol* 354:22-28.
- Montgomery DE, Tardiff JC, Chandra M (2001) Cardiac troponin T mutations: correlation between the type of mutation and the nature of myofibrillar dysfunction in transgenic mice. *J Physiol* 536:583-592.
- Nakamura K, Kusano KF, Matsubara H, Nakamura Y, Miura A, Nishii N, Banba K, Nagase S, Miyaji K, Morita H, Saito H, Emori T, Ohe T (2005) Relationship between oxidative stress and systolic dysfunction in patients with hypertrophic cardiomyopathy. *J Card Fail* 11:117-123.
- O'Hanlon R, Grasso A, Roughton M, Moon JC, Clark S, Wage R, Webb J, Kulkarni M, Dawson D, Sulaibeekh L, Chandrasekaran B, Bucciarelli-Ducci C, Pasquale F, Cowie MR, McKenna WJ, Sheppard MN, Elliott PM, Pennell DJ, Prasad SK (2010) Prognostic significance of myocardial fibrosis in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 56:867-874.
- Olivotto I et al. (2018) Efficacy of Ranolazine in Patients With Symptomatic Hypertrophic Cardiomyopathy: The RESTYLE-HCM Randomized, Double-Blind, Placebo-Controlled Study. *Circ Heart Fail* 11:e004124.
- Olivotto I et al. (2020) Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 396:759-769.
- Ostman-Smith I, Wettrell G, Riesenfeld T (1999) A cohort study of childhood hypertrophic cardiomyopathy: improved survival following high-dose beta-adrenoceptor antagonist treatment. *J Am Coll Cardiol* 34:1813-1822.



- Ostman-Smith I, Wettrell G, Keeton B, Holmgren D, Ergander U, Gould S, Bowker C, Verdicchio M (2008) Age- and gender-specific mortality rates in childhood hypertrophic cardiomyopathy. *Eur Heart J* 29:1160-1167.
- Pacileo G, De Cristofaro M, Russo MG, Sarubbi B, Pisacane C, Calabro R (2000) Hypertrophic cardiomyopathy in pediatric patients: effect of verapamil on regional and global left ventricular diastolic function. *Can J Cardiol* 16:146-152.
- Penicka M, Gregor P, Kerekes R, Marek D, Curila K, Krupicka J, Candesartan use in H, Non-obstructive Cardiomyopathy Estate Study I (2009) The effects of candesartan on left ventricular hypertrophy and function in nonobstructive hypertrophic cardiomyopathy: a pilot, randomized study. *J Mol Diagn* 11:35-41.
- Petersen SE, Watkins H, Neubauer S (2009) Impaired left ventricular energy metabolism in hypertrophic cardiomyopathy is not due to fibrosis. *Heart* 95:505; author reply 505.
- Pollick C (1988) Disopyramide in hypertrophic cardiomyopathy. II. Noninvasive assessment after oral administration. *Am J Cardiol* 62:1252-1255.
- Prajapati C, Ojala M, Aalto-Setälä K (2018) Divergent effects of adrenaline in human induced pluripotent stem cell-derived cardiomyocytes obtained from hypertrophic cardiomyopathy. *Dis Model Mech* 11.
- Previs MJ, O'Leary TS, Morley MP, Palmer BM, LeWinter M, Yob JM, Pagani FD, Petucci C, Kim MS, Margulies KB, Arany Z, Kelly DP, Day SM (2022) Defects in the Proteome and Metabolome in Human Hypertrophic Cardiomyopathy. *Circ Heart Fail* 15:e009521.
- Prondzynski M et al. (2019) Disease modeling of a mutation in alpha-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. *EMBO Mol Med* 11:e11115.
- Pua CJ et al. (2020) Genetic Studies of Hypertrophic Cardiomyopathy in Singaporeans Identify Variants in TNNI3 and TNNT2 That Are Common in Chinese Patients. *Circ Genom Precis Med* 13:424-434.
- Qiu H, Sun Y, Pan Z, Zhou J, Wang H, Wang X, Cai D, Fu G, Gong T, Jiang C, Liang P (2021) Inhibition of HSC70 alleviates hypertrophic cardiomyopathy pathology in human induced pluripotent stem cell-derived cardiomyocytes with a MYBPC3 mutation. *Clin Transl Med* 11:e647.
- Ramachandra CJA, Mai Ja KPM, Lin YH, Shim W, Boisvert WA, Hausenloy DJ (2019) Induced Pluripotent Stem Cells for Modelling Energetic Alterations in Hypertrophic Cardiomyopathy. *Cond Med* 2:142-151.
- Ramachandra CJA, Cong S, Chan X, Yap EP, Yu F, Hausenloy DJ (2021a) Oxidative stress in cardiac hypertrophy: From molecular mechanisms to novel therapeutic targets. *Free Radic Biol Med* 166:297-312.
- Ramachandra CJA, Chua J, Cong S, Kp MMJ, Shim W, Wu JC, Hausenloy DJ (2021b) Human-induced pluripotent stem cells for modelling metabolic perturbations and impaired bioenergetics underlying cardiomyopathies. *Cardiovasc Res* 117:694-711.
- Ramachandra CJA, Kp MMJ, Chua J, Hernandez-Resendiz S, Liehn EA, Knoll R, Gan LM, Michaelsson E, Jonsson MKB, Ryden-Markinhulta K, Bhat RV, Fritsche-Danielson R, Lin YH, Sadayappan S, Tang HC, Wong P, Shim W, Hausenloy DJ (2022) Inhibiting cardiac myeloperoxidase alleviates the relaxation defect in hypertrophic cardiomyocytes. *Cardiovasc Res* 118:517-530.
- Raman B, Ariga R, Spartera M, Sivalokanathan S, Chan K, Dass S, Petersen SE, Daniels MJ, Francis J, Smillie R, Lewandowski AJ, Ohuma EO, Rodgers C, Kramer CM, Mahmood M, Watkins H, Neubauer S (2019) Progression of myocardial fibrosis in hypertrophic cardiomyopathy: mechanisms and clinical implications. *Eur Heart J Cardiovasc Imaging* 20:157-167.
- Ranjbarvaziri S et al. (2021) Altered Cardiac Energetics and Mitochondrial Dysfunction in Hypertrophic Cardiomyopathy. *Circulation* 144:1714-1731.
- Reichart D, Newby GA, Wakimoto H, Lun M, Gorham JM, Curran JJ, Raguram A, DeLaughter DM, Conner DA, Marsiglia JDC, Kohli S, Chmatal L, Page DC, Zabaleta N, Vandenberghe L, Liu DR, Seidman JG, Seidman C (2023) Efficient in vivo genome editing prevents hypertrophic cardiomyopathy in mice. *Nat Med* 29:412-421.
- Revera M, van der Merwe L, Heradien M, Goosen A, Corfield VA, Brink PA, Moolman-Smook JC (2008) Troponin T and beta-myosin mutations have distinct cardiac functional effects in hypertrophic cardiomyopathy patients without hypertrophy. *Cardiovasc Res* 77:687-694.
- Rohde JA, Roopnarine O, Thomas DD, Muretta JM (2018) Mavacamten stabilizes an autoinhibited state of two-headed cardiac myosin. *Proc Natl Acad Sci U S A* 115:E7486-E7494.
- Rosing DR, Kent KM, Maron BJ, Condit J, Epstein SE (1980) Verapamil therapy: a new approach to pharmacologic treatment of hypertrophic cardiomyopathy. *Chest* 78:239-247.
- Rosing DR, Idanpaan-Heikkilä U, Maron BJ, Bonow RO, Epstein SE (1985) Use of calcium-channel blocking drugs in hypertrophic cardiomyopathy. *Am J Cardiol* 55:185B-195B.
- Rovner A, Smith R, Greenberg NL, Tuzcu EM, Smedira N, Lever HM, Thomas JD, Garcia MJ (2003) Improvement in diastolic intraventricular pressure gradients in patients with HOCM after ethanol septal reduction. *Am J Physiol Heart Circ Physiol* 285:H2492-2499.
- Ryba DM, Warren CM, Karam CN, Davis RT, 3rd, Chowdhury SAK, Alvarez MG, McCann M, Liew CW, Wiczorek DF, Varga P, Solaro RJ, Wolska BM (2019) Sphingosine-1-Phosphate Receptor Modulator, FTY720, Improves Diastolic Dysfunction and Partially Reverses Atrial Remodeling in a Tm-E180G Mouse Model Linked to Hypertrophic Cardiomyopathy. *Circ Heart Fail* 12:e005835.
- Sabater-Molina M, Perez-Sanchez I, Hernandez Del Rincon JP, Gimeno JR (2018) Genetics of hypertrophic cardiomyopathy: A review of current state. *Clin Genet* 93:3-14.
- Saberi S, Cardim N, Yamani M, Schulz-Menger J, Li W, Florea V, Sehnert AJ, Kwong RY, Jerosch-Herold M, Masri A, Owens A, Lakdawala NK, Kramer CM, Sherrid M, Seidler T, Wang A, Sedaghat-Hamedani F, Meder B, Havakuk O, Jacoby D (2021) Mavacamten Favorably Impacts Cardiac Structure in Obstructive Hypertrophic Cardiomyopathy: EXPLORER-HCM Cardiac Magnetic Resonance Substudy Analysis. *Circulation* 143:606-608.
- Senthil V, Chen SN, Tsybouleva N, Halder T, Nagueh SF, Willerson JT, Roberts R, Marian AJ (2005) Prevention of cardiac hypertrophy by atorvastatin in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circ Res* 97:285-292.
- Shimada YJ, Raita Y, Liang LW, Maurer MS, Hasegawa K, Fifer MA, Reilly MP (2021) Comprehensive Proteomics Profiling Reveals Circulating Biomarkers of Hypertrophic Cardiomyopathy. *Circ Heart Fail* 14:e007849.
- Shimada YJ, Raita Y, Liang LW, Maurer MS, Hasegawa K, Fifer MA, Reilly MP (2022) Prediction of Major Adverse Cardiovascular Events in Patients With Hypertrophic Cardiomyopathy Using Proteomics Profiling. *Circ Genom*

- Precis Med 15:e003546.
- Shimada YJ, Passeri JJ, Baggish AL, O'Callaghan C, Lowry PA, Yannekis G, Abbara S, Ghoshhajra BB, Rothman RD, Ho CY, Januzzi JL, Seidman CE, Fifer MA (2013) Effects of losartan on left ventricular hypertrophy and fibrosis in patients with nonobstructive hypertrophic cardiomyopathy. *JACC Heart Fail* 1:480-487.
- Sieverding L, Jung WI, Breuer J, Widmaier S, Staubert A, van Erckelens F, Schmidt O, Bunse M, Hoess T, Lutz O, Dietze GJ, Apitz J (1997) Proton-decoupled myocardial <sup>31</sup>P NMR spectroscopy reveals decreased PCr/Pi in patients with severe hypertrophic cardiomyopathy. *Am J Cardiol* 80:34A-40A.
- Sitbon YH, Kazmierczak K, Liang J, Yadav S, Veerasammy M, Kanashiro-Takeuchi RM, Szczesna-Cordary D (2020) Ablation of the N terminus of cardiac essential light chain promotes the super-relaxed state of myosin and counteracts hypercontractility in hypertrophic cardiomyopathy mutant mice. *FEBS J* 287:3989-4004.
- Smith JGW, Owen T, Bhagwan JR, Mosqueira D, Scott E, Mannhardt I, Patel A, Barriales-Villa R, Monserrat L, Hansen A, Eschenhagen T, Harding SE, Marston S, Denning C (2018) Isogenic Pairs of hiPSC-CMs with Hypertrophic Cardiomyopathy/LVNC-Associated ACTC1 E99K Mutation Unveil Differential Functional Deficits. *Stem Cell Reports* 11:1226-1243.
- Spertus JA, Fine JT, Elliott P, Ho CY, Olivetto I, Saberi S, Li W, Dolan C, Reaney M, Sehnert AJ, Jacoby D (2021) Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): health status analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 397:2467-2475.
- Spindler M, Saupé KW, Christie ME, Sweeney HL, Seidman CE, Seidman JG, Ingwall JS (1998) Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. *J Clin Invest* 101:1775-1783.
- Spoladore R, Maron MS, D'Amato R, Camici PG, Olivetto I (2012) Pharmacological treatment options for hypertrophic cardiomyopathy: high time for evidence. *Eur Heart J* 33:1724-1733.
- Sugiura K, Kubo T, Ochi Y, Miyagawa K, Baba Y, Noguchi T, Hirota T, Yamasaki N, Doi YL, Kitaoka H (2022) Very long-term prognosis in patients with hypertrophic cardiomyopathy: a longitudinal study with a period of 20 years. *ESC Heart Fail* 9:2618-2625.
- Suwa M, Hirota Y, Kawamura K (1984) Improvement in left ventricular diastolic function during intravenous and oral diltiazem therapy in patients with hypertrophic cardiomyopathy: an echocardiographic study. *Am J Cardiol* 54:1047-1053.
- Talreja DR, Nishimura RA, Edwards WD, Valeti US, Ommen SR, Tajik AJ, Dearani JA, Schaff HV, Holmes DR, Jr. (2004) Alcohol septal ablation versus surgical septal myectomy: comparison of effects on atrioventricular conduction tissue. *J Am Coll Cardiol* 44:2329-2332.
- Teekakirikul P, Eminaga S, Toka O, Alcalai R, Wang L, Wakimoto H, Nayor M, Konno T, Gorham JM, Wolf CM, Kim JB, Schmitt JP, Molkentin JD, Norris RA, Tager AM, Hoffman SR, Markwald RR, Seidman CE, Seidman JG (2010) Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-beta. *J Clin Invest* 120:3520-3529.
- Toepfer CN, Wakimoto H, Garfinkel AC, McDonough B, Liao D, Jiang J, Tai AC, Gorham JM, Lunde IG, Lun M, Lynch TL, McNamara JW, Sadayappan S, Redwood CS, Watkins HC, Seidman JG, Seidman CE (2019) Hypertrophic cardiomyopathy mutations in MYBPC3 dysregulate myosin. *Sci Transl Med* 11.
- Toepfer CN et al. (2020) Myosin Sequestration Regulates Sarcomere Function, Cardiomyocyte Energetics, and Metabolism, Informing the Pathogenesis of Hypertrophic Cardiomyopathy. *Circulation* 141:828-842.
- Tsybouleva N, Zhang L, Chen S, Patel R, Lutucuta S, Nemoto S, DeFreitas G, Entman M, Carabello BA, Roberts R, Marian AJ (2004) Aldosterone, through novel signaling proteins, is a fundamental molecular bridge between the genetic defect and the cardiac phenotype of hypertrophic cardiomyopathy. *Circulation* 109:1284-1291.
- Vakrou S, Fukunaga R, Foster DB, Sorensen L, Liu Y, Guan Y, Woldemichael K, Pineda-Reyes R, Liu T, Tardiff JC, Leinwand LA, Tocchetti CG, Abraham TP, O'Rourke B, Aon MA, Abraham MR (2018) Allele-specific differences in transcriptome, miRNome, and mitochondrial function in two hypertrophic cardiomyopathy mouse models. *JCI Insight* 3.
- Vander Roest AS, Liu C, Morck MM, Kooiker KB, Jung G, Song D, Dawood A, Jhingran A, Pardon G, Ranjbarvaziri S, Fajardo G, Zhao M, Campbell KS, Pruitt BL, Spudich JA, Ruppel KM, Bernstein D (2021) Hypertrophic cardiomyopathy beta-cardiac myosin mutation (P710R) leads to hypercontractility by disrupting super relaxed state. *Proc Natl Acad Sci U S A* 118.
- Viswanathan SK et al. (2018) Association of Cardiomyopathy With MYBPC3 D389V and MYBPC3Delta25bpIntronic Deletion in South Asian Descendants. *JAMA Cardiol* 3:481-488.
- Wheeler MT, Jacoby D, Elliott PM, Saberi S, Hegde SM, Lakdawala NK, Myers J, Sehnert AJ, Edelberg JM, Li W, Olivetto I (2023) Effect of beta-blocker therapy on the response to mavacamten in patients with symptomatic obstructive hypertrophic cardiomyopathy. *Eur J Heart Fail* 25:260-270.
- Wilder T, Ryba DM, Wiczorek DF, Wolska BM, Solaro RJ (2015) N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy. *Am J Physiol Heart Circ Physiol* 309:H1720-1730.
- Wu G, Liu L, Zhou Z, Liu J, Wang B, Ruan J, Yang Q, Kanchwala M, Dai P, Zhang C, Wang D, Kang L, Wang S, Hui R, Zou Y, Xing C, Song L, Wang J (2020) East Asian-Specific Common Variant in TNNI3 Predisposes to Hypertrophic Cardiomyopathy. *Circulation* 142:2086-2089.
- Wu H, Yang H, Rhee JW, Zhang JZ, Lam CK, Sallam K, Chang ACY, Ma N, Lee J, Zhang H, Blau HM, Bers DM, Wu JC (2019) Modelling diastolic dysfunction in induced pluripotent stem cell-derived cardiomyocytes from hypertrophic cardiomyopathy patients. *Eur Heart J* 40:3685-3695.
- Yadav S, Kazmierczak K, Liang J, Sitbon YH, Szczesna-Cordary D (2019) Phosphomimetic-mediated in vitro rescue of hypertrophic cardiomyopathy linked to R58Q mutation in myosin regulatory light chain. *FEBS J* 286:151-168.
- Yuan CC, Kazmierczak K, Liang J, Ma W, Irving TC, Szczesna-Cordary D (2022) Molecular basis of force-pCa relation in MYL2 cardiomyopathy mice: Role of the super-relaxed state of myosin. *Proc Natl Acad Sci U S A* 119.
- Zhou W, Bos JM, Ye D, Tester DJ, Hrstka S, Maleszewski JJ, Ommen SR, Nishimura RA, Schaff HV, Kim CS, Ackerman MJ (2019) Induced Pluripotent Stem Cell-Derived Cardiomyocytes from a Patient with MYL2-R58Q-Mediated Apical Hypertrophic Cardiomyopathy Show Hypertrophy, Myofibrillar Disarray, and Calcium Perturbations. *J Cardiovasc Transl Res* 12:394-403.