Preventative therapeutic approaches for hypertrophic cardiomyopathy

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Abstract Sarcomeric gene mutations are associated with the development of hypertrophic cardiomyopathy (HCM). Current drug therapeutics for HCM patients are effective in relieving symptoms, but do not prevent or reverse disease progression. Moreover, due to heterogeneity in...
the clinical manifestations of the disease, patients experience variable outcomes in response to therapeutics. Mechanistically, alterations in calcium handling, sarcomeric disorganization, energy metabolism and contractility participate in HCM disease progression. While some similarities exist, each mutation appears to lead to mutation-specific pathophysiology. Furthermore, these alterations may precede or proceed development of the pathology. This review assesses the efficacy of HCM therapeutics from studies performed in animal models of HCM and human clinical trials. Evidence suggests that a preventative rather than corrective therapeutic approach may be more efficacious in the treatment of HCM. In addition, a clear understanding of mutation-specific mechanisms may assist in informing the most effective therapeutic mode of action.

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Abstract figure legend Sarcomeric gene mutations are associated with the development of hypertrophic cardiomyopathy (HCM). Mechanistically, alterations in calcium handling, sarcomeric disorganization, energy metabolism and contractility participate in HCM disease progression. These alterations may precede or proceed development of the pathology. This review assesses the efficacy of preventative versus corrective HCM therapeutics from studies performed in animal models of HCM and human clinical trials.

Introduction

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant cardiovascular disease that affects 1:200 of the general population (Semsarian et al. 2015). It is well-documented that the clinical characteristics of HCM consist of left ventricular wall thickening in the absence of increased haemodynamic workload, diastolic dysfunction and in other cases left ventricular outflow tract (LVOT) obstruction, mitral valve abnormalities and left atrial enlargement (Maron et al. 2006; Marian & Braunwald, 2017). At the cellular level, HCM is characterized by cardiac myocyte remodelling, disorganization of sarcomeric proteins, interstitial fibrosis and altered energy metabolism (Watkins et al. 2011). The literature to date suggests that HCM occurs primarily due to genetic mutations in sarcomeric proteins, which demonstrate variable penetrance and heterogeneous phenotypic expression in patients (Marian & Braunwald, 2017).

In cardiac muscle, calcium influx through the L-type calcium channel (I_{Ca,L}) in response to depolarization of the plasma membrane initiates contraction which leads to complex interactions between sarcomeric proteins and sarcoplasmic reticulum Ca^{2+} release. Genetic studies have identified over 1500 different mutations in genes of sarcomere proteins that have been associated with the development of HCM (Marian & Braunwald, 2017). The most common mutations appear to be cardiac myosin binding protein-C (MYBPC3), β-myosin heavy chain (MYH7), troponin I (TNNI3), troponin T (TNNT2) and α-tropomyosin (TPM1) (Watkins et al. 1995; Seidman & Seidman, 2001; Sabater-Molina et al. 2018). Regarding function, β-myosin heavy chain (β-MHC) is a sarcomeric protein that consists of a myosin carboxyl terminal rod domain, and an amino terminal globular head domain that interacts with actin filaments during muscle contraction (Fig. 1) (Rayment et al. 1993; Sata et al. 1997). Actin–myosin interactions that occur during excitation–contraction coupling, are regulated by the cardiac troponin (cTn) complex (Chandra et al. 2007). Cardiac troponin is composed of three regulatory sub-units: cardiac troponin I (cTnI), cardiac troponin T (cTnT) and cardiac troponin C (cTnC). Cardiac troponin I regulates cardiac contraction and relaxation in response to alterations in intracellular calcium (Ca^{2+}), while cTnT anchors the entire cTn complex to tropomyosin (Cheng & Regnier, 2016). During relaxation, cTnI inhibits the actin–myosin interaction, but when Ca^{2+} binds to the cTnI Ca^{2+} binding site (cTnC), cTnI undergoes a conformational change that allows the actin–myosin interaction and as a result, contraction (Cheng & Regnier, 2016). Cardiac myosin binding protein-C (cMyBP-C) is a thick filament associated protein that is believed to have structural importance by binding to actin, myosin and titin, as well as functional importance, through regulation of cross-bridge cycling and cardiac muscle contractility (Freiburg & Gautel, 1996; Sequeira et al. 2014).

Evidence suggests that HCM-causing sarcomeric gene mutations are associated with disorganization of sarcomere proteins, alterations in Ca^{2+} handling, myofilament Ca^{2+} sensitivity and mitochondrial metabolic function (for a review, see Viola & Hool, 2019). Indeed, patients expressing sarcomeric gene mutations are found to have decreased myocardial energy efficiency, which is thought to play an important role in the molecular pathway of the disease. However, although some similarities exist, each mutation appears to result in mutation-specific pathophysiology (Ferrantini et al. 2017;
β-adrenergic blockers

The cardiac L-type calcium channel is comprised of α_1c, α_2δ and β_2 subunits. Upon β-adrenergic receptor stimulation, calcium (Ca^{2+}) influx through the pore-forming α_1c subunit initiates ‘Ca^{2+}-induced-Ca^{2+}-release’ from sarcoplasmic reticulum (SR) stores via the Ryanodine receptor (RyR). ATP production occurs through Ca^{2+}-dependent mitochondrial oxidative phosphorylation, a process involving Ca^{2+} uptake by the mitochondrial Ca^{2+} uniporter (MCU), subsequent activation of the tricarboxylic acid (TCA) cycle and movement of electrons down complexes I–V of the electron transport chain. The β_2 subunit of the L-type calcium channel is anchored to F-actin via subsarcolemmal stabilizing protein AHNAK. Mitochondria also associate with F-actin via mitochondrial docking proteins. Mechanism for calcium-independent regulation of mitochondrial membrane potential (ψm) by the L-type calcium channel is shown in green. Concurrently, Ca^{2+} binds to thin filaments, which, powered by ATP, results in contraction. During the course of contraction, ATP is converted to ADP via ATPase, and back to ATP via the conversion of phosphocreatine (PCr) to creatine (Cr). Actin–myosin interaction is regulated by the cardiac troponin (cTn) complex, which is anchored to tropomyosin by cardiac troponin T (cTnT). When Ca^{2+} binds to cardiac troponin C (TnC), cTnI undergoes a conformational change that allows actin–myosin interaction, and therefore contraction. During relaxation, cTnI inhibits actin–myosin interaction. Cardiac myosin binding protein-C (cMyBP-C) binds to actin, myosin and titin and plays a role in regulating actin–myosin cross-bridge cycling and contractility. Hypertrophic cardiomyopathy (HCM) is caused by mutations in sarcomeric proteins. Existing therapeutic targets for treatment of HCM include β-adrenergic receptor (blockers), L-type calcium channel blockers (diltiazem, verapamil and nisoldipine), and β-myosin heavy chain (β-MHC)-actin binding (Mavacamten, MYK-461). AC, adenylyl cyclase; AID, alpha-interaction domain; cAMP, cyclic adenosine monophosphate; CSQ, calsequestrin; Gs, G-stimulatory protein; NCX, sodium/calcium exchanger; P, phosphorylation; PKA, protein kinase A; PLN, phospholamban; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase. Adapted from Viola & Hool (2019).
Viola & Hool, 2019). Additionally, these alterations may precede or proceed development of HCM pathology. This may contribute to the observed phenotypic variability in sarcomeric-related HCM, and as a result, provide an additional challenge to the design of effective drug therapy. Recent findings indicate that the cardiac L-type calcium channel (ICa-L) and mitochondria may play a collaborative role in the development of HCM (Viola & Hool, 2019). Interestingly, this appears to occur before the development of the pathology. In the present article, we assess the current knowledge regarding hypertrophic cardiomyopathy therapeutics in order to develop an understanding of the efficacy of preventative compared to corrective approaches (Abstract Figure).

**Role of the L-type calcium channel in cardiac function**

Calcium entry into cardiac myocytes through the ICa-L is critical for maintaining cardiac excitation and contraction (Bodi et al. 2005). The ICa-L is a heterotetrameric structure consisting of the pore-forming α1C and the accessory β2 and α2δ subunits (Fig. 1). The α1C subunit is a transmembrane structure consisting of four homologous motifs that regulate ion conductance and voltage sensing and contains binding sites for channel-modifying second messengers, toxins and drugs (Bodi et al. 2005). The β2 subunit of the ICa-L is entirely intracellular and assists with trafficking and insertion of the α1C subunit in the cell membrane (Burrai & Yang, 2013). The β2 subunit is bound to the cytoplasmic I–II linker of the α1C subunit of the channel called the alpha-interaction domain (AID) and undergoes conformational movement during channel activation and inactivation (Bodi et al. 2005).

The ICa-L regulates mitochondrial function via both Ca2+-dependent and Ca2+-independent mechanisms (Fig. 1). *In vitro* studies using intact quiescent cardiac myocytes, demonstrate that activation of the ICa-L by voltage-clamp of the plasma membrane, or the ICa-L agonist BayK(−), leads to increased intracellular Ca2+, increased mitochondrial Ca2+ uptake and superoxide production, and increased mitochondrial metabolic activity (Viola et al. 2009). While each of these responses is Ca2+-dependent, there is also evidence that activation of the ICa-L results in increased mitochondrial membrane potential (Ψm) that occurs in a Ca2+-independent manner (Viola et al. 2009, 2016a). This response may be in part dependent on a structural–functional interaction between the ICa-L and mitochondria that is transmitted via sarcomeric proteins.

In cardiac myocytes, microtubules (tubulin), microfilaments (actin) and intermediate filaments, extend from the plasma membrane to traverse cellular organelles including the t-tubules, sarcoplasmic reticulum and mitochondria (Tokuyasu et al. 1983). The β2 subunit of the ICa-L is anchored to F-actin networks (Fig. 1) (Rueckschloss & Isenberg, 2001; Hohaus et al. 2002). Changes in actin filament organization are sufficient to alter channel kinetics (Haase et al. 1999; Hohaus et al. 2002; Leach et al. 2005). Mitochondria also associate with sarcomeric proteins via mitochondrial docking proteins (Rappaport et al. 1998). We have identified that alterations in cardiac ICa-L activity can regulate Ψm via sarcomeric proteins in a Ca2+-independent manner (Viola et al. 2009). Preventing movement of the β2 subunit with application of a peptide derived specifically against the AID region of the ICa-L attenuates increases in Ψm caused by application of BayK(−) (Viola et al. 2009). Additionally, exposure of cardiac myocytes to F-actin depolymerizing agent latrunculin A also attenuates the response (Viola et al. 2014). These findings suggest that the ICa-L may influence cardiac mitochondrial function through a structural–functional communication. In support of this concept, the actin cytoskeleton plays an important role in mediating regulation of mitochondrial function by neuronal ICa-L (Johnson & Byerly, 1993; de Oliveira et al. 2019; Hotka et al. 2020). In neurons of the locus coeruleus, application of the mitochondrial protonophore carbonyl cyanide m-chlorophenylhydrazone has been demonstrated to induce a hyperpolarizing response that can be inhibited by application of either ICa-L blockers (nifedipine or nicardipine) or the actin depolymerizing agent cytochalasin D (de Oliveira et al. 2019). These findings suggest that a structural–functional communication between ICa-L and mitochondria may also play a role in regulating neuronal function.

**Role of the L-type calcium channel and mitochondria in hypertrophic cardiomyopathy disease progression**

Mutations in MYBPC3 genes coding for cMyBP-C are the most abundant, including primarily heterozygous nonsense mutations, insertions or deletions, and splicing point mutations (Carrier et al. 2015). Generally, these mutations result in C-terminally truncated cMyBP-C that lacks binding sites for sarcomeric proteins myosin and titin (Carrier et al. 2015). Studies performed in murine models expressing these mutations reveal that the absence of cMyBP-C protein is associated with increased actin–myosin cross-bridge cycling, myocyte disarray and fibrosis (Harris et al. 2002; Carrier et al. 2004). HCM patients with these mutations present with a mild disease phenotype and late onset of disease (Barefield et al. 2014). Since HCM is associated with disorganization of sarcomeric proteins and altered energy metabolism, it may be reasonable to postulate that a communication ‘break-down’ between the cardiac ICa-L and mitochondria may be involved in progression of HCM.

Transgenic mouse models of HCM are a useful tool to gain further insight into HCM pathophysiology. However,
a clear understanding of the underlying mechanisms of disease progression, from a pre- to post-hypertrophic state, has been difficult to ascertain from the current literature. This is in part due to the lack of clarity of cohort age (Viola & Hool, 2019). Therefore, the most valuable knowledge on the role of the $\text{ICa-L}$ and mitochondria in early and late stage HCM has been gained from studies performed in mouse models of the disease resulting from sarcomeric gene mutations.

In humans, a missense mutation in the TNNI3 gene encoding the cTnI protein (Gly203Ser) is characterized primarily by the development of apical hypertrophy, and in some cases supraventricular and ventricular arrhythmias (Kimura et al. 1997). Transgenic mice with a human disease-causing Gly203Ser mutation (cTnI-G203S) develop similar characteristic HCM features by 21 weeks of age, including hypertrophy, hypercontractility, cardiac myocyte disorganization and interstitial fibrosis (Tsoutsman et al. 2006; Viola et al. 2016a).

Cardiac myocytes isolated from 25- to 30-week-old cardiomyopathic cTnI-G203S mice exhibit significantly faster $\text{ICa-L}$ inactivation rates compared to wild-type myocytes (Viola et al. 2016a). In addition, consistent with the human phenotype, cardiac myocytes exhibit a hypermetabolic state compared to wild-type myocytes, as evidenced by significantly larger increases in mitochondrial activity and $\Psi_m$ in response to exposure of myocytes to BayK$(-)$ (Viola et al. 2016a). Interestingly, the increase in $\Psi_m$ was not due to further increases in mitochondrial Ca$^{2+}$ uptake in myocytes. We proposed that a structural–functional ‘breakdown’ between the cardiac $\text{ICa-L}$ and mitochondria may be involved in progression of the disease state. Furthermore, the same responses were observed in myocytes isolated from 10- to 15-week-old pre-hypertrophic cTnI-G203S mice (Viola et al. 2016a), indicating that altered metabolism appears to occur before the onset of clinical manifestations of HCM.

Patients carrying the Arg403Gln missense mutation in the MYH7 gene progressively develop septal hypertrophy and myocardial dysfunction and have a high incidence of sudden cardiac death (SCD) (Geisterfer-Lowrance et al. 1990; McConnell et al. 2001). There are two cardiac isoforms of MHC: $\alpha$-MHC and $\beta$-MHC. The predominant isoform in humans is $\beta$-MHC, accounting for >90% of ventricular myosin (Gupta, 2007). In neonatal mice the predominant isoform is $\beta$-MHC, but expression of $\beta$-MHC is silenced after birth and the predominant isoform transcribed shifts to $\alpha$-MHC in adult mice (Gupta, 2007). Heterozygous mice expressing the human Arg403Gln $\beta$-MHC mutation ($\alpha$MHC$^{403/+}$) gradually develop hypertrophy, myocyte disarray and increased myocardial fibrosis, mimicking the human disease (Geisterfer-Lowrance et al. 1996; Fatkin et al. 2000). Myocyte disarray appears to be an early cellular response, while histopathological features such as the development of hypertrophy and fibrosis occur after haemodynamic abnormalities (Geisterfer-Lowrance et al. 1996).

Similar to findings observed in myocytes isolated from cTnI-G203S mice, myocytes isolated from 30- to 50-week-old $\alpha$MHC$^{403/+}$ mice with established hypertrophy and fibrosis exhibit faster $\text{ICa-L}$ inactivation rates, and a hypermetabolic state compared to wild-type myocytes (Viola et al. 2016b). Additionally, myocytes isolated from 10- to 15-week-old pre-hypertrophic $\alpha$MHC$^{403/+}$ mice exhibit alterations in $\text{ICa-L}$ inactivation rates, mitochondrial activity and $\Psi_m$ which were comparable to those observed in post-hypertrophic $\alpha$MHC$^{403/+}$ mice (Viola et al. 2016b). Consistent with this, ex vivo studies assessing pre-cardiomyopathic 20- to 24-week-old Arg403Gln mice demonstrate lower cardiac phosphocreatine (PCr) to ATP (PCr/ATP) ratio, indicative of inefficient metabolic energetics (Spindler et al. 1998). Overall, data from both cTnI-G203S and $\alpha$MHC$^{403/+}$ mice suggest that alterations in $\text{ICa-L}$ kinetics, and a resulting hypermetabolic state, manifest before the development of the cardiomyopathy. Therefore, targeting the $\text{ICa-L}$ as a means of normalizing mitochondrial metabolic activity may be an attractive therapeutic approach for the treatment of HCM.

### Evaluation of current hypertrophic cardiomyopathy therapeutics

Clinical studies examining phenotypic heterogeneity in HCM have established that the disease ranges from asymptomatic or mildly symptomatic to severe manifestations (Marian & Braunwald, 2017). The presentation of HCM is age-dependent and while most patients have a normal life-expectancy with manageable symptoms, some are at increased risk of heart failure (HF) and SCD (Marian & Braunwald, 2017). Clinical features in patients with HCM, in addition to left ventricular hypertrophy, include altered ejection fraction, atrial fibrillation, ventricular arrhythmias and mitral regurgitation (Marian & Braunwald, 2017). One-third of patients present with LVOT obstruction at rest, and it can be induced in another third by increased cardiac workload (e.g. exercise) (Maron et al. 2006). To date, common therapeutics for patients with HCM focus on symptom management and the prevention of thrombotic events and SCD. These treatment strategies consist primarily of pharmacological therapies, and in more severe cases, surgical interventions including septal reduction and implantable cardioverter-defibrillators (Spoladore et al. 2012). Septal reduction methods such as septal myectomy or septal ablation, can improve function by relieving LVOT obstruction (Spoladore et al. 2012). However, these surgical procedures are invasive, target only symptomatic features, are not widely accessible
and carry risk to the patients. Additionally, implantable cardioverter-defibrillators are only used in high-risk patients, or those with very severe symptoms for the prevention of SCD.

Current pharmacological treatments in patients with HCM mainly aim to reduce LVOT obstruction and increase filling capacity (Ammirati et al. 2016). The most widely used therapeutics for HCM include β-adrenergic receptor blockers and Ca\textsuperscript{2+} channel blockers. Despite some management of symptoms with these drugs, their use can have pleiotropic effects and inconsistent therapeutic responses in patients (Ammirati et al. 2016). Given mutation-specific variations in disease progression (Ferrantini et al. 2017; Viola & Hool, 2019), we examined the current knowledge gained from studies performed in both animal models of HCM and clinical trials to develop an understanding of the efficacy of preventative versus corrective approaches.

β-Adrenergic receptor blockers

β-Adrenergic receptor blockers (β-blockers) have been described extensively in the literature as a treatment of symptomatic HCM since the 1960s. β-Blockers are capable of reducing LVOT obstruction, angina, dyspnoea and the risk of ventricular arrhythmias (Spoladore et al. 2012). β-Blockers inhibit sympathetic stimulation by binding to β-AR (β-adrenergic receptors) (Fig. 1). Downstream effects include decreased heart rate, contractility and LVOT obstruction (Spoladore et al. 2013). Studies performed in human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) demonstrate some therapeutic effects of β-blockers on myocardial hypertrophy, arrhythmia and Ca\textsuperscript{2+} handling abnormalities (Lan et al. 2013; Han et al. 2014; Toepfer et al. 2019a). Clinical studies in HCM patients indicate that β-blockers reduce left ventricular diastolic pressures and improve left ventricular filling; however there appears to be little beneficial impact regarding long-term effects on disease progression (Marian, 2009; Spoladore et al. 2012). Additionally, as β-blockers are a broad class of therapeutics and are used for a variety of heart conditions, they carry the potential for adverse side effects (Farzam & Jan, 2020). Recent reviews on their efficacy indicate that chronic use of β-blockers may induce additional side effects such as bradycardia, hypotension and atrioventricular nodal conduction block (Farzam & Jan, 2020).

Metabolic modulating agents

While the healthy adult heart utilizes fatty acid oxidation as a primary source of energy production, hypertrophic and failing hearts shift toward glucose and lactate metabolism (Lopaschuk et al. 2010; Vakrou & Abraham, 2014). Additionally, reduced PCr/ATP ratios have been reported in HCM patients with established left ventricular hypertrophy and in patients before the development of the pathology (Jung et al. 1998; Crilley et al. 2003; Timmer et al. 2011; Abraham et al. 2013). These findings support the notion that excessive ATP utilization and subsequent energy deficiency is an early mechanism in the development of HCM pathology. With this, a number of studies have investigated the use of metabolic therapies to target energetic deficits (Lee et al. 2005; Abozguia et al. 2010; Horowitz & Chirkov, 2010; van Driel et al. 2019).

Over the past decade, metabolic modulating agents such as perhexiline, trimetazidine and ranolazine, which were initially developed as therapeutic agents for angina, have been examined as potential HCM therapeutics (Abozguia et al. 2010; Olivotto et al. 2018). Perhexiline is thought to bind to and inhibit mitochondrial carnitine palmitoyltransferase enzymes, shifting myocardial substrate utilization from fatty acid oxidation to glucose metabolism (Ashrafian et al. 2007). In a Phase 2 clinical trial (METAL-HCM trial), perhexiline treatment (100 mg administered for 3–6 months) appeared to improve myocardial ratios of PCr/ATP ratio (indicative of improved energetics), diastolic dysfunction and \( P_{\text{O}_{2}} \) during exercise in a cohort of symptomatic HCM patients (Abozguia et al. 2010). Trimetazidine, a metabolic modulator and anti-ischaemic agent, is believed to act via inhibition of fatty acid β-oxidation, shifting metabolism from fatty acid oxidation to glucose oxidation (Dezsi, 2016; Steggall et al. 2017). In a Phase 2b clinical trial, trimetazidine (20 mg administered 3 times a day for 3 months) was shown to be ineffective in improving exercise capacity in symptomatic patients with non-obstructive HCM (Coats et al. 2019). Ranolazine acts to inhibit fatty acid β-oxidation and late inward sodium channels (Ardehali et al. 2012; Steggall et al. 2017). In a Phase 4 clinical trial, ranolazine (500 mg administered for 60 days) was used in the treatment of non-obstructive HCM, and although it effectively relieved some symptomatic features (angina and dyspnoea), it was demonstrated to have no overall effect on exercise performance or diastolic dysfunction (Gentry et al. 2016; Olivotto et al. 2018). Although some improvements have been observed, there is conflicting evidence in relation to improvements in overall functional capacity of HCM patients, with a small number of studies reporting adverse side effects (Abozguia et al. 2010; Gentry et al. 2016; Olivotto et al. 2018). Overall, it would appear that enhancing myocardial glucose metabolism may not be an efficacious approach in the treatment of HCM.

Calcium channel inhibitors

Calcium channel inhibitors target the pore-forming α\textsubscript{1C} subunit of the I\textsubscript{Ca-L} and have been used as an alternative treatment to β-blockers in clinical settings (Fig. 1)
(Striessnig et al. 2015). Calcium channel blockers are used in a similar manner to \( \beta \)-blockers in that they reduce heart rate and contractility, leading to improved diastolic filling and outflow; however, they are primarily administered in patients that exhibit non-obstructive HCM, or as an alternative in those experiencing adverse side effects with \( \beta \)-blockers (Spoladore et al. 2013; Striessnig et al. 2015). Calcium channel blockers such as diltiazem interrupt \( Ca^{2+} \) dysregulation processes through attenuation of \( Ca^{2+} \)-induced \( Ca^{2+} \) release, and subsequent restriction of \( Ca^{2+} \) uptake by the mutated sarcomere (Semsarian et al. 2002). Calcium channel inhibitors also cause greater negative ionotrophic effects compared to \( \beta \)-blockers due to the inhibition of \( Ca^{2+} \) through the channel pore and thereby tend to lead to poor clinical outcomes (Braunwald et al. 2002; Ho et al. 2015).

**Animal studies.** Studies performed in a mouse model of HCM due to a \( Tnnt2 \) mutation have revealed that in this model diastolic dysfunction occurs in the absence of significant hypertrophy (Westermann et al. 2006). Hypertrophy develops later in the pathogenesis of the disease. Under resting conditions, 21- to 30-week-old pre-hypertrophic mice with \( Tnnt2 \) mutation \( cTnT-Ile79Asn \), demonstrate left ventricular diastolic dysfunction, hypercontractility, enhanced myofilament \( Ca^{2+} \) sensitivity and cardiac stiffness, in the absence of hypertrophy or cardiac interstitial fibrosis. In response to \( \beta \)-adrenergic stimulation (isoproterenol), \( cTnT-Ile79Asn \) mice exhibit diastolic HF and SCD (Westermann et al. 2006). However, when pre-treated with diltiazem (25 mg kg\(^{-1}\) day\(^{-1}\)), isoproterenol-induced HF and SCD was prevented (Table 1). It was proposed that this effect may have been due to acute inhibition of \( I_{Ca-L} \) current, resulting in reduced \( Ca^{2+} \) influx into myocytes, and subsequent alterations in diastolic \( Ca^{2+} \) (Westermann et al. 2006). Certainly, it would appear that pre-treatment of the \( cTnT-Ile79Asn \) mice with diltiazem prevented isoproterenol-induced HF and SCD.

Although the \( I_{Ca-L} \) is the primary target of diltiazem, it is also known to have other cellular targets including the mitochondrial \( Na^{+}/Ca^{2+} \) exchanger (Striessnig et al. 2015). With this, it has been proposed that diltiazem may reduce hypertrophic presentation by normalizing alterations in mitochondrial \( Ca^{2+} \) concentration, thereby improving cardiac energetics (Semsarian et al. 2002). In a recent study, diltiazem was assessed as an HCM therapeutic in homozygous \( Mybpc3 \)-targeted knock-in (KI) mice carrying a \( c.772G>A \) transition on the last nucleotide of exon 6 (\( Mybpc3 \) KI (\( c.772G>A \)) (Frayssé et al. 2012; Flenner et al. 2017). \( Mybpc3 \) KI (\( c.772G>A \)) mice exhibit increased systolic and diastolic dysfunction and myofilament \( Ca^{2+} \) sensitivity followed by cardiac hypertrophy (Frayssé et al. 2012; Flenner et al. 2017). Cardiac myocytes were isolated from cardiac myopathic \( Mybpc3 \) KI (\( c.772G>A \)) mice, and exposed to isoproterenol and high pacing frequency stress conditions (Flenner et al. 2017). Under these conditions, myocytes exhibited decreased diastolic sarcomere length, increased \( Ca^{2+} \) transient rise, and arrhythmias (Flenner et al. 2017). Each of these observations was normalized in the presence of diltiazem (Table 1). In *vivo* studies were also performed in 6- to 8-week-old pre-cardiomyopathic \( Mybpc3 \) KI (\( c.772G>A \)) mice, treated with diltiazem for 6 months (Flenner et al. 2017). Diltiazem treatment did not prevent activation of the fetal gene programme, cardiac hypertrophy and dysfunction, or fibrosis (Table 1) (Flenner et al. 2017). These data suggest that while acute diltiazem treatment in post-hypertrophic \( Mybpc3 \) KI (\( c.772G>A \)) mice may be beneficial in prevention of stress-induced contractile abnormalities, chronic administration of diltiazem treatment does not appear to reverse or prevent development of HCM pathology.

Studies performed in \( \alpha MHC^{403/+} \) mice have indicated abnormal \( Ca^{2+} \) handling and reduced \( Ca^{2+} \)-binding and storage protein levels in this model, including calsequestrin, junctin, triadin and ryanodine receptor 2 (RyR2) compared to control mice (Semsarian et al. 2002). This occurs before the onset of the disease phenotype. In the same study, 15- to 20-week-old pre-hypertrophic \( \alpha MHC^{403/+} \) mice were treated with diltiazem (25 mg kg\(^{-1}\) day\(^{-1}\)). Following 7 weeks of treatment, \( Ca^{2+} \)-binding and storage protein levels were restored (Table 1) (Semsarian et al. 2002). Interestingly, histological features such as fibrosis, myocyte hypertrophy and disarray were also abated with early diltiazem treatment (Semsarian et al. 2002). Similar studies were performed in 30- to 50-week-old post-hypertrophic \( \alpha MHC^{403/+} \) mice. Following 7 weeks of diltiazem treatment, \( \alpha MHC^{403/+} \) mice demonstrated reduced expression of hypertrophic molecular markers, reduced left ventricular wall thickness, improved end-diastolic and end-systolic volumes, and reduced fibrosis, compared to untreated mice (Table 1) (Semsarian et al. 2002). However, fractional shortening (FS) was not improved. These data indicate that an early, pre-treatment approach may be more effective in preventing HCM pathology.

In *vivo* studies performed in cardiac myocytes isolated from both pre- and post-cardiomyopathic \( \alpha MHC^{403/+} \) mice provide additional support for an early intervention approach. Myocytes isolated from both pre- and post-cardiomyopathic \( \alpha MHC^{403/+} \) mice exhibit a significantly faster \( I_{Ca-L} \) inactivation rate, and subsequently a hypermetabolic mitochondrial state in response to BayK(−), compared to myocytes isolated from age-matched wild-type mice (Viola et al. 2016b). Exposure of \( \alpha MHC^{403/+} \) myocytes to diltiazem or nisoldipine (15 \( \mu M \)) normalized mitochondrial metabolic activity in both pre- and post-cardiomyopathy \( \alpha MHC^{403/+} \) myocytes.
| Gene      | Mutation/Model | Pre/Post HCM | Characteristics                  | Treatment | Outcomes                                | Ref                  |
|-----------|----------------|--------------|----------------------------------|-----------|-----------------------------------------|----------------------|
| **Animal models** |              |              |                                  |           | (Continued)                              |                      |
| **TNNT2** | Tnn2-TnT-179N mice | in vivo      | Pre (21–30 week) ISO            | HFSCD: ↑  | Diltiazem (25 mg kg⁻¹ day⁻¹, 50 days)  | Westermann et al. (2006) |
| **MYBPC3** | Homozygous Mybpc3 KI (c.772G>A) mice | in vitro     | Post (32–34 week ISO/Paced       | Sarcomere length: ↓, Ca²⁺ transient time to peak: ↑, Arrhythmias: ↑ | Diltiazem (1 µM) Sarcomere length: ↓, Ca²⁺ transient time to peak: ↓, Arrhythmias: ↓ | Flenner et al. (2017) |
| **in vitro** | Post (6–8 week) | Hypertrophy & Dysfunction: ↑ | Diltiazem (5 mg kg⁻¹ day⁻¹, 6 months) | Hypertrophy and Dysfunction: ↑ | (no ∆) | |
| **Mybpc1h/ Mybpc1h** | mice | in vitro     | Post (8–20 week) | Cell shortening: ↑, MYBPC3t/t Cell shortening: ↑, Relaxation time: ↑ | MYK-461 (0.15 µM, 0.3 µM) | Toepfer et al. (2019b) |
| **TNNT2** | Tnn13-Gly203Ser mice | in vitro      | Pre (10–15 week) MMA: ↑, ψm: ↑ | Nisoldipine (15 µM) | MMA: ↓, ψm: ↓ | Viola et al. (2016a) |
| **in vivo** | Post (25–30 week) | MMA: ↑, ψm: ↑ | Diltiazem (15 µM) MMA: ↓, Nisoldipine (15 µM) | MMA: ↓, ψm: ↓ | | |
| **in vivo** | Pre (20 week) | I ca-L inactivation rate: ↑, MMA: ↑, ψm: ↑ | AID-TAT (10 µM, 3 × week/5 week) | I ca-L inactivation rate: ↓, MMA: ↓, ψm: ↓, Myocyte hypertrophy: ↓, HW:BW: ↓, IVST: ↓, LVEDD/LVESD: ↑, FS: ↓ | Viola et al. (2020) |
| **Post (30 week)** | I ca-L inactivation rate: ↑, MMA: ↑, ψm: ↑, Myocyte hypertrophy: ↑, HW:BW: ↑, IVST: ↑, LVEDD/LVESD: ↓, FS: ↑ | AID-TAT (10 µM, 3 × week/5 week) | MMA: ↑ (no ∆), ψm: ↑ (no ∆), Myocyte hypertrophy: ↑ (no ∆), HW:BW: ↑ (no ∆), IVST: ↑ (no ∆), LVEDD/LVESD: ↓ (no ∆), FS: ↑ (no ∆) | | |
| (Continued) | | | | | | |
| Gene   | Mutation/Model   | Pre/Post HCM | Characteristics    | Treatment       | Outcomes                           | Ref               |
|--------|------------------|--------------|--------------------|-----------------|------------------------------------|-------------------|
| MYH7   | Arg403Gln mice   | Pre (10–15 week) | MMA: ↑ ✓         | Nisoldipine (15 µM) | MMA: ↓ ✓                          | Viola et al. (2016b) |
|        |                   | Post (30–50 week) | ⊳Psi1 m: ↑ ✓     | Diltiazem (15 µM) | ⊳Psi1 m: ↓ ✓                       |                   |
|        |                   |               | ⊳MMA: ↑ ✓         | Nisoldipine (15 µM) | MMA: ↓ ✓                          |                   |
|        |                   | Pre (10–15 week) | Ca²⁺-binding proteins: ↓ | Diltiazem (25 mg kg⁻¹ day⁻¹, 7 weeks) | Ca²⁺-binding proteins: ↑ Fibrosis: ↓ Myocyte hypertrophy and disarray: ↓ | Sensarian et al. (2002) |
|        |                   | Post (30–50 week) | Hypertrophic markers: ↑ Myocyte disarray: ↑ Fibrosis: ↑ ESV and EDV: ↓ | | Hypertrophic markers: ↓ Myocyte disarray: ↓ Fibrosis: ↓ ESV and EDV: ↑ FS: ↑ (no Δ) |                   |
| MYH7 – | Arg403Gln Arg719Trp Arg453Cys mice | Pre (6–15 week) | NA | MYK-461 (2.5 mg kg⁻¹ day⁻¹, 20–26 weeks) | LVWT: improved (✓) FS: improved (✓) Fibrosis: improved (✓) Myocyte disarray: improved (✓) | Green et al. (2016) |
|        |                   | Post (30–35 week) | LVWT: ↑ FS: ↑ Fibrosis: ↑ Myocyte disarray: ↑ | MYK-461 (2.5 mg kg⁻¹ day⁻¹, 4 weeks) | LVWT: partial ↓ FS: partial ↓ Fibrosis: ↑ (no Myocyte disarray: ↑ (no Δ) |                   |
| Not specified | Idiopathic HCM felines | Post (5.7–10.8 years) | LVWT: ↑ IVST: ↑ LVEDD: ↓ FS: ↑ | Diltiazem (~5.34 mg kg⁻¹, 6 months) | LVWT: ↓ IVST: ↓ LVEDD: ↑ FS: ↑ (no Δ) Adverse effects or HF/SCD | Bright et al. (1991) |
| Not specified | Idiopathic HOCM felines | Post (0.9–3.7 years) | ISO | FS: ↑ LVOT obstruction: ↑ SAM: ↑ | MYK-461 (0.12–0.36 mg kg⁻¹ h⁻¹) | FS: ↓ LVOT obstruction: ↓ SAM: ↓ LVWT: not reported | Stern et al. (2016) |

(Continued)
| Gene          | Mutation/Model | in vitro/in vivo | Pre/Post HCM | Characteristics                      | Treatment                  | Outcomes                                      | Ref                  |
|--------------|----------------|------------------|--------------|--------------------------------------|---------------------------|----------------------------------------------|----------------------|
| MYBPC3       | Mybp<sup>c1h</sup> | in vitro         | 30 days post-differentiation | Cell shortening: ↑ | Propanolol (0–10 μM l⁻¹) | Cell shortening: ↓ | Toepfer et al. (2019a) |
|              |                 |                  |              | Contractility: ↑                     | Verapamil (0–10 μM l⁻¹)  | Contractility and relaxation time: ↑  |                      |
|              |                 |                  |              | Relaxation time: ↑                    | MYK-461 (0–10 μM l⁻¹)    | Cell shortening: ↓ |                      |
| MYH7         | MYH7 – Arg663His | in vitro         | 20–40 days post-differentiation | Hypertrophic markers: ↑ | Propanolol (400 nM)          | Myocyte hypertrophy: ↓ | Lan et al. (2013) |
|              |                 |                  |              | Myocyte hypertrophy: ↑                | Verapamil, (50–100 nM)   | Ca<sup>2+</sup> handling abnormalities: ↓ |                      |
|              |                 |                  |              | Ca<sup>2+</sup> handling abnormalities: ↑ | Diltiazem, (50–100 nM)  | Arrhythmia: ↓   |                      |
|              |                 |                  |              | Arrhythmia: ↑                          | Metapropolol (10 μM)     |                                | Han et al. (2014) |
|              | MYH7 – Arg442Gly | in vitro         | 30 days post-differentiation | Contractility: ↑ | Verapamil (100 nM)          | Ca<sup>2+</sup> handling abnormalities: ↓ |                      |
|              |                 |                  |              | Myocyte hypertrophy & disarray: ↑     |                           | Arrhythmia: ↓ |                      |
|              |                 |                  |              | Ca<sup>2+</sup> handling abnormalities: ↑ |                           |                          |                      |

(Continued)
Table 1. Continued

| Gene            | Mutation/Model       | in vitro/in vivo | Pre/Post HCM | Characteristics | Treatment                          | Outcomes                                         | Ref                      |
|-----------------|----------------------|------------------|--------------|----------------|------------------------------------|--------------------------------------------------|--------------------------|
| Human studies   | **MYBPC3**           | *in vivo*        | Pre (20–55 years) | $S'$: ↓ | Diltiazem (240 mg day\(^{-1}\), 8 weeks) | $S'$: ↑ | McTaggart (2004) |
|                 | MYBPC3–O969X, N755K human patients |                 |              | $E'$: ↓       |                                    |                                                  |                          |
|                 | **MYH7**             | Mixture of 25 mutations | in vivo | Pre (5–39 years) | NA | Diltiazem (5 mg kg\(^{-1}\) day\(^{-1}\), 12–42 months) | LVWT dimension: improved (↓) | Ho et al. (2015) |
|                 | **MYBPC3**           |                  |              |               |                                    |                                                  |                          |
|                 | **TNNT2**            |                  |              |               |                                    |                                                  |                          |
| Not specified   | Human HCM patients   | *in vivo*        | Post (22–70 years) | PIONEER HCM TRIAL (Phase 2) | Resting LVEF: ↑ Post-exercise LVOT gradient: ↑ | MYK-461 (10–20 mg day\(^{-1}\), 12 weeks) | Ho et al. (2019) |
| LVWT: not reported |                  |                  |              |               |                                    |                                                  |                          |
| Not specified   | Human HCM patients   | *in vivo*        | Post (mean age: 54 years) | MAVERICK HCM trial (Phase 2) | LVEF: ↑ NT-proBNP (wall stress): ↑ | 19, 21, 19 patients to 200 ng ml\(^{-1}\), 500 ng ml\(^{-1}\), or placebo, respectively | Ho et al. (2020a) |
| LVWT: not reported |                  |                  |              |               |                                    |                                                  |                          |
| Unknown         | Human HCM patients   | *in vivo*        | >18 years | EXPLORER HCM trial (Phase 3) | LVEF: ↑ $P_{O2}$: ↑ LVOT gradient: ↑ | 2.5, 5.0, 10.0 or 15 mg day\(^{-1}\), 30 weeks | Ho et al. (2020b), Myokardia (2020) |

$E'$, peak velocity of early diastolic mitral annular motion; $E/E'$, ratio of peak velocity of early diastolic transmural flow to mitral annular motion; EDV, end-diastolic volume; ESV, end-systolic volume; FS, fractional shortening; HW:BW, heart weight to body weight ratio; HOCM, obstructive hypertrophic cardiomyopathy; HF, heart failure; I\(_{Ca-L}\), L-type calcium channel; ISO, isoproterenol; IVST, Intraventricular septum thickness; LVD, left ventricular diameter; LVEDD, left ventricular end-diastolic diameter; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVOT, left ventricular outflow tract obstruction; LVWT, left ventricular wall thickness; MMA, mitochondrial metabolic activity; NA, not applicable; $\Delta$, change; $\Psi_1$, mitochondrial membrane potential; $P_{O2}$, peak oxygen consumption; $S'$, systolic velocity peak; SAM, systolic anterior motion of the mitral valve; SCD, sudden cardiac death.
arrhythmias, while diltiazem exposure ameliorated Ca\textsuperscript{2+} including cellular enlargement, contractile arrhythmia, demonstrate numerous disease features of HCM, and human clinical studies indicate that early treatment may be beneficial to prevent some HCM-associated characteristics.

**hiPSC-CM studies.** *In vitro* experiments utilizing hiPSC-CMs expressing a missense mutation in \(\beta\)-MHC demonstrate numerous disease features of HCM, including cellular enlargement, contractile arrhythmia, Ca\textsuperscript{2+} dysregulation and sarcomeric disorganization (Lan et al. 2013; Han et al. 2014; Tanaka et al. 2014). In a hiPSC-CM model expressing MYH7 gene mutation Arg663His, *in vitro* verapamil prevented myocyte hypertrophy and abolished Ca\textsuperscript{2+} dysregulation and arrhythmias, while diltiazem exposure ameliorated Ca\textsuperscript{2+} handling abnormalities and arrhythmias, in single and multi-cell preparations (Lan et al. 2013) (Table 1). Similar findings were observed in hiPSC-CMs expressing MYH7 gene mutation Arg442Gly, whereby *in vitro* exposure to verapamil normalized Ca\textsuperscript{2+} handling abnormalities and arrhythmias (Han et al. 2014).

**Human studies.** Transgenic rabbit models of HCM and human clinical studies indicate that early diastolic velocities are abnormally low in MYBPC3 gene mutation carriers before the development of left ventricular hypertrophy (Nagueh et al. 2001; Ho et al. 2002). Therefore, utilizing tissue Doppler, the effects of diltiazem treatment have been assessed in patients carrying MYBPC3 gene mutations (Gln969X or Asn755Lys) (McTaggart, 2004). Patients were pre-hypertrophic, with no symptomatic manifestations of HCM assessed by echocardiography and electrocardiography. Patients administered diltiazem (240 mg day\textsuperscript{−1}) for 8 weeks, exhibited increases in both systolic velocity peak (\(S^\prime\)) and early diastolic velocity peak (\(E^\prime\)) that appeared to normalize cardiac flow, as compared to patients receiving placebo treatment (Table 1) (McTaggart, 2004). These data indicate a potential benefit of early diltiazem treatment in the pre-hypertrophic stages of the disease in patients carrying MYBPC3 gene mutations. Interestingly, the greatest improvements were in the youngest patients who may have had fewer structural changes present.

More recently, a pilot study was undertaken to assess the efficacy of diltiazem in preventing the phenotypic presentation of HCM in 38 patients carrying MYBPC3, MYH7 and TNNT2 gene mutations (Ho et al. 2015). Mutation carriers with no clinical diagnosis of HCM (specifically left ventricular hypertrophy as assessed by echocardiography) received chronic diltiazem treatment (5 mg kg\textsuperscript{−1} day\textsuperscript{−1}, 12–42 months) or an equivalent placebo. Patients treated with diltiazem exhibited improved LVWT-to-dimension ratio, and LVEDD, compared to the placebo group (Table 1) (Ho et al. 2015). Within the diltiazem-treated MYBPC3 mutation carriers, LVWT, diastolic filling (reflected by \(E/E^\prime\)) and cardiac troponin I levels were improved compared to the placebo group (Ho et al. 2015). Interestingly, four unrelated patients, three with MYH7 mutations and one with a TNNT2, did not respond to diltiazem treatment.

Overall, studies performed in animal models of HCM and human clinical studies indicate that early treatment with diltiazem may be beneficial to prevent some HCM-associated characteristics. Mechanistically, this may occur by normalizing cellular Ca\textsuperscript{2+} handling, and/or by restoring structural–functional communication between the I\textsubscript{Ca-L} and mitochondria and subsequently normalizing mitochondrial metabolic activity. Certainly, treatment efficacy appears to vary depending on the underlying gene mutation.

**MYK-461**

Patients with HCM often present with early hypercontractility that stems from a high degree of actin–myosin cross-linking (Heitner et al. 2019). Recent studies have identified a cardiac-specific small-molecule, mavacamten (MYK-461), that directly targets the sarcomere by modulating \(\beta\)-MHC (Green et al. 2016; Stern et al. 2016; Kawas et al. 2017). This reversibly inhibits \(\beta\)-MHC–actin binding, and subsequently reduces sarcomere force output and contractility (Fig. 1) (Heitner et al. 2019). Over the past 5 years, several studies have investigated the efficacy of MYK-461 as a potential HCM therapeutic.
Animal models. The effectiveness of MYK-461 treatment has been assessed in murine models of HCM expressing cMyBP-C gene mutations (Mybpc3\(^{3/4}\) and Mybpc3\(^{3/5}\)) (Toepfer et al. 2019b). Echocardiography studies have revealed that Mybpc3\(^{3/4}\) (endogenous heterozygous) mice exhibit minimal increases in left ventricular posterior wall thickness, and depressed cardiac contractility compared to wild-type mice (Toepfer et al. 2019b) (Table 1). On the other hand, Mybpc3\(^{3/5}\) (homozygous truncated) mice exhibit significantly increased left ventricular volumes and mass, but depressed contractile function (Toepfer et al. 2019b). However, studies performed in isolated cardiac myocytes revealed contractile differences that were not apparent from in vivo echocardiography. Utilizing isolated cardiac myocytes, sarcomere length in vivo that were not apparent from in vitro contractility and relaxation (defined as proxies for systolic and diastolic function respectively). Cardiac myocytes isolated from Mybpc3\(^{3/4}\) and Mybpc3\(^{3/5}\) mice exhibited significantly increased cell shortening compared to wild-type myocytes (Toepfer et al. 2019b). Relaxation time was significantly increased in Mybpc3\(^{3/5}\) myocytes, but not significantly altered in Mybpc3\(^{3/4}\) myocytes. These data are consistent with a hypercontractile state. Acute exposure to MYK-461 (0.15–0.3 \(\mu\)M) significantly reduced cell shortening in both Mybpc3\(^{3/4}\) and Mybpc3\(^{3/5}\), and normalized relaxation times in Mybpc3\(^{3/5}\) myocytes (Toepfer et al. 2019b). These in vitro data indicate that MYK-461 may normalize contractile function.

Studies have also been performed in mice expressing \(\beta\)-MHC mutations to investigate the efficacy of MYK-461 in both preventing and reversing associated HCM (Green et al. 2016). Treatment of 6- to 15-week-old pre-hypertrophic mice expressing \(\beta\)-MHC mutations (Arg403Gln, Arg719Trp or Arg453Cys) with MYK-461 (0.12–0.36 mg kg\(^{-1}\) h\(^{-1}\)) before isoproterenol treatment exhibited a significantly reduced FS compared to vehicle treatment, without negatively impacting heart rate (Stern et al. 2016) (Table 1). In addition, in post-hypertrophic felines exposed to isoproterenol, MYK-461 treatment reduced systolic anterior motion of mitral valves and prevented worsening of LVOT obstruction (Stern et al. 2016). Overall, early MYK-461 treatment appeared to improve contractility and relieve inducible HOCM (Stern et al. 2016).

hiPSC-CM studies. An in vitro model utilizing hiPSC-CMs that express a heterozygous truncation variant in the MYBPC3 gene (Mybpc3\(^{3/4}\)) recapitulates aspects of the HCM phenotype including hypercontractility, cell shortening and impaired relaxation (Toepfer et al. 2019a). Consistent with observations in animal models of the disease, exposure of hiPSC-CMs to MYK461 resolved contractile abnormalities, specifically low doses (1 \(\mu\)mol l\(^{-1}\)) normalized hypercontractility, whereas higher doses (2–4 \(\mu\)mol l\(^{-1}\)) were required to normalize relaxation times (Toepfer et al. 2019a) (Table 1).

Human studies. In a Phase 2 clinical trial (PIONEER HCM trial), patients with symptomatic HOCM presenting with elevated resting left ventricular ejection fraction (LVEF), post-exercise left ventricular outflow tract (LVOT) obstruction and LVWT, received MYK-461 (10–20 mg day\(^{-1}\)) for 12 weeks (Heitner et al. 2019). Patients receiving MYK-461 treatment demonstrated improved resting LVEF (reduced), improved peak oxygen consumption (increased), and reduced post-exercise LVOT gradients (Heitner et al. 2019) (Table 1). The effect of MYK-461 on LVWT was not reported.

In another Phase 2 clinical trial (MAVERICK-HCM trial), patients with symptomatic non-obstructive HCM (characterized by the presence of hyper-contractility and impaired relaxation but no significant LVOT obstruction at rest or with provocation) were treated with 200 ng ml\(^{-1}\), 500 ng ml\(^{-1}\) or a placebo for 16 weeks (Ho et al. 2020a). Compared to placebo-treated individuals, patients receiving MYK-461 treatment exhibited improved LVEF (reduced), improved peak oxygen consumption (\(P_{\text{VO}_2}\)) and decreased wall stress as indicated by reduced levels of serum biomarkers such as N-terminal pro-B-type natriuretic peptide (NT-proBNP) (Ho et al. 2020a) (Table 1). No data has been released regarding the effect of MYK-461 on LVWT.

A recent Phase 3 trial (EXPLORER HCM trial) was undertaken involving patients with HOCM presenting with LVOT obstruction and associated left ventricular hypertrophy, being administered MYK-461 at a range of doses (2.5, 5, 10 and 15 mg day\(^{-1}\)) over a 30-week period (Ho et al. 2020b). Primary and secondary efficacy assessments included post-exercise LVOT peak...
improvements in cellular Ca-L kinetics, mitochondrial treatment with AID-TAT peptide resulted in significant improvements in $P_{\text{VO}_2}$ and LVOT gradient (Ho et al. 2020b) (Table 1). To date, patients receiving MYK-461 have been reported to display improvements in $P_{\text{VO}_2}$ and LVOT gradient (decreased) (Myokardia, 2020). No data have been released regarding the effect on LVWT (Myokardia, 2020).

The use of hiPSC-CMs has been an important development in the field of cardiovascular disease modelling to further understand pathophysiological mechanisms of cardiovascular diseases in vitro, and develop novel therapeutic treatments for cardiovascular diseases such as HCM (Lodrini et al. 2020). The recent application of genome editing to hiPSC-CMs has enabled further investigation on the genetic causation of HCM. However, limitations exist, as hiPSC-CMs are structurally and functionally immature in comparison to human adult cardiac myocytes and therefore do not fully recapitulate their complex physiological properties (Lan et al. 2013; Han et al. 2014; Ramachandra et al. 2019). Nonetheless, when considered together, studies performed in animal models of HCM, hiPSC-CM and human clinical studies indicate that early therapeutic intervention with MYK-461 may be effective in normalizing HCM-associated hypercontractility, and relieve inducible HOCM, by inhibiting $\beta$-MHC–actin binding and subsequently, reducing sarcomere force output.

**AID-TAT peptide**

Studies utilizing transgenic mouse models of HCM indicate that a structural–functional ‘breakdown’ between the cardiac $I_{\text{Ca-L}}$ and mitochondria via sarcomeric proteins may lead to the development of a hypermetabolic mitochondrial state, which precedes development of HCM pathology (Viola et al. 2016a,b). Recent studies have investigated the use of AID-TAT peptide as a potential HCM therapeutic (Viola et al. 2020). Unlike $\beta$-blockers and Ca$^{2+}$ channel blockers, AID-TAT peptide specifically targets the AID region of the cardiac $I_{\text{Ca-L}}$, immobilizing movement of the $I_{\text{Ca-L}}$ $\beta_2$ subunit (Fig. 1) (Hohaus et al. 2000; Viola et al. 2020).

Twenty-week-old pre-cardiomyopathic cTnI-G203S mice were treated with AID-TAT peptide (10 $\mu$m) three times a week for 5 weeks (Viola et al. 2020). Treatment with AID-TAT peptide resulted in significant improvements in cellular $I_{\text{Ca-L}}$ kinetics, mitochondrial metabolic activity and cell size (decreased), and a significant decrease in heart weight to body weight ratio (Viola et al. 2020). In vivo echocardiography revealed a significant improvement in LVEDD/LVESD (increase), and IVST and FS (decrease) in cTnI-G203S mice treated with AID-TAT peptide. Treatment of 30-week-old post-cardiomyopathic cTnI-G203S mice with established hypertrophy with AID-TAT peptide did not significantly improve mitochondrial metabolic activity, cell size, heart weight to body weight ratio (HW:BW) or echocardiographic parameters (Viola et al. 2020). These studies indicate that early therapeutic intervention with AID-TAT peptide may represent a viable approach to restore structural–functional communication between $I_{\text{Ca-L}}$ and mitochondria, normalize metabolic activity and prevent the development of HCM.

**Conclusion**

Conventionally, HCM is characterized by cardiac myocyte remodelling, disorganization of sarcomeric proteins, interstitial fibrosis and altered energy metabolism. There is now evidence to suggest that alterations in Ca$^{2+}$ handling, energy metabolism, contractility and sarcomeric disorganization may precede the presentation of hypertrophy and fibrosis. Indeed, here we find that a preventative rather than corrective therapeutic approach may be more efficacious in the treatment of HCM. However, while some similarities exist, each mutation appears to lead to mutation-specific pathophysiology, which may contribute to the observed clinical phenotypic variability in sarcomere-related HCM (Viola & Hool, 2019). A clear understanding of early mutation-specific mechanisms may be required, on a cellular level, in order to determine the most effective therapeutic mode of action. Studies investigating the efficacy of diltiazem or AID-TAT peptide indicate that early treatment may be beneficial in preventing hypertrophy by normalizing cellular Ca$^{2+}$ handling, and/or normalizing mitochondrial metabolic activity. On the other hand, early therapeutic intervention with MYK-461 may be effective in normalizing hypercontractility and relieve inducible HOCM, by reducing sarcomere force output. In addition to mutation-specific pathophysiology, epigenetic differences, genetic modifiers and environmental factors can also influence HCM morphology, producing a variety of clinical phenotypes from the same gene mutation (Burke et al. 2016). Therefore, an understanding of the physiological mechanisms underlying patient-specific pathology will also be an important consideration in the design of personalized treatment approaches, or ‘precision medicine’ (Dainis & Ashley, 2018), for HCM patients.

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### Additional information

#### Competing interests

None.

#### Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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#### Keywords

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