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**Myosin inhibitors in Hypertrophic
cardiomyopathy: efficacy and safety of a new
class of drugs**

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Contents

PART 1	3
Hypertrophic cardiomyopathy: pathophysiology, natural history and therapeutic approaches	3
Introduction	3
Genetic basis of hypertrophic cardiomyopathy	3
Genetic testing in HCM	4
Current challenges in genetic testing and next steps	5
HCM Pathophysiology	5
The sarcomere, hypercontractility and hypertrophy	5
Microvascular dysfunction and fibrosis	6
Metabolic derangements in HCM	6
Ion channel involvement	6
Left ventricular outflow tract obstruction	6
Definition and diagnostic criteria	7
Diagnostic strategies	7
Multimodality imaging for HCM diagnosis	8
Differential diagnosis	8
Natural history from phenotype-positive individuals to end-stage	9
Stage I: Pre-phenotype expression and HCM development	9
Stage 2. Overt HCM phenotype	9
Stage 3: Adverse remodeling	9
Stage IV: Overt dysfunction	10
Peculiarities of pediatric HCM	10
Epidemiology	10
Aetiology	10
Natural history	11
Clinical assessment and management	11
Sudden cardiac death risk prediction and prevention	12
Sports participation	13
Pregnancy	13
Specific therapy including myosin inhibitors and gene therapy	14
Therapeutic approaches for LVOTO	14
New approaches for non-obstructive HCM	16
Gene therapy	17
Conclusion	18
PART 2:	49
Real-world experience using myosin inhibitors for the treatment of hypertrophic cardiomyopathy	49
	1

Introduction	49
Methods	49
Study population	49
Electrocardiography and echocardiography	49
Statistical analysis	50
Results	50
Myosin inhibitors effect on echocardiography and ECG parameters over time	53
Correlation analysis of echocardiography and electrocardiography parameters	65
Adverse events and response to therapy	72
Limitations	73
Discussion	73
Myocardial structural remodeling associated with myosin inhibitor therapy	73
Electrical normalization associated with myosin inhibitors therapy	74
Adverse events	74
Supplementary material	86

PART 1

Hypertrophic cardiomyopathy: pathophysiology, natural history and therapeutic approaches

Abbreviations: APD: action potential duration; AS: aortic stenosis, BBs: beta-blockers; CCBs: calcium channel blockers; CMR: cardiac magnetic resonance; DRX: disordered relaxed state; HCM: hypertrophic cardiomyopathy; HF: heart failure, ICD: implantable cardioverter defibrillator; ECG: electrocardiography; LV: left ventricle, left ventricular; LVOTO: left ventricular outflow tract obstruction; LVSD: LV systolic dysfunction; N-terminal pro-brain natriuretic peptide: NTproBNP; SCD: sudden cardiac death; P/LP: pathogenic/likely pathogenic; SAM: systolic anterior motion, SRX: super relaxed state; VUS: variant of uncertain significance

Introduction

Hypertrophic Cardiomyopathy (HCM) is the most common inherited cardiomyopathy with an estimated prevalence of one in 200-500 adults (0.2-0.5%) worldwide.[1] HCM is characterized by increased left ventricular (LV) wall thickness in the absence of abnormal loading conditions, such as hypertension or aortic stenosis, capable of causing that degree of hypertrophy.[2] Epidemiologically, HCM represents a leading cause of morbidity and mortality in young patients, with 60% of patients diagnosed before 40 years presenting with at least a heart failure-related event by 70 years. Ventricular arrhythmias and sudden cardiac death are also complications that can present in youth; however, the overall risk is smaller than previously thought.

HCM is a paradigm for the development of targeted therapies in heart diseases. The recently achieved deep understanding of the pathological mechanisms underlying the disease has allowed the development of new therapeutic approaches that could potentially change the natural history of the disease including myosin inhibitors and gene therapy. In this section, we will review the pathophysiology, clinical characteristics, traditional and emergent therapies for this condition.

Genetic basis of hypertrophic cardiomyopathy

HCM is widely regarded as a disease of the sarcomere. There are currently 8 'definitive' sarcomere genes with evidence of causation for disease including *MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL2* and *MYL3* (Table 1), with the *MYBPC3* and *MYH7* genes the most common described in HCM populations worldwide.[3] Screening of these 8 genes typically identifies a disease-causing variant in 20-30% of sporadic cases and up to 60% of familial cases. Other more recent genes are classified as having 'moderate' evidence, including *FLNC*, *ALPK3*, *PLN*, *CSRP3*, *TNNC1*, *ACTN2* and *JPH2*, while other genes have been classified at this stage as having 'limited or no evidence' of disease association (Figure 1, Table 1). These classifications are based on guidelines published by the American College of Medical Genetics and Association of Molecular Pathology in 2015 that have provided a framework for sequence variant interpretation in Mendelian disorders.[4] The variant classifications include 'pathogenic' (P), 'likely pathogenic' (LP), 'variant of uncertain significance' (VUS), 'likely benign' (LB), and 'benign' (B) (Figure 2). The list of causative genes in HCM continues to expand as more evidence is collated from clinical and research groups worldwide.

Most causative gene variants are of the missense type, where a single base pair change can result in the change, or replacement, of one amino acid.[5] Other variant types exist that can disrupt the encoded protein, called "frameshift" or "truncation" variants, which can lead to major changes in the protein

sequence or loss of amino acids, resulting in shortened proteins. Variable penetrance can result in differences in the onset and severity of clinical manifestations and are likely to be the result of both genetic factors and other environmental influences.[6]

Genetic testing in HCM

Apart from the genetic testing of established sarcomere genes outlined previously, a growing list of non-sarcomere HCM genes are also being recommended as part of the genetic testing process in HCM.[7] Furthermore, genes associated with HCM phenocopies (Table 2), where other disease-causing variants lead to clinical presentations resembling classical HCM, yet have a different aetiology[8,9], can be included in first tier genetic testing if there is a clinical suspicion of a syndromic disorder.[9,10] These genes include *PRKAG2* for glycogen storage disease, *LAMP2* for Danon Disease, and *GLA* for Fabry Disease (Table 2). It is crucial to identify Danon Disease promptly, as individuals and potentially positive family members identified in subsequent cascade testing require aggressive clinical management and possibly transplant. Individuals with Fabry Disease can be treated with enzyme replacement therapy. Other relevant genes may include those for transthyretin amyloid cardiomyopathy.[8,11]

The overall process of genetic testing in HCM is summarized in Figure 3, and is recommended in current European and US guidelines, with very similar approaches.[10,11] Targeted gene testing can then be perceived as having one of the following outcomes: identification of a variant that is P, LP, VUS, LB or B (previously defined). If a P or LP (i.e. disease-causing) variant is identified, this is a clinically actionable result that provides diagnostic clarification in the proband and information relevant to therapeutic choices, and the option of cascade (predictive) genetic testing in the family. In contrast, if a LB or B (i.e. not disease-causing) variant is identified, then family members should undergo clinical surveillance only (no genetic testing), with at least an ECG and an echocardiogram.

Currently, a VUS is not considered a clinically actionable result but can be investigated further at a clinical or research level to further clarify variant pathogenicity. If a VUS is identified, guidelines suggest regular re-evaluation of probands for variant reclassification. As in Figure 3, reclassification may then result in clinically actionable results.[12] An experienced cardiac genetics team should be involved to convey results accurately and in a clinically meaningful way to individuals who have variants reclassified, particularly when this process can be challenging and result in psychological sequelae.[10] If no variant is identified, second tier testing in the proband and consideration of testing for HCM phenocopies may be important.

Cascade genetic testing can be performed in 'at risk' family members who do not have a clinical phenotype at time of presentation or family members presenting with clinical evidence of HCM (Figure 3).[10] Cascade genetic testing can only be performed after a disease-causing variant is identified in the proband. Family members who don't carry the disease-causing family variant can be released from life-long clinical surveillance, while those who do carry the variant are followed up at appropriate time intervals and regular screening is recommended with ECG and 2D echocardiogram. Importantly, it is strongly recommended that this process of genetic evaluation in HCM patients and their families be performed in the setting of a specialized multidisciplinary HCM centres where adequate support, counselling and guidance can be provided.[10] Predictive genetic testing in children related to an affected individual is recommended in those aged over 10-12 years of age, however this can be performed earlier if there is a family history of early disease onset. Additionally, ongoing clinical screening is not recommended in genotype-negative relatives in most families with genotype-positive HCM.

Current challenges in genetic testing and next steps

Several challenges to genetic testing are important for clinicians treating patients with HCM and their families to be aware of. These include the reclassification of genetic variants and the approach to VUS findings, the ethics of access to testing and how genomic information varies across ethnicities and patient populations, the psychological impact of testing, and family counselling especially related to reproductive decisions. With regards to reproductive challenges, pre-conception and pre-natal genetic counselling and testing should be offered. Specifically, pre-natal risk of inheriting a potential variant and reproductive options including genetic screening, pre-implantation genetic diagnosis and post-natal genetic testing should be discussed.

Genetic testing on HCM is now common practice and has clear benefits in term of diagnosis, family screening, guiding therapies, and in facilitating preimplantation genetic diagnosis.[11] Despite these advances, many patients, sometimes up to 60%, are found not to carry a genetic cause.[13] Some of these genetically unexplained HCM patients may have disease-causing variants in currently unknown genes, and yet to be discovered. Furthermore, emerging evidence suggests there are non-Mendelian causes of HCM, whereby a combination of genetic variants, rather than a single genetic variant, result in the HCM phenotype, including non-familial HCM and polygenic and complex genetic models of disease.[13–15]

HCM Pathophysiology

Historically, the pathophysiology of HCM was described in terms of the direct hemodynamic consequences of left ventricular outflow tract obstruction and myocardial hypertrophy[16], observed initially as a “tumor” of the left ventricle.[17] As genetic sequencing technologies became mainstream in academic research, families with HCM caused by damaging genetic variants in critical sarcomere genes were increasingly reported[18,19], shifting our understanding of the underlying pathophysiology of HCM from a hypertrophic tumor-like overgrowth to a hypertrophic response of the cardiac muscle to hypercontractility caused by inborn errors in the sarcomere (Figure 4).

The sarcomere, hypercontractility and hypertrophy

The sarcomere is the molecular motor of the cardiomyocyte, contracting in response to calcium-induced calcium release triggered during the plateau phase of the myocyte action potential. Calcium released from the sarcoplasmic reticulum leads to retraction of tropomyosin away from the actin scaffold, which allows the binding of the myosin head, conversion of ATP to ADP + Pi, and the release of the Pi generates the “power stroke” in which the myosin head pulls the edges of the sarcomere together along the actin scaffold. The ATPase activity of myosin presents three distinct rates: actin- activated activity (fast), the disordered relaxed (DRX) state (~100- fold slower than actin- activated activity) and the super- relaxed (SRX) state (~10- fold slower than DRX). Myosin heads not bound to actin are in a dynamic equilibrium between the DRX and SRX states. The DRX state is defined by the basal ATPase activity of myosin, in which the myosin heads are available for interaction with actin, on the contrary, the SRX state is defined by an energy- conserving ATPase activity of myosin.[20]

HCM variants cause increased contractility by several mechanisms. Missense, gain of function variants in the converter domain of the myosin heavy chain 7 (MYH7) increase the number of myosin heads in the DRX state, thus ready to engage actin per sarcomere unit.[21] Truncating and non-truncating variants in *MYBPC3*, lead to myosin binding protein C (MyBP- C) haploinsufficiency, destabilization of the SRX population, and increase in myofilament sliding velocity.[22,23] Variants in thin filaments genes increase the sarcomere’s calcium sensitivity, leading to enhanced contractility in response to less calcium-induced calcium release and severe diastolic dysfunction.[24,25] Systolic hypercontractility participates in the development of LVOT obstruction, while the hypercontractile state during diastole is responsible for diastolic dysfunction.

Microvascular dysfunction and fibrosis

Structural and functional abnormalities of the microcirculation are associated with the development of ischemia without coronary artery disease, fibrotic remodeling and ventricular arrhythmias. The presence of small vessels with thickened walls and a reduced density is a hallmark of HCM histology.[26] Furthermore, hypercontractility and impaired relaxation cause excessive microcirculatory compression in systole and impaired decompression in diastole which can be associated with an impaired coronary vasodilator reserve.[27] Abrupt increases in energetic demands may trigger ventricular arrhythmias, while chronic ischemia may cause myocyte death and replacement fibrosis eventually leading to wall thinning and systolic dysfunction. Besides replacement fibrosis, an extracellular matrix expansion (interstitial fibrosis) may be associated with the increased TGF- β signaling due to sarcomeric variants. [26] The presence of surviving myocardial bundles separated by insulating layers of collagen may favor the formation of re-entry circuits and the development of ventricular arrhythmias. Thus, the presence of extensive fibrosis (>15% of LV mass) has been associated with an increased risk of SCD. [2]

Metabolic derangements in HCM

Because HCM is caused by variants resulting in increased force generation, it leads to significant metabolic demands and reliance on mitochondrial function. This leads to impaired calcium cycling, increased intracellular ADP levels, and, consequently, dysfunction of the mitochondrial electron transport chain, all of which likely contribute to myocardial stiffness (diastolic dysfunction), fibrosis burden, and arrhythmia. In turn, this leads to the accumulation of reactive oxygen species (ROS). Recent comprehensive multiomic profiling of HCM energetics from myocardial samples revealed major dysregulation in fatty acid metabolism, reduction of acylcarnitines, and accumulation of free fatty acids. This was coupled with evidence of global energetic decompensation manifested by a decrease in high energy phosphate metabolites (e.g. ADP) and electron microscopic evidence of mitochondrial disarray and degradation without upregulation of expected mitophagic clearance.[28] Recent data from myectomy samples showed reduced oxidative phosphorylation and fatty acid oxidation capacity in both genotype-positive and genotype-negative patients.[29] In genotype-negative patients, mitochondrial dysfunction parameters were strongly associated with LV hypertrophy, probably playing an important role in hypertrophic remodeling similarly to mitochondrial cardiomyopathies.[29]

Ion channel involvement

HCM arrhythmic propensity may be partially explained by the presence of an acquired channelopathy. Action potential duration (APD) is consistently prolonged in HCM myocytes, due to a combination of increased late sodium current (I_{NaL}), increased L-type calcium channel (I_{CaL}) amplitude, slower I_{CaL} inactivation, and decreased potassium currents. APD prolongation increases the likelihood of early after depolarizations (EADs), while calcium overload is associated with enhanced spontaneous Ca-release from the sarcoplasmic reticulum, thus increasing the probability of calcium waves and delayed after depolarizations (DADs). Indeed, sarcomeric variants may promote CaMKII hyperactivation determining post-translational modifications of channel proteins responsible for the above-mentioned abnormalities.[30]

Left ventricular outflow tract obstruction

LVOTO is likely the best recognized feature of HCM pathophysiology, and is caused by hypercontractility in conjunction with several LV anatomical abnormalities (Figure 5). LV outflow tract obstruction may be found either at rest (14%) or after provocative measures (42%), including the Valsalva maneuver, administration of nitrates, standing or exercise echocardiography) [31], defining the obstructive phenotype if the outflow gradient is higher than 50 mmHg. LVOTO occurs during ventricular systole when hypercontractility combined with hypertrophy generates drag forces (though Venturi forces are also present, which create lift in systems with low pressure due to increased flow velocity) that push the mitral leaflets towards the LV

septum hindering the blood outflow. Obstruction is exacerbated by an altered mitral apparatus including elongated mitral valve leaflets, anteriorized and hypertrophic papillary muscles, long and lax chordae that force the coaptation point of the mitral valve anteriorly, possibly leading to functional mitral regurgitation.[32] The obstruction is typically dynamic and can be relieved by increased LV volume load (“stenting” open the outflow tract), but is worsened by increased contractility (e.g. from increased myocardial calcium load after a premature ventricular contraction, as is observed in the Brockenbrough-Braunwald-Morrow effect), and hypovolemia (dehydration, postprandial gastrointestinal hyperemia).

Definition and diagnostic criteria

In adults, HCM is diagnosed by the finding of an otherwise unexplained LV wall thickness ≥ 15 mm in at least one myocardial segment. The diagnosis can also be established with lower degrees of wall thickening (13–14 mm) if other features are present, including family history of HCM, specific genetic findings, and ECG abnormalities.[2] The most commonly accepted threshold for the diagnosis of HCM in children is a maximal left ventricular wall thickness of >2 standard deviations above the predicted body surface area-corrected mean (z-score >2). However, care must be taken when using z-scores to diagnose HCM, as there are inherent limitations, including the existence of many different published normative data, that can result in significant variation in z-scores for the same patient, and a z-score >2 may not correlate with the accepted value of 15mm as the diagnostic cut-off for adults.[33] In addition, there are no normative data for wall thickness other than at the basal interventricular septum or posterior wall, which may not necessarily represent the area of maximal wall thickness in an individual. There are no accepted diagnostic criteria for paediatric relatives of individuals with HCM, but the presence of an abnormal ECG or associated morphological abnormalities (e.g. mitral valve abnormalities, exaggerated systolic thickening) in a child relative with a maximal left ventricular wall thickness <2 z-scores should raise the suspicion of early phenotypic expression.[2]

In addition to LV hypertrophy diagnostic criteria, a phenotype-derived score to predict a positive HCM genetic testing has been proposed including the echocardiographic reverse curve morphological, age at diagnosis < 45 years, a maximum left ventricular wall thickness ≥ 20 mm, a family history of HCM, and a family history of sudden cardiac death as positive predictors and hypertension as a negative predictor, with an 80% probability of positive genetic testing in presence of 5 positive predictors.[34]

Diagnostic strategies

Patients with HCM may be symptomatic but HCM can be also an incidental diagnosis due to an abnormal ECG during occupational, sports or family screening after the discovery of an index case. A complete diagnostic workup foresees accurate family history, electrocardiography, echocardiography, cardiac magnetic resonance (CMR) imaging, and laboratory workup including genetic testing.

Electrocardiography in HCM

ECG changes like inverted T waves in the inferolateral leads might precede the appearance of measurable hypertrophy. The majority of HCM patients have repolarization abnormalities, electrocardiographic elements of LV hypertrophy, atrial enlargement and pseudo-necrosis Q waves.[35] Giant negative T waves in precordial leads are characteristic of apical HCM.[36] However, around 5-10% of HCM cases have a normal ECG, reminding us of the possibility of false negative tracings.[37]

Deep learning algorithms for ECG interpretation may help in screening and detection of HCM in large patient cohorts [38], as well as in reliably identifying patients with high-risk imaging features.[39]

Several ECG features might suggest the presence of an HCM phenocopy.[40] For example, a short PR with right bundle branch block can be found in Fabry disease, while in case of a WPW preexcitation pattern, signs of severe LV hypertrophy and early AV blocks a PRKAG2 syndrome or Danon disease can be suspected. Also,

discrepant ECG voltage with LV hypertrophy, atrial fibrillation, and AV blocks in elderly patients can be suggestive of cardiac amyloidosis.

Multimodality imaging for HCM diagnosis

Multimodality imaging is instrumental for the diagnosis of HCM, as well as retrieving information necessary for therapeutic management (Table 3). Among the various techniques, echocardiography is a readily available, first-line imaging modality in this setting, with class I level of evidence B indication in all patients with HCM, at initial evaluation, at rest and during Valsalva maneuver in the sitting and semi-supine positions, and then on standing if no gradient is provoked, to detect LVOT obstruction.[2] Postprandial exercise can also lead to higher LVOT obstruction, and is particularly useful in patients describing increased symptoms after meals.[41]

Several imaging phenotypes of HCM were described based on the various septal morphologies of HCM, where septal contour was classified as reverse curve (in 30-40% cases), sigmoidal (40-50%), apical, and neutral (in 10% respectively) contour HCM.[42]

Exercise echocardiography is used to elicit an LVOT gradient in symptomatic patients without significant obstruction at rest or after other maneuvers [2], being the most physiologic form of provocative maneuver. Genotype positive-phenotype negative family members should be evaluated by echocardiography regularly. Diagnostic imaging usually begins in pediatric age continuing annually until age 18-21 years. Screening can be extended to every 5 years in adulthood.[43]

Transoesophageal echocardiography should be considered in patients with HCM and LVOTO if the mechanism of obstruction is unclear or when assessing the mitral valve apparatus before a septal reduction procedure, or when severe mitral regurgitation caused by intrinsic valve abnormalities is suspected (class IIa level of evidence C) (Figure 6).[2] Echocardiography is an essential aid to septal reduction therapies: during alcohol septal ablation, contrast echocardiography helps determine the distribution of the target septal branches and its relation to the obstruction (SAM contact) site.

CMR imaging is recommended for the assessment of myocardial structure and function as well as for myocardial tissue characterization [2] (Table 3). CMR may unveil HCM phenocopies like Fabry disease or amyloidosis and highlight the presence of myocardial fibrosis and edema (Figure 7). Moreover, CMR may be considered in genotype positive, phenotype negative family members to detect early disease features before echocardiography.[2]

Differential diagnosis

Hypertensive heart disease can mimic HCM[44], but usually displays only moderate LV hypertrophy of less than 15 mm (LV thickness>15mm could be present in patients with chronic kidney disease or Afro-Americans), usually concentric, or mildly asymmetric (with a septal/posterior wall thickness <1/3), as opposed to the more asymmetric forms found in sarcomeric HCM.[45]

In athletes, HCM is suggested by an LV wall thickness above 16 mm, especially with asymmetric, segmental hypertrophy, reduced LV cavity size, associated LV systolic dysfunction (even subclinical with decreased global longitudinal strain), diastolic dysfunction, left atrial enlargement, cryptae and/or mitral valve anomalies with systolic anterior motion and LV obstruction.[46]

Patients with moderate to severe valvular or sub/supravalvular aortic stenosis (AS) usually develop LV hypertrophy in time. The differential diagnosis is necessary when hypertrophy appears disproportionate to AS severity, also keeping in mind the possibility of the concomitance of AS and HCM, as well as the occurrence of ATTR CA reported in 10% of AS patients.[47,48]

Differential diagnosis should also take into account age at diagnosis. Early HCM diagnosis (especially below 10 years old) is usually the result of phenocopies, with more frequent etiologies like RASopathies, metabolic and mitochondrial disorders, while in later life the cause is more frequently related to sarcomeric disorders, followed by Fabry disease or amyloidosis. [49]

Natural history from phenotype-positive individuals to end-stage

Stage I: Pre-phenotype expression and HCM development

Patients with subclinical HCM may present multiple myocardial crypts and trabecular complexity, histological abnormalities like cardiomyocyte disarray [50], microvascular remodelling and perfusion defects[51], smaller systolic cavity, anterior mitral valve leaflet elongation, hypercontractility and ECG abnormalities.[51–53]. Myocardial disarray and microvascular dysfunction may manifest on CMR-guided electrocardiographic mapping in patients with subclinical HCM with a globally slowed conduction and more spatially heterogeneous repolarization compared to healthy volunteers even in the absence of abnormal ECG. In overt HCM the electrophysiological alterations are more severe with regionally slowed conduction, prolonged and spatially heterogeneous repolarization, the latter associated with scar extent and presence of non-sustained VT. [54]

Except for some neonatal forms of HCM where infants carry bi-allelic mutations[55], LVH typically appears for the first time in adolescence or early adult life.[56] Due to incomplete penetrance and age-related onset, however, genotype-positive individuals can develop LV hypertrophy as late as the 6th or 7th decade.[57] The penetrance in nonproband relatives carrying P/LP variants identified during cascade screening is ~60%.[58] In genotype-positive/ phenotype negative patients, male sex and abnormal ECG are associated with a higher risk of developing HCM.[59]

Stage 2. Overt HCM phenotype

In this stage, the hypertrophic phenotype is fully expressed and hypercontractility in the absence of extensive fibrotic changes is the main characteristic. A common cause of symptoms is represented by LV outflow tract obstruction (LVOTO) and patients may complain about dyspnea on exertion, angina and syncope. Patients with non-obstructive HCM may present dyspnea and angina symptoms related to microvascular ischemia and diastolic dysfunction, and although they present a different pathophysiology, no difference in sudden or heart failure-associated deaths compared with obstructive disease has been shown.[60] On the other hand, genetic testing may help in risk stratification, since patients with P/LP variants, VUS and sarcomere-negative patients present the highest, intermediate and low risk of developing arrhythmias and heart failure-related outcomes, respectively.[61]

Stage 3: Adverse remodeling

Up to 15% of patients may develop a functional impairment of the “classic” HCM phenotype characterized by worsening systolic and/or diastolic function and underlying development of LV fibrosis. This phenotype should prompt close clinical surveillance, since an EF between 50% and 60% is associated with substantial presence of late gadolinium enhancement (>15% of the LV) on cardiac magnetic resonance (CMR), and may predict progression to overt LV systolic dysfunction.[62] A common complication is represented by atrial fibrillation, associated with a high risk of thromboembolic complications and demanding anticoagulation independent of the CHA(2)DS(2)-VASc score.[2] Nonetheless, patients may suffer from ischemic stroke even if in sinus rhythm being severe LA dilation (>48mm) associated with ischemic complications.[63]

Stage IV: Overt dysfunction

Overt LV systolic dysfunction (LVSD, EF<50%) develops in ~8% of patients with HCM. In a large cohort of patients with HCM-LVSD, 75% of them developed clinically relevant events, and 53% met a composite outcome including all-cause death, cardiac transplantation or LVAD implantation after a median follow-up time of 8 years. Predictors of incident HCM-LVSD include greater LV cavity size and wall thickness, LVEF between 50-60%, presence of fibrosis on CMR, and presence of P/LP particularly in thin filament genes.[62]

Patients with thin filament-HCM indeed present a distinct clinical presentation and natural history. Compared to thick-filament HCM patients with thin-filament HCM present milder and atypically distributed LV hypertrophy, but more frequent progression to NYHA class III/IV and a higher risk of developing severe diastolic dysfunction and overt dysfunction.[64]

Peculiarities of pediatric HCM

Epidemiology

The true prevalence of HCM in children is unknown, but is estimated to be in the region of 3:100,000[65], and epidemiological studies from Australia[66], North America[67] and Finland[65] have suggested an annual incidence between 0.24 and 0.47 per 100,000. These figures are likely to be an underestimate of the true incidence and prevalence, largely reflecting data from an era before routine availability of genetic testing and multimodality imaging.

Aetiology

As in adults, most cases of childhood-onset HCM, even in preadolescence, are caused by disease-causing variants in the genes encoding components of the cardiac sarcomere, inherited as autosomal dominant traits, and accounting for 55-75% of childhood-onset HCM cases beyond infancy (<1 year of age).[68] Even in infant-onset HCM, sarcomeric disease accounts for up to 40% of cases.[68,69] While previous international recommendations had suggested that clinical expression of sarcomeric disease was rare below the age of 10-12 years, recent studies have challenged this paradigm, demonstrating that sarcomeric HCM can present in child relatives in at least 10% of families, with the diagnosis resulting in a change in management.[70] As a result, more recent North American and European guidelines now recommend clinical and genetic screening of paediatric first-degree relatives at any age.[2,10]

The aetiology of infant-onset HCM, in contrast, is highly heterogeneous. Over 30% of cases are caused by malformation syndromes (e.g. Noonan syndrome and associated RASopathies), inborn errors of metabolism (e.g. Pompe disease) and mitochondrial cytopathies [69] (Figure 8). Neuromuscular disorders, such as Friedreich ataxia, more commonly manifest in adolescence.[69] A systematic approach to diagnostic workup is required to identify clinical cardiac and extracardiac red flags that may point towards specific aetiological diagnoses (Tables 4 and 5), and help guide management (Figure 9).[2] It is important to exclude potentially reversible causes (e.g. maternal gestational diabetes, twin–twin transfusion syndrome, neonatal corticosteroid use), as well as early-onset sarcomeric disease (including double/compound variants), even in the absence of a family history for HCM, which can often present with severe heart failure symptoms and generally do not survive beyond the first year of life. In infants with HCM, in the presence of biventricular outflow tract obstruction and at least 1 red flag for a neurocardiofaciocutaneous syndrome (e.g. dysmorphism, cutaneous or skeletal anomalies), a diagnosis of Noonan syndrome or a related RASopathy should be suspected. Biventricular hypertrophy with systolic impairment may be a manifestation of an inborn error of metabolism, particularly in the presence of additional extracardiac red flags (e.g. hypotonia, elevated CK levels, parental consanguinity) – Figure 10.

Natural history

HCM in children often represents two opposite ends of a broad spectrum of disease: (1) severe, early-onset disease, often with rapid disease progression and a poor prognosis, similar to the most severe presentations in adults; and (2) mild, early phenotypic expression of adult cardiomyopathy phenotypes, which are increasingly identified as a result of more widespread family screening practices.[2] The natural history of childhood-onset HCM is highly dependent on the age at presentation and underlying aetiology: children with inborn errors of metabolism and malformation syndromes often present with symptoms of heart failure in the first few months of life and have a very high mortality rate in the first 2 years, most commonly due to heart failure-related death, whereas sudden cardiac death (SCD) is the most common mode of death during childhood and adolescence.[66,67,69] Five-year survival rates for childhood-onset HCM according to underlying aetiological groups and age of diagnosis are shown in (Table 6). Importantly, in non-syndromic HCM, children diagnosed in preadolescence (<12 years of age) have similar outcomes and mortality rates than older teenagers.[71]

Early, highly selected cohort studies reported SCD rates of up to 10% per year[72,73], but more recent population-based studies suggest annual SCD rates in the region of 1.2-1.5%.[67,74] Whilst substantially lower than previously thought, this is still approximately 50% higher than reported rates in adult-onset HCM. Importantly, although most cases of SCD occur in children with sarcomere protein gene variants, metabolic and syndromic HCM can also be associated with SCD[74,75], highlighting the importance of systematic risk stratification in all children with HCM. There is substantial lifelong morbidity and mortality associated with childhood-onset HCM, with the natural history curves shifted to the left compared to adult-onset disease, and, in many cases, a more rapid rate of disease progression.[56,71] Furthermore, although SCD is the most common cause of death throughout childhood and adolescence in HCM, longer-term morbidity and mortality is largely related to heart failure, atrial fibrillation and stroke.[56]

Clinical assessment and management

The clinical assessment and management of childhood-onset HCM is largely the same as for adult-onset disease. The systematic clinical diagnostic workflow approach recommended by the 2023 ESC Guidelines for the Management of Cardiomyopathies applies equally to adult and paediatric onset disease, and includes thorough assessment of the cardiac phenotype using multimodality imaging, resting and ambulatory ECG, cardiopulmonary exercise testing, laboratory testing and evaluation of extracardiac manifestations.[2] The aim is to arrive at a phenotype-based aetiological diagnosis to guide management decisions.

Children with HCM may present with symptoms of chest pain, exertional dyspnoea, palpitation, presyncope or syncope, as a result of family screening, or following the incidental detection of a murmur or abnormal ECG. Symptoms in infants can be non-specific, and include poor weight gain and diaphoresis during feeding. As in adults, the assessment of resting and provokable LVOTO with resting and, in symptomatic children or teenagers without a resting or Valsalva-induced gradient, exercise stress echocardiography is a cornerstone of clinical evaluation of childhood-onset HCM. Assessment of diastolic dysfunction, microvascular ischaemia and left atrial function is also important.

Clinical management is primarily aimed at symptom palliation. Beta-blockers and calcium channel blockers, in isolation or combined with disopyramide, are first-line drugs for symptoms related to LVOTO. Cardiac myosin inhibitors (mavacamten and aficamten) are currently undergoing clinical trials in adolescents but are not yet licensed for use in patients under the age of 18 years.[76] In children who remain symptomatic despite maximal medical therapy, surgical myectomy is a class I indication. Alcohol septal ablation is generally not recommended in children and adolescents who are candidates for septal reduction therapy, due to unknown long-term effects. The treatment of non-obstructive symptoms is empirical and often suboptimal, but, as in adults, includes beta-blockers, calcium channel blockers, ranolazine and cautious use of diuretics, with conventional heart failure medication and cardiac transplantation infrequently required,

usually for progression to end-stage disease (characterised by systolic dysfunction) but occasionally for intractable chest pain or ventricular arrhythmia.

Sudden cardiac death risk prediction and prevention

SCD is one of the most feared complications of HCM.[77] Young individuals are at risk, but SCD may occur at any age.[78,79] Although HCM was deemed as a predominant cause of SCD in young athletes[80], more recent reports suggest that HCM is a possible (but not the most common) cause underlying these tragic events.[81–83] Contemporary series of adult patients with HCM show an annual cardiovascular death of 1–2%, with SCD, heart failure, and thrombo-embolism being the main causes of death.[84]

European and American guidelines recommend an ICD following a major arrhythmic event, such as ventricular fibrillation-driven cardiac arrest or sustained ventricular tachycardia, but the recommendations differ for primary prevention.[2,10] The European guidelines rely on the validated HCM Risk-SCD tool, which considers several variables (age, maximal wall thickness, left atrial diameter, LV outflow tract gradient, family history of SCD, NSVT and unexplained syncope) and provides a linear regression risk score predicting SCD event rates at 5 years.[85] The ACC/AHA recommends an ICD in primary prevention in the presence of at least one major clinical marker. While the ACC/AHA guidelines emphasize variables such as apical aneurysms, extensive late gadolinium enhancement at CMR and LV ejection fraction $\leq 50\%$, the ESC guidelines assign less level of evidence to the same variables (IIa, IIb and IIb respectively). These differences have significant repercussions on numbers of ICD implanted per patient with HCM, which is higher in the United States (US) than in Europe. A recent study suggests a lower rate of appropriate ICD therapy in US sites, consistent with a lower-risk population, and no significant difference in the rate of SCD in the US compared with non-US sites.[86] Furthermore, the ACC/AHA guidelines propose the use of the risk calculator tool in support of a shared decision-making process for ICD implantation, the same tool appears more center-staged and first step for SCD prevention according to ESC guidelines.[2,10]

Strategies aimed at preventing SCD in HCM should be balanced with competing risks. These may include age and comorbidities, which impact on the general life expectancy of the individual and therefore on the choice of implanting an ICD.[87]

Most patients with HCM with an ICD are exposed to long-term ICD-related issues, due to their long-life expectancy and active lifestyle. Subcutaneous ICD (S-ICD) offers the opportunity to avoid complications due to transvenous ICDs intracardiac leads. Therefore, when the risk of bradyarrhythmias and/or T wave oversensing is deemed low, S-ICDs are a reliable option for safe and effective termination of potentially life-threatening ventricular tachyarrhythmias.[88]

Implantation of a secondary prevention implantable cardioverter-defibrillator (ICD) in children with HCM who have previously experienced a malignant ventricular arrhythmia-related cardiac arrest is a class I indication in European and North American guidelines.[2,10] Data from the UK have shown that over 60% of patients undergoing secondary prevention ICD implantation received an appropriate ICD therapy within 5 years of follow-up.[89] The identification of individuals who have not yet had a sustained ventricular arrhythmia but are at increased risk of SCD, and would therefore benefit from primary prevention ICD implantation, is more challenging and a major aspect of the clinical management of childhood-onset HCM. The approach to risk stratification in paediatric HCM has changed significantly in the last decade, with the development of paediatric-specific risk prediction models. Previous European (and current American) guidelines recommended the use of individual risk factors, largely extrapolated from adult data, to identify children at high risk of SCD by providing relative (rather than absolute) estimates of risk,[2,10] but the accuracy of this approach has now been shown to be poor.[90] In 2019, the first paediatric-specific risk prediction model was developed, using clinical parameters (maximal left ventricular wall thickness z-score, left atrial diameter z-score, left ventricular outflow tract gradient, presence of nonsustained ventricular tachycardia and unexplained syncope) to provide a personalised estimate of SCD risk at 5 years.[91] This model (HCM Risk-Kids – <https://hcmriskkids.org>) has been externally validated[92] and incorporated into

European guidelines as the recommended method to evaluate risk in children <16 years with HCM (Class I, LOE B), as part of shared decision making discussions with patients and families.[2] A second paediatric model (PRIMaCY) has also been reported[93], with similar discriminatory ability but poorer calibration (resulting in apparent overestimation of risk for some patients) compared to HCM Risk-Kids.[94] The development of paediatric-specific individualised risk prediction models represents a significant advance in the management of childhood-onset HCM; future work will attempt to refine the performance of these models and provide real-world validation.

Sports participation

International guidelines were traditionally restrictive for sports participation in athletes with HCM and competitive exercise was categorically prohibited in the whole spectrum of patients with HCM.[95,96] This stance found its roots in autopsy studies on young athletes who died suddenly, which reported a high prevalence of HCM at post-mortem examination.[80] Recent studies have challenged this notion, emphasizing that SCD in athletes may be due to a plethora of cardiac conditions and HCM does not appear to be the most common.[81–83,97,98] Moreover, over the past decade, there have been several observations indicating that physical activity and exercise of moderate intensity should be promoted, rather than discouraged, in patients with HCM. An Italian study showed no excess of SCD in athletes with HCM who continued to exercise, despite receiving this diagnosis.[99] Data from a multi-national sports safety ICD registry which included 65 individuals with HCM showed no adverse events during a 31-month follow-up period.[100] Although more individuals received appropriate and inappropriate shocks during physical activity than during resting conditions, there was no difference in appropriate ICD discharges between recreational physical activity and competitive sport. This study suggests that even individuals deemed to be at high risk may be able to participate in vigorous exercise with an ICD in situ. The study was underpowered to assess the true impact of vigorous exercise, and while dynamic sports involving explosive sprints such as basketball and soccer were included, few athletes participated in sports with high risk of collision such as American football or ice hockey. The prospective registry, “Lifestyle and Exercise in HCM” (LIVE-HCM) included a large cohort of exercising individuals with HCM and showed no increased risk of SCD or life-threatening arrhythmias among individuals with HCM who exercised vigorously in comparison with those exercising moderately or those who were sedentary.[101] The RESET-HCM trial randomized 136 patients with HCM to 16 weeks of moderate-intensity exercise training or usual activity. Moderate exercise appeared safe in these patients with a small increase in exercise capacity at 16 weeks (+1.35 (95% CI, 0.50 to 2.21) mL/kg/min vs +0.08 (95% CI, -0.62 to 0.79) mL/kg/min).[102]

The heterogeneous morphology and pathophysiology of HCM means that some individuals can participate in vigorous exercise including high-intensity competitive sports. Most recent guidelines have embraced a more liberal attitude and promote a shared decision-making approach in the absence of clinical markers of increased risk.[103,104] This is particularly important as many factors may impact a patient’s perception of risk. As medicine progresses from the outdated paternalistic approach to a physician-patient relationship based on autonomy where patients are decision-makers of their own health, subjectivity of risk becomes relevant.[105]

Pregnancy

Pregnancy, contraception, and heritability of a genetic disease to future offsprings are delicate matters, that often require appropriate counselling and management in expert centers.

Most women with HCM tolerate pregnancy well and in a recent report, in most patients, pregnancies occurred before HCM diagnosis.[106,107] The incidence of maternal death and neonatal death in HCM is low and reported as 0.2% each.[108] However, patients with NYHA functional class of ≥II and signs of HF before pregnancy, are at higher risk of adverse events during pregnancy, and the development of severe HF during pregnancy is associated with death or advanced heart failure over 20 years of follow-

up.[106,109] No significant differences in pregnancy outcome were found between women with obstructive and non-obstructive HCM.[106] Indeed, the pregnancy-related increase in plasma volume and LV dimensions may reduce LVOT obstruction and result in a favorable hemodynamic effect. Pregnant women with HCM should be assessed at each trimester with an ECG and echocardiogram with other tests (for example ECG ambulatory monitoring or CPET) and frequency of follow-up determined by the risk according to the World Health Organization (WHO) classification. Bleeding rates are higher in women who undergo cesarean section, which therefore should be reserved only for obstetric reasons. [10]

Specific therapy including myosin inhibitors and gene therapy

Therapeutic approaches for LVOTO

For decades nonspecific negative inotropes/chronotropes (nonvasodilating beta-blockers, non-dihydropyridine calcium channel blockers and disopyramide) have been the mainstay of LVOTO (Table 7).[110,111] In a double-blind, placebo-controlled randomized crossover trial, 29 patients with symptomatic obstructive HCM were treated with metoprolol or placebo for 2 weeks. Treatment with metoprolol was associated with a reduction in LVOT gradients and a reduced degree of exertional dyspnea and angina, but no change in maximum exercise capacity (peak VO₂).[112] Invasive investigations did not show improvements in pulmonary capillary wedge pressure (PCWP); nevertheless, metoprolol reduced the degree of mitral regurgitation, increased LV end-diastolic volume and stroke volume at rest and during exercise.[113] Non-dihydropyridine CCBs may be an alternative to BBs when ineffective or poorly tolerated.[10] However, in patients with very high resting gradients (eg, >100 mmHg), CCB should be avoided due to the possible vasodilator effect.

Disopyramide is a class Ia antiarrhythmic drug, used preferably as an add-on therapy to BB or CCB for refractory symptoms in obstructive HCM. Disopyramide determines a negative inotropic effect mediated by a multichannel inhibition and improvement of calcium homeostasis, and has proven effective and safe in large multicenter registries.[114]

However, almost 40% of patients with oHCM are still symptomatic on BB/CCBs± disopyramide[115], being candidates for septal myectomy or alcohol septal ablation (ASA), invasive procedures not free from complications, and not widely available. Myectomy is considered the gold standard, preferred in young patients including those with multiple abnormalities requiring correction (markedly elongated mitral leaflets, anomalous papillary muscles, chordal slack, coronary artery bridging). ASA is preferred in fragile patients with high surgical risk, or when surgical expertise is not available. Although very effective in relieving symptoms associated with obstruction, SRTs do not impact the subsequent progression of the cardiomyopathic process.

Allosteric cardiac myosin inhibitors, including Mavacamten and Aficamten, have been recently developed to normalize excessive contractility in HCM and recent clinical trials showed functional capacity, hemodynamic and symptomatic improvements (Tables 8-9, Figure 11).

Myosin inhibitors slow the release kinetics of inorganic phosphate from myosin, responsible for the development of power stroke, thus reducing the actomyosin cross-bridges formation and contractility. [116] For mavacamten, this effect is mediated by the shift of myosin equilibrium towards the SRX at the expense of the DRX, reducing the number of actin-myosin cross-bridges and restoring the physiologic sarcomere function.[117] Aficamten likely stabilizes a small but significant fraction of SRX state heads into a third 'ultrarelated' state in which myosins have a greater than 10-fold lower ATP turnover rate than those in the usual SRX state.[118]

In the phase 3 randomized, double-blind, placebo-controlled trial EXPLORER-HCM, 251 symptomatic patients (NYHA class II-III) with LVOTO and exercise-induced gradients ≥ 50 mmHg were randomized to mavacamten or placebo on top of BB or CCB therapy. At the end of the 30 weeks of treatment twice as

many patients on mavacamten met the primary endpoint compared to placebo (≥ 1.5 mL/kg/min increase in pVO₂ with ≥ 1 NYHA class improvement or ≥ 3.0 mL/kg/min increase in pVO₂ with no worsening of NYHA class, 37% vs. 17%, $p < 0.01$). The mean difference between treatment and placebo in the change in postexercise LVOT gradient was -35.6 mmHg (-43.2--28.1), the mean difference in pVO₂ change was 1.4 (0.6-2.1), and 65% of patients on Mavacamten compared to 31% on placebo had ≥ 1 NYHA class improvement. N-terminal pro-brain natriuretic peptide (NTproBNP) and troponin I showed respectively an 80% and 41% greater reduction compared to placebo. Seven patients on Mavacamten and 2 on placebo had a transient LV ejection fraction (EF) reduction $< 50\%$. [119] Secondary analyses of the EXPLORER-HCM trial are reported in Table 10. Ninety-four percent of patients remained on treatment in the MAVA-LTE study (A Long-Term Safety Extension Study of Mavacamten in Adults Who Have Completed EXPLORER-HCM) showing sustained improvement in LVOT gradient (week 48 compared to baseline resting: -35.6 ± 32.6 mm Hg; Valsalva: -45.3 ± 35.9 mm Hg) and NT-proBNP (week 48 compared to baseline median: -480 ng/L; Q1-Q3: -1,104 to -179 ng/L). Decreases in LVEF were observed at week 4 and plateaued by week 16 (LVEF at week 48 compared to baseline was $-7.0\% \pm 8.3\%$), 11% of patients halved or discontinued BB or CCBs with stable EF and LVOT gradients, while 33% had no change or worsened NYHA class. The most common treatment-emergent adverse events were fatigue, hypertension, and atrial fibrillation, each reported in $\sim 10\%$ of patients. Twelve patients (5%) presented a transient LVEF reduction $< 50\%$ and 8 of them resumed mavacamten treatment, while 10 patients (4%) permanently discontinued the treatment. [120]

In the VALOR-HCM trial (A Study to Evaluate Mavacamten in Adults With Symptomatic Obstructive HCM Who Are Eligible for Septal Reduction Therapy) 112 patients with NYHA class III/IV despite maximal medical therapy (BB/CCB $\pm$ disopyramide) were randomized to Mavacamten or placebo. After 16 weeks, $\sim 20\%$ of patients on mavacamten versus $\sim 80\%$ on placebo (difference 58.9%, 95% CI 44%-74%, $p < 0.01$) were still candidates for or underwent SR therapy. Virtually all patients agreed to participate in the long term study, therapeutic benefits were persistent at 56 weeks, however, 11% of patients presented a transient LVEF $< 50\%$, one patient permanently discontinued the drug due to EF $< 30\%$, atrial fibrillation and heart failure. [121] The reason for the increased number of patients experiencing LVEF reduction in this population is unclear and may be due to the presence of a more advanced disease in this population compared to EXPLORER-HCM trial. Mavacamten proved safe in the Chinese population, characterized by higher rates of poor CYP2C19 metabolizers and lower BMI; no patients developed EF $< 50\%$ and one patient had a temporary dose interruption due to a pre-dose plasma mavacamten concentration > 1000 ng/mL with normal LVEF. [122]

Mavacamten is approved by FDA and EMA for patients with obstructive HCM and NYHA class II/III. The EMA requires genotyping for CYP2C19 since poor metabolizers may have up to 3 times increased mavacamten exposure. Special warnings reported by FDA and EMA are reported in Table 11.

Following the 2023 ESC guidelines, mavacamten administration should be considered in addition to BB or CCB (Classe IIa, A) or in monotherapy (Class IIa, B) when other therapeutic options are not feasible to improve symptoms in patients with obstructive HCM, while septal reduction therapy is reserved for patients unresponsive to medical treatment. [2] The update of the AHA guidelines recommends in patients with persistent symptoms despite BBs or CCBs, adding a myosin inhibitor (adult patients only), or disopyramide, or septal reduction therapy performed at experienced centers. [104]

In real-world data, among 150 consecutive obstructive HCM patients (mean age 65 years), after a mean follow-up of 8 months, 64% had > 1 NYHA class improvement, there was a significant reduction of LVOT gradient (mean drop in resting LVOT gradient from baseline to last follow-up -26 ± 3 mmHg, for Valsalva LVOT gradient -43 ± 4 mmHg), and no patients underwent septal reduction therapy. However, 26% didn't have a symptomatic improvement despite a significant reduction of LVOT gradients, highlighting the non-predictable correlation between gradient and symptoms. Three patients (2%) had a temporary discontinuation of the drug and 2 patients had a permanent interruption for non-cardiac causes. [123]

The myosin inhibitor aficamten was developed to improve pharmacokinetic and pharmacodynamic performance (Table 12). [124]

In the SEQUOIA-HCM trial, 282 patients with obstructive HCM were randomized to placebo or Aficamten, 40% of patients were women, ~80% were white and at least 90% were on background therapy with BB or CCBs, ~13% were on disopyramide. At week 24, the placebo-corrected least-squares mean difference between the groups in the peak oxygen uptake was 1.7 ml per kilogram per minute (95% CI, 1.0 to 2.4; $P < 0.001$). The results were consistent across prespecified subgroups including BBs use. All the secondary endpoints were met with a least-squares mean difference in the KCCQ-CSS of 7 points (95% CI, 5 to 10), an improvement from baseline of ≥ 1 NYHA class in 58.5% of the patients as compared with 24.3% of those in the placebo group, a least-squares mean difference in the left ventricular outflow tract gradient after the Valsalva maneuver of -50 mm Hg (95% CI, -57 to -44), and a left ventricular outflow tract gradient of less than 30 mm Hg after the Valsalva maneuver in 49.3% of the patients as compared with 3.6% of those in the placebo group. The effect on the LVOT gradient after the Valsalva maneuver was rapid, with a least-squares mean difference between the groups of -20 mm Hg (95% CI, -27.3 to -13.3) after 2 weeks and associated with a reduction in cardiac biomarkers. The number of severe adverse events was similar between the treatment group and placebo and none of them were deemed associated with the study drug. Atrial fibrillation was present in both treatment and placebo arm (2.8% and 2.9%, respectively), palpitations and hypertension were more common with Aficamten compared to placebo. The reduction in LVEF was modest (least-squares mean difference, -4.8 percentage points; 95% CI, -6.3 to -3.2), a transient reduction $< 50\%$ in LVEF occurred in 5 patients compared to 1 in the placebo group, but none of them interrupted the treatment due to heart failure (Table 13). [125]

Several questions are still open regarding the use of myosin inhibitors. Around 30% of patients in myosin inhibitors trials continued to have significant LVOT gradient, and predictors of therapeutic response are yet to be defined, long-term efficacy and safety, effects on children and non-white patients, and an actual modification of the natural history of the disease are still to be determined.

New approaches for non-obstructive HCM

In non-obstructive HCM, hypercontractility, small LV cavity and diastolic dysfunction may lead to increased intraventricular pressures and reduced stroke volume. Several therapeutic approaches have been tested and are under study (Table 14).

The dose-ranging phase II MAVERICK-HCM trial showed a 53% reduction in NTproBNP and 34% reduction in Troponin I in patients with non-obstructive HCM treated with mavacamten. One-third of patients with a more severe phenotype (elevated Troponin I or E/e') improved in terms of NYHA class and pVO₂ but, over 16 weeks of treatment, 12.5% of patients presented transient LVEF $< 45\%$. [126] This effect could be partially explained by a less hypercontractile phenotype in the non-obstructive HCM population compared to the obstructive (mean (SD) ejection fraction EXPLORER-HCM 75(10.8) vs 68 (5) in MAVERICK). The phase 3 ODYSSEY-HCM trial (NCT05582395) is evaluating the efficacy and safety of mavacamten in symptomatic non-obstructive HCM patients.

Cohort 4 of REDWOOD HCM trial included 41 patients with non-obstructive HCM treated with aficamten (NYHA class II/III, NT-proBNP > 300 pg/mL, and LVEF $> 60\%$), including 7 patients with mid-cavitary obstruction. By week 10, 55% improved ≥ 1 NYHA class, KCCQ-CS improved ≥ 5 points in 56%, NT-proBNP decreased by 56% and TnI by 22%. Three (8%) patients had LVEF $< 50\%$ without signs of heart failure, and no patient discontinued the treatment due to severe adverse events. [127]

In the Phase 2 trial IMPROVE-HCM (NCT04826185), 67 patients with non-obstructive HCM were randomized to ninerafaxstat or placebo. Ninerafaxstat is a novel cardiac mitotrope that partially inhibits fatty acid oxidation via direct competitive inhibition of 3-ketoacyl-CoA thiolase (3-KAT), shifting cardiac energy metabolism from free fatty acid oxidation to glucose oxidation, reducing the amount of oxygen required per

mole of ATP generated, eventually increasing cardiac efficiency. Nineraxstat seemed safe and well tolerated and the treatment was associated with a better ventilatory efficiency (VE/VCO₂ slope) compared to placebo. A phase 3 study will assess the efficacy of this approach.[128]

SGLT2 inhibitors proved safe and effective in reducing the risk of worsening HF and cardiovascular death across all the spectrum of HF phenotypes, but patients with HCM were excluded from RCTs.[129] In a real-world population the administration of SGLT2 inhibitors in patients with obstructive and non-obstructive HCM seemed safe with a signal towards improved outcomes.[130]

Sotagliflozin is an SGLT2 inhibitor that also provides some gastrointestinal SGLT1 inhibition, and its efficacy in improving quality of life will be evaluated in a phase 3 study including patients with obstructive and non-obstructive HCM.

Gene therapy

Gene therapy consists in the use of genetic material for the treatment of a specific disease.[131]

The genetic material should be introduced into the target cells via an appropriate vector. Currently, due to their cardiotropism and low immunogenicity, the most used vectors in cardiology are adeno-associated viruses. Gene replacement aims to provide a functional copy of a defective gene, while gene editing directly modifies a targeted native DNA sequence. Gene editing tools introduce double- or single-stranded breaks at specific DNA sites to allow for DNA nucleobases conversion, deletion or insertion of DNA base pairs.[131]

Gene replacement techniques are being investigated for the treatment of Danon Disease and MYBPC3-related HCM. Danon disease is caused by loss of function variants in *LAMP2* gene on the X chromosome that lead to LAMP2B deficiency, macroautophagy impairment, and the development of severe, pediatric-onset hypertrophic cardiomyopathy in males.[132] RP-A501 (AAV9 vector enclosing LAMP2B) is under study in male patients (NCT03882437). Preliminary results of the phase 1 study have shown that gene therapy mediated LAMP2 expression was associated with improved cardiac histology, improvement or stabilization of clinical status, reduction of left ventricular hypertrophy and cardiac biomarkers.[133,134]

Preliminary data on *Mybpc3*^{-/-} mice shows that TN-201 (AAV9- delivering *MYBPC3* transgene) improved LV hypertrophy, cardiac function, and lifespan relative to vehicle-treated *Mybpc3*^{-/-} mice. The treatment was associated with a dose-dependent increase in *MYBPC3* RNA, restoration of wild-type levels of cardiac *MYBPC3* protein, and proper sarcomeric incorporation of the protein.[135] In October 2023 the first patient participating in the MyPeak-1 Phase 1b clinical trial evaluating safety and tolerability of a one-time intravenous infusion of TN-201 has been dosed.[136]

BMN 293 (AAV vector encoding a wild-type human *MYBPC3*) transduction of human iPSC *MYBPC3*^{-/-} cardiomyocytes and engineered heart tissues resulted in high levels of human *MYBPC3* mRNA and cMyBP-C protein, correct incorporation of cMyBP-C into the sarcomere, and complete normalization of contractile kinetics. BMN 293 was well tolerated in *MYBPC3*^{-/-} mice and resulted in uniform restoration of cMyBP-C expression throughout the heart and significant correction of structural and functional cardiac abnormalities.[137] Preclinical models for CRISPR-Cas9 gene editing are also under study for HCM.

Chai et al.[138] used a dual AAV9 delivery system enclosing an adenine base editing (ABEmax-VRQR, which mediates the conversion of A_T to G_C without DNA cleavage) and a guide RNA (h403_sgRNA) to target the pathogenic missense variant in the *MYH7* gene p.Arg403Gln. The treatment corrected the target pathogenic adenine with a 98% efficiency in induced pluripotent stem cells derived from patients with HCM with minimal bystander editing of nearby adenines and low DNA off-target editing. The differentiated cardiomyocytes had a normal contractile force and cellular energetics compared with uncorrected cardiomyocytes. In a humanized mouse model of HCM, intrathoracic injection of a single dose of dual AAV9 encoding ABEmax-VRQR and the h403_sgRNA resulted in correction of the pathogenic allele, reduction in pathogenic transcripts, and reduction in cardiac hypertrophy and histopathologic remodeling.

Reichart et al.[139] used a similar dual AAV-based strategy to deliver an ABE and gRNA to correct the same pathogenic variant in a non-humanized mice model, obtaining a correction of the pathogenic variant in ~ 70% of ventricular cardiomyocytes and preventing the development of the pathological phenotype. As an alternative approach, AAV9 delivery of RNA-guided Cas9 nuclease was tested. The technique effectively inactivated the pathogenic allele, but there was also reduced contractile function with higher doses, demonstrating accidental editing of the wild-type allele.

Conclusion

A new era of great interest for genetic diseases has begun, with cardiomyopathies becoming a model for drug development from bench to bedside. HCM takes the lead in this revolution, with new approaches in development targeting the pathophysiology of the disease and enriching the landscape of therapeutic options for our patients.

Table 1. Genetic causes of hypertrophic cardiomyopathy

Gene Symbol	Gene Name	Inheritance Pattern	Frequency (%)
MYBPC3	Myosin Binding Protein C 3	AD, AR	40-45
MYH7	Myosin Heavy Chain 7	AD	15-25
<i>TNNT2</i>	Troponin T, Type 2	AD	1-7
<i>TNNI3</i>	Troponin I, Type 3	AD	1-7
<i>TPM1</i>	Tropomyosin 1	AD	1-2
<i>ACTC1</i>	Actin Alpha Cardiac Muscle 1	AD	1-2
<i>MYL2</i>	Myosin Light Chain 2	AD	1-2
<i>MYL3</i>	Myosin Light Chain 3	AD, AR	1-2
<i>FLNC</i>	Filamin C	AD	<1
<i>PLN</i>	Phospholamban	AD	<1
<i>ALPK3</i>	Alpha Kinase 3	AR	<1
<i>CSRP3</i>	Cysteine & Glycine Rich Protein 3	AD	<1
<i>JPH2</i>	Junctophilin 2	AD	<1
<i>TNNC1</i>	Troponin C, Type 1	AD	<1
<i>MYOZ2</i>	Myozenin 2	AD	<1
<i>TCAP</i>	Titin-Cap	AD	<1
<i>FHOD3</i>	Formin Homology 2 Domain Containing 3	AD	<1

<i>Actin polymerization</i>			
<i>ACTN2</i>	Actinin Alpha 2	AD	<1

Table 2. Common “HCM phenocopy” disease genes

Gene Symbol	Gene Name	Disease	Inheritance Pattern	Treatments
<i>PRKAG2</i>	Protein Kinase AMP-activated non-catalytic subunit gamma 2	Wolff-Parkinson-White (WPW) Syndrome	Autosomal Dominant	Anti-arrhythmic drugs, WPW ablation
<i>LAMP2</i>	Lysosomal-associated membrane protein 2	Danon disease	X-linked	ICD implantation, early assessment for heart transplantation
<i>GLA</i>	Galactosidase alpha	Fabry disease	X-linked	Chaperone therapy, Enzyme-replacement therapies, antiplatelet/anticoagulant therapies, analgesics for symptomatic neuropathy
<i>GAA</i>	Alpha glucosidase	Pompe disease	Autosomal recessive	Enzyme-replacement therapies; non-invasive ventilation
<i>TTR</i>	Transthyretin	Transthyretin amyloidosis	Autosomal Dominant	Transthyretin stabilizers, Antisense Oligonucleotides , Small Interfering RNA , gene therapy , antibodies Heart failure support therapy, Heart transplant

Table 3. Multimodality imaging in HCM

Feature	Imaging technique	Observations
LV wall thickness	Echo/CMR	Precise values for LV wall thickness are used in the HCM definition as well as the ESC SCD risk estimation calculator Distribution of hypertrophied segments defines HCM type and aids in planning septal reduction techniques. Extreme concentric hypertrophy (more than 30-35 mm) can be suggestive of Danon or Pompe diseases.
LV myocardium tissue characterization	CMR	Native T1 mapping can characterize the myocardium: high T1 can be the result of high ECV due to diffuse fibrosis or amyloid deposition; low T1 values can appear very early in Fabry cardiomyopathy. T2 mapping can find edema as a sign of active injury/inflammation – which can correlate with low grade troponin release and worse prognosis [doi: 10.1093/ehjci/jead065]. LGE presence, localization and extent are described and can aid the arrhythmic risk stratification [doi.org/10.1093/eurheartj/ehad581]. LGE distribution in sarcomeric HCM usually follows the hypertrophied segments. Concentric diffuse subendocardial LGE is suggestive for CA. Inferolateral basal LGE distribution can be seen more often in FD.
RV wall thickness	Echo/CMR	RV hypertrophy is often significant in CA, FD
Mitral valve and apparatus	Echo/CMR	Mitral valve elongation (mainly the anterior leaflet) and systolic anterior motion participate to the LVOT obstruction mechanisms. Dyskinesia of the aorto-mitral apparatus, including SAM of the elongated AMVL can occur in very early disease stages [doi: 10.1093/ehjci/jead186] Anterior displacement of the papillary muscles as well as other possible anomalies of the mitral apparatus can be seen [doi: 10.1093/ehjci/je095].
LVOT obstruction	Echo	Evaluated at rest or during provocative maneuvers (e.g. Valsalva, standing, exercise, administration of sublingual nitrates) – the value of LVOT gradient is a determinant of symptoms and a part of the sudden cardiac death risk calculator. A maximum provoked peak LVOTO ≥ 50 mmHg in symptomatic patients represent an indication for LVOTO reduction therapies.
LV ejection fraction	Echo/CMR	Usually, LVEF in HCM is normal or supra-normal. However, during evolution towards heart failure phenotype (or “burnout phase”) LVEF can progressively decrease. LV systolic dysfunction can occur in mitochondrial cardiomyopathy.

LV longitudinal strain	Echo	Longitudinal myocardial velocities and strain are usually abnormal especially at the level of the hypertrophied segments – with early changes during hypertrophy development. Apical sparing pattern of GLS is suggestive for cardiac amyloidosis.
LV diastolic function	Echo	Diastolic dysfunction can be of grade I, II or III. Some patients with nonobstructive HCM develop a restrictive physiology phenotype or triphasic pattern with severe biatrial enlargement and heart failure with preserved EF.
LA atrium	Echo/CMR	LA anteroposterior diameter is a marker of risk for both atrial and ventricular arrhythmias, being a part of the ESC SCD risk estimation calculator
Interatrial septum	Echo/CMR	Hypertrophy of the interatrial septum and atrial walls is usually seen in CA
Pericardial fluid	Echo/CMR	Pericardial effusion associated to LV hypertrophy often appears in CA

Table 4: Electrocardiographic red flags in childhood-onset HCM

	<i>PRKAG2</i>	<i>Mitochondrial</i>	<i>Danon</i>	<i>GSD</i>	<i>Noonan</i>
Short PR/pre-excitation	✓	✓	✓	✓	
AV conduction disease	✓	✓	✓		
Superior QRS axis					✓
Biventricular hypertrophy				✓	✓
Extreme LVH			✓	✓	

Table 5: Echocardiographic red flags in childhood-onset HCM

	<i>PRKAG2</i>	<i>Mitochondrial</i>	<i>Danon</i>	<i>GSD</i>	<i>Noonan</i>	<i>Friedreich ataxia</i>
Concomitant RVH			✓	✓	✓	
Concentric LVH		✓		✓		✓
Extreme concentric LVH			✓	✓		
Global hypokinesia (with/without LV dilatation)	✓	✓	✓	✓		(✓)

Table 6: 5-year survival of childhood-onset HCM by age and aetiology [PMID 17261650, PMID 30535072]

	5yr survival
MFS	74-91%
IEM	42-66%
NMD	97-98%
Non-syndromic	92.7%
Age at diagnosis	<1y
	81-82%
	>1y
	94%

MFS: malformation syndromes, IEM: inborn errors of metabolism, NMD: neuromuscular disorders.

Drug	Indication	Startin g dose	Maximu m dose	Notes	Side effects
Beta blockers					
Nadolol	Symptomatic treatment of LVOTO Rate control in AF VT	20mg qd	80mg bid	Hydrophilic, long acting (24 hours half life) non selective beta blocker Beta blocker of choice in LQTS/CPVT Blocks peak sodium current reducing the risk for delayed afterdepolarizations	Chronotropic incompetence AV conduction prolongation Asthma
Metoprolol	Symptomatic treatment of LVOTO Rate control in AF VT	50mg qd	100 mg bid	Reduces LVOT obstruction at rest and during exercise, provides symptom relief, and improves quality of life in patients with obstructive HCM Half life: 3-7 hours	Chronotropic incompetence AV conduction slowing Asthma
Bisoprolol	Heart failure with reduced ejection fraction	1.25mg qd	15mg qd	Good tolerability	Chronotropic incompetence AV conduction slowing Asthma
Calcium channel blockers					
Verapamil	Symptomatic treatment of LVOTO Rate control in AF	40 mg bid	240 mg bid	Potentially harmful due to vasodilating effect in severe	negative inotrope AV conduction slowing

				LVOTO (>100) and congestive heart failure	
Diltiazem	Symptomatic treatment of LVOTO Rate control in AF	60 mg bid	180 mg bid	Less prominent vasodilator and myocardial depressant compared to verapamil	negative inotrope AV conduction slowing
Antiarrhythmics					
Disopyramide	Symptomatic treatment of LVOTO in association with BB or CCBs AF rhythm control, control of SVT/NSVT/ventricular ectopic beats	125 mg bid	250 mg tid	Negative inotrope Multiple ion channel inhibition reduces arrhythmic propensity Safe and well tolerated in large studies	Qt interval prolongation xerostomy, accommodation disturbances, lower urinary tract symptoms/prostatism
Amiodarone	AF rhythm control, control of SVT/NSVT/ventricular ectopic beats	200 mg qd	200 mg qd	Incomplete efficacy for SCD prevention despite reduction of NSVT	QTc prolongation Photosensitivity Thyroid dysfunction Pulmonary interstitial disease
Sotalol	prevent recurrence of sustained VT or ventricular fibrillation rhythm control in AF	40mg bid	80 mg tid	modest success on cardioversion of AF	QTc prolongation fatigue Bradycardia Proarrhythmia (APD prolongation and delayed afterdepolarization induction) Asthma
Flecainide	rhythm control in AF prevention of ventricular arrhythmias, ventricular ectopic beats	50 bid	200bid	Contraindicated in Heart failure and ischemic heart disease Not generally recommended in the absence of an ICD	Prolonged QT AV conduction slowing organization of atrial fibrillation into atrial flutter and facilitation of 1:1 conduction (association with beta blocker recommended)
Myosin inhibitors					
Mavacamten	LVOTO	2.5mg qd	15mg qd	Long half life (6-9 days)	Negative inotrope Pharmacodynamic interactions Contraindicated in pregnancy

Table 7. Commonly used drugs for hypertrophic cardiomyopathy. AF, atrial fibrillation; AV, atrioventricular; HF, heart failure; HR, heart rate; ICD, implantable cardioverter defibrillator; LVOTO, left

ventricular outflow tract obstruction; PAF, paroxysmal atrial fibrillation; NSVT, non-sustained ventricular tachycardia; qd, once a day; SVT, sustained ventricular tachycardia. (Modified from Ammirati et al. doi.10.1002/ejhf.541)

STUDY NAME AND STATUS	STUDY DESIGN	POPULATION	PRIMARY ENDPOINT	SECONDARY ENDPOINTS
Obstructive HCM				
PIONEER-HCM Status: completed	multi-center phase II open-label non-randomized 2 sequential cohorts (A and B) 12-weeks treatment phase followed by a 4-weeks post-treatment phase	21 oHCM patients Cohort A (10 to 20mg without background medications): 56y Cohort B (2 to 5 mg with BB allowed): 58y 57% men 57% NYHA II 43% NYHA III	Cohort A: mean post-exercise LVOT gradient decreased from 103±50 mmHg to 19±13 mmHg at 12 weeks (p = 0.008) Cohort B: mean LVOT gradient decreased from 86±43 mmHg to 64±26 mmHg (p= 0.020)	Cohort A: Resting LVEF reduction -15% (CI, -23% to -6%). Peak VO ₂ increased by a mean of 3.5 mL/kg/min (CI, 1.2 to 5.9 mL/kg/min). Cohort B: mean change in resting LVEF -6% (CI, -10% to -1%). Peak VO ₂ increased by a mean of 1.7 mL/kg/min (CI, 0.03 to 3.3 mL/kg/min). Dyspnea scores improved in both cohorts.
EXPLORER-HCM Status: completed	multi-center phase III randomised double-blind placebo-controlled Treatment duration: 30w	251 oHCM, NYHA class II/III Mean age 58±11 years, 59% men Dose: 2.5 to 15 mg, BB and CCB allowed	≥1.5 ml/kg/min increase in pVO ₂ with ≥1 NYHA class improvement OR ≥3.0 ml/kg/min increase in pVO ₂ with no worsening of NYHA class in 37% of pt in MG vs 17% in PG (p <0.0005)	Post-exercise LVOT gradient -47 mmHg in MG vs -10 mmHg in PG (p<0.0001) pVO ₂ +1.4 ml/kg/min in MG vs -0.05 ml/kg/min in PG (p<0.0006) ≥ NYHA class improvement 65% pt in MG vs 31% pt in PG (p<0.0001)

				KCCQ-CSS (n): + 14 in MG vs +4 in PG (p<0.0001) HCMSQ-SoB (n) (negative better): -2.8 in MG vs -0.9 in PG (p<0.0001) 9 patients with LVEF <50% (7 on mavacamten)
VALOR-HCM Status: completed Duration 16 week	Multi-center phase III randomized double-blind placebo-controlled	112 oHCM NYHA III/IV or NYHA II with exertional syncope or near syncope Referred for SRT in the past 12 months mean age 60 ± 12 years 51% men 2.5 to 10 mg BB, CCB, DPY allowed	18% in MG vs 77% in PG (difference 58.9%, 95% CI 44%-74%, p<0.01) were still candidates for or underwent SR therapy	Change from baseline in post-exercise LVOT gradient, mm Hg Treatment difference -37.2 mmHg(-48.1 to -26.2) p<0.001 At least 1 class of NYHA improvement Treatment difference 41.1 (24.5 to 57.7) p<0.001 Change from baseline in KCCQ-23 CSS, Treatment difference 9.4 (4.9 to 14.0), p<0.01 Change from baseline in NT-proBNP, ng/L Treatment difference Geometric mean ratio 0.33 (0.26 to 0.42), p<0.0 Change from baseline in cardiac troponin I, ng/L Treatment difference Geometric mean ratio 0.53 (0.41 to 0.70 p<0.001 2 patients on M temporary LVEF <50%
VALOR-HCM Week 56 results	Multi-center phase III randomized	Obstructive HCM (New York Heart Association class III/IV) referred for SRT	5/56 patients (8.9%) in MG and 10/52 (19.2%) in placebo crossover group met the	reduction in resting (MG mean difference, -34.0 mm Hg; 95% CI, -43.5 to -24.5 mm

	double-blind placebo-controlled placebo crossover at 16 weeks	108 (mean [SD] age, 60.3 [12.5] years; 54 male [50.0%])	composite end point (patients undergoing SRT, remaining guideline eligible or unevaluable)	Hg and PG –33.2 mm Hg; 95% CI, –41.9 to –24.5 mm Hg) LVOT gradient. Improvement in NYHA class ≥ 1 in (93%) in MG and 73% in PG. 12/108 patients (11.1%; 95% CI, 5.87%–18.60%), had an LVEF less than 50% (2 with LVEF $\leq 30\%$, one of whom died), and 9/12 patients (75%) continued treatment
MAVA-LTE (A Long-Term Safety Extension Study of Mavacamten in Adults Who Have Completed EXPLORER-HCM data cutoff (August 31, 2021) Status: ongoing	Multi-center	mavacamten 5 mg once daily adjustments made based on site-read echocardiograms 231/ 244 patients (94.7%) enrolled in MAVA-LTE (mean age: 60 years; 39% female) 217 (93.9%) remained on treatment (median time in study: 62.3 weeks)	At 48 weeks improvements in gradients (mean change $\pm$ SD from baseline: resting: -35.6 ± 32.6 mm Hg; Valsalva: -45.3 ± 35.9 mm Hg), N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (median: -480 ng/L; Q1-Q3: $-1,104$ to -179 ng/L), and NYHA functional class (67.5% improved by ≥ 1 class).	8 pt had cardiac failure, 21 patients had atrial fibrillation, 12 (5.2%) had temporary LVEF<50%
EXPLORER CN 12 hospitals in China	multi-center phase III randomised 2:1 M or P double-blind placebo-controlled Treatment duration: 30w	oHCM and LVOT gradient >50 mmHg and NYHA class II or III symptoms Starting dose 2.5 mg 81 patients (mean [SD] age, 51.9 [11.9] years; 58 men [71.6%])	Improvement in Valsalva LVOT peak gradient least-squares mean [LSM] difference, -70.3 mmHg; 95%CI, -89.6 to -50.9 mmHg; 1-sided $P < .001$)	resting LVOT peak gradient (LSM difference, -55.0 mmHg; 95%CI, -69.1 to -40.9 mmHg KCCQ-CS: LSM difference, 10.2; 95%CI, 4.4-16.1 NTproBNP: proportion of geometric mean ratio, 0.18; 95%CI, 0.13-0.24

				Hs TnI: proportion of geometric mean ratio, 0.34; 95%CI, 0.27-0.42 no EF<50%
Non-obstructive HCM				
MAVERICK-HCM Status: completed	multi-center phase II randomized double-blind placebo-controlled	59 non-oHCM, mean age 54±14 y, 58% women	Serious adverse events occurred in 10% pt in MG and in 21% in PG 5 pt in MG had reversible reduction in LVEF ≤45%.	NTproBNP decreased by 53% in the MG vs 1% in the PG, (p=0.0005). Troponin I decreased by 34% in the MG vs a 4% increase in the PG, (p = 0.009). One-third of patients with a more severe phenotype (elevated Troponin I or E/e') improved in terms of NYHA class and pVO2 bu
A Study of Mavacamten in Non-Obstructive Hypertrophic Cardiomyopathy (ODYSSEY-HCM)	Randomized, Double-blind, Placebo-controlled Clinical Study	Non obstructive HCM NYHA class II/III	Change in KCCQ-CSS Change in Pvo2	Change from baseline in ventilatory efficiency (VE/VCO2 slope) Proportion of participant increasing ≥1 NYHA class Change from baseline in NTproBNP Change from baseline in HCMSQ-SoB Time to first major adverse CV events

Table 8. Performed and ongoing trials evaluating Mavacamten.

STUDY NAME AND STATUS	STUDY DESIGN	POPULATION	MAIN FINDINGS	SECONDARY ENDPOINTS
REDWOOD-HCM Cohorts 1/2 Status: ongoing	Multi-center phase II	oHCM NYHA III/IV	resting LVOT gradients mean difference: -40 ± 27 mm Hg in	EF mean difference -6 ± 7.5 in cohort 1 and -12 ± 6 in cohort 2<0.001)

	randomized double-blind placebo-controlled 10 weeks	LVOT gradient ≥ 50 mmHg N=28 Cohort 1 (n=14), 5mg to 10mg Cohort 2 (N=14), 10 mg to 14mg Placebo n=13	cohort 1 and - 43 ± 37 mm Hg in cohort 2 (pvs placebo <0.001) Valsalva LVOT gradients mean difference: -36 ± 27 mm Hg in cohort 1 and - 53 ± 44 mm Hg in cohort 2 (<0.001)	NYHA ≥ 1 43% in cohort 1, 64% in cohort 2, 31 % on placebo (non significant) 62% proportional reduction in NT-proBNP levels at Week 10 compared with placebo ($P < 0.001$) 18% relative reduction in hsTnl $P = 0.29$ compared with placebo no serious adverse events and no treatment interruptions or discontinuations
REDWOOD-HCM Cohort 3	Multi-center phase II randomized double-blind placebo-controlled 10 weeks	oHCM NYHA III/IV LVOT gradient ≥ 50 mmHg On BB and/or CCBs + Disopyramide N=13 Dose 5 to 15mg	week 10 resting LVOT-G $\downarrow 27$ (22) mmHg ($P < 0.0001$), and Valsalva LVOT-G $\downarrow 28$ (32) mmHg ($P = 0.0002$)	Mean (SD) LVEF $\downarrow 74\%$ (7.5%) to 69% (7.2%) ($P = 0.018$), and it returned to baseline after 2 weeks washout 85% ≥ 1 NYHA class improvement mean (SD) changes relative to baseline in NT-proBNP levels -42.5% (30.7; $P < 0.01$) and hsTnl of -9.1% (32.8; $P = NS$) no dosage interruptions, treatment discontinuations or serious adverse events. No patients LVEF $< 50\%$
REDWOOD-HCM Cohort 4 N= 41	Multi-center phase II randomized double-blind placebo-controlled	Non-obstructive HCM NYHA II/III LVEF $\geq 60\%$ Resting or provoked	By week 6 no drug discontinuations due to adverse events 3 (7.3%) had temporary LVEF $< 50\%$ at Week 10 that	Mean improvement in KCCQ-CSS of 10.6 points ($p < 0.0001$ for change from baseline to 10 weeks) 56% ≥ 1 NYHA class improvement

	10 weeks Dose 5 to 15mg	LVOT<30 mmHg NTproBNP≥300 pg/mL	regressed after the 2-week washout period	Mean (SE) relative reduction in hsTnI of 21% by Week 10 with an absolute reduction of -24.8 ng/L (11.6) Mean (SE) relative reduction in NT-proBNP of 55% by Week 10 with an absolute reduction of -870 pg/mL (155.3)
FOREST HCM N=45	Open-Label, Research Evaluation of Sustained Treatment with Aficamten in HCM	oHCM who completed a parent study of aficamten On BB and/or CCBs + Disopyramide Dose 5 to 20mg	At week 48 ↓ in peak resting and Valsalva LVOT-G from baseline to Week 48 [Δ Resting mean (SD): -32 ± 28 mmHg; Δ Valsalva mean (SD): -47 ± 28 mmHg]	LVEF from baseline to Week 48 (Δ -5 ± 3%) 88% of patients experienced ≥1 NYHA 42% were eligible for SRT and none was eligible at 48 weeks NT-proBNP ↓ from baseline to Week 48 (from a geometric mean [CV%] of 651.0 [160.4] to 111.1 [93.4] pg/mL), (-70% from baseline) no aficamten-related reduction in LVEF <50%
CMR substudy FOREST HCM N=16	CMR at baseline and week 48	oHCM or nonobstructive HCM who completed a parent study of aficamten On BB and/or CCBs + Disopyramide Dose 5 to 20mg	↓ left ventricular mass index (-11.4 g/m ² ±19.4, p=0.03), ↓ maximum left ventricular septal wall thickness (-1.3 mm ±1.8, p=0.02),	↓ left atrial volume (-16.3 ml/m ² ±26.4, p=0.05), and ↓ mitral regurgitant volume (-12.9 ml ±15.1, p=0.01) no increase in absolute mass of late gadolinium enhancement (-0.6 g ± 5.0, p=0.64).
SEQUOIA HCM N=282	Multi-Center Randomized Double-blind	Obstructive HCM resting LVOT-G ≥30 mm Hg	↑ pVO ₂ least square mean difference (95% CI) of 1.74 (1.04 - 2.44)	Statistically meaningful Improvement in all secondary endpoints

	Placebo-controlled	and post-Valsalva LVOT-G ≥ 50 mm Hg RER ≥ 1.05 and $pVO_2 \leq 90\%$ predicted on the screening CPET BMI < 35 kg/m²	mL/kg/min ($p < 0.001$) vs 0 (95% CI, -0.5 to 0.5) in placebo group	a least-squares mean difference in the KCCQ-CSS of 7 points (95% CI, 5 to 10), an improvement from baseline of ≥ 1 NYHA class in 58.5% of the patients as compared with 24.3% of those in the placebo group, a least-squares mean difference in LVOT mavergradient after the Valsalva maneuver of -50 mm Hg (95% CI, -57 to -44), and a left ventricular outflow tract gradient of less than 30 mm Hg after the Valsalva maneuver in 49.3% of the patients as compared with 3.6% of those in the placebo group. Treatment emergent serious adverse events in 8 (5.6%) and 13 (9.3%) patients on <i>aficamten</i> and placebo, respectively. LVEF was observed to be $< 50\%$ in 5 patients (3.5%) on <i>aficamten</i> compared to 1 patient (0.7%) on placebo.
Phase 3 Trial to Evaluate the Efficacy and Safety of Aficamten Compared to Metoprolol Succinate in Adults With Symptomatic oHCM (MAPLE-HCM)	Multi-Center Randomized Double-blind Placebo-controlled Aficamten up to 20 mg plus placebo for metoprolol Metoprolol succinate up to	oHCM NYHA II/III Resting LVOT-G > 30 mm Hg and/or post-Valsalva LVOT-G ≥ 50 mmHg at screening AND LVEF $\geq 60\%$	Change in peak oxygen uptake (pVO_2) by cardiopulmonary exercise testing	Proportion of patients with ≥ 1 class improvement in NYHA class Change in KCCQ Change in LV mass Change in LAVi Change in NTproBNP Change in psot Valsalva LVOT-G

	200 mg plus placebo for aficamten			
Phase 3 Trial to Evaluate the Efficacy and Safety of Aficamten Compared to Placebo in Adults With Symptomatic nHCM (ACACIA-HCM)	Phase 3 Double blind Placebo controlled trial	Nhcm NYHA II/III KCCQ-CSS score of ≥ 30 and ≤ 85 NT-pro BNP ≥ 300 pg/mL or NT-proBNP ≥ 900 pg/mL if in atrial fibrillation	Change in KCCQ-CS	Change in composite of two Z-scores of CPET parameters (pVO2 and VE/VCO2 slope) Proportion of participants with ≥ 1 class improvement in NYHA Functional Class
Clinical Evaluation of Dosing with <i>Aficamten</i> to Reduce Obstruction in a Pediatric Population in HCM (CEDAR HCM)	multi-center, randomized, double-blind, placebo-controlled and open-label extension clinical trial to evaluate the efficacy, pharmacokinetics (PK) and safety of <i>aficamten</i> in a pediatric population with symptomatic obstructive HCM	age 12-17, EF $\geq 60\%$, Valsalva LVOTG $\geq 50\%$ & NYHA class ≥ 2	change in Valsalva left ventricular outflow tract gradient (LVOT-G) from baseline to Week 12	change from baseline to Week 12 in resting LVOT-G, New York Heart Association (NYHA) Functional Class, pharmacokinetics and cardiac biomarkers including NT-proBNP and hs-cTnI.

Table 9 Performed and ongoing trials evaluating Aficamten.

Substudy	Main results
Effect of Mavacamten on Echocardiographic Features in Symptomatic Patients With Obstructive Hypertrophic Cardiomyopathy	M was associated with favorable LV remodeling, improvement in diastolic function and improvement in MR Significant Least squares Mean Difference M vs P at W 30: LVEF, % -4.0 (-5.5 to -2.5) LV end-systolic dimension, mm 1.0 (0.2 to 1.9)

	Interventricular septal thickness, mm -1.2 (-1.6 to -0.9) Inferolateral wall thickness, mm -0.8 (-1.2 to -0.4) LVMI, g/m² -15.5 (-19.0 to -11.9) LAVI, mL/m² -7.5 (-9.4 to -5.5) Lateral e', cm/s 1.3 (0.9 to 1.8) Septal e', cm/s 0.7 (0.4 to 1.0) Complete resolution of SAM 80.9% on M compared with 34.0% on P ($p < 0.001$) Complete resolution of MR 9% on M vs 0 on P ($p < 0.001$)
Mavacamten Favorably Impacts Cardiac Structure in Obstructive Hypertrophic Cardiomyopathy: EXPLORER-HCM Cardiac Magnetic Resonance Substudy Analysis N=35	M was associated with favorable LV remodeling Mean difference from baseline to W30: LV mass index -15.8 g/m² [95% CI, -22.6 to -9.0] LV mass -30.0 g [95% CI, -43.3 to -16.7] Max LV wall thickness -2.4 mm [95% CI, -3.9 to -0.9] maximum left atrial volume index -10.3 mL/m² [95% CI, -16.0 to -4.6] no notable within- or between-group changes in late gadolinium enhancement
Effect of beta-blocker therapy on the response to mavacamten in patients with symptomatic obstructive hypertrophic cardiomyopathy Beta blocker n=142 No Beta blocker n=45	M had less impact in patients on BB on heart rate-dependent measures (Pvo2, peak METs, peak HR, peak exercise time), but similar benefit on heart rate-independent measures in patients on BB (VE/VCO2 slope, LVOT gradients, NYHA class, KCCQ-CSS, NTproBNP) Mean difference from baseline to W30: Improvements in peak VO₂ with M vs P lower with beta-blockers (mean difference [95% confidence interval (CI)]: 1.04 [0.12, 1.95] mL/kg/min) than without beta-blockers (mean difference [95% CI]: 2.69 [1.29, 4.09] mL/kg/min) improvements in non-heart rate-dependent parameters (V_E/VCO₂ slope) appeared unaffected by beta-blockers
Effects of Mavacamten on Measures of Cardiopulmonary Exercise Testing Beyond Peak Oxygen Consumption N=251	M associated with improvements in peak and submaximal exercise parameters Least squares mean difference: peak V_E/VCO₂ ratio -2.2; 95% CI, -3.05 to -1.26; $P < .001$ peak METs 0.4; 95% CI, 0.17-0.60; $P < .001$ peak circulatory power 372.9 mL/kg/min $\times$ mm Hg; 95% CI, 153.12-592.61; $P = .001$ peak PETCO₂ 2.0 mm Hg; 95% CI, 1.12-2.79; $P < .001$ peak exercise time 0.7 minutes; 95% CI, 0.13-1.24; $P = .02$

	V_E/VCO_2 slope -2.6; 95% CI, -3.58 to -1.52; $P < .001$ ventilatory power 0.6 mm Hg; 95% CI, 0.29-0.90; $P < .001$
Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): health status analysis of a randomised, double-blind, placebo-controlled, phase 3 trial N=251	Mavacamten markedly improved the health status of patients with symptomatic obstructive hypertrophic cardiomyopathy compared with placebo Mean change at 30 weeks: KCCQ-OS 14.9 [SD 15.8] vs 5.4 [13.7]; difference $+9.1$ [95% CI 5.5-12.8]; $p < 0.0001$ Proportion of patients with a very large change (KCCQ-OS ≥ 20 points) 36% on M vs 15% on P, with an estimated absolute difference of 21% (95% CI 8.8-33.4)
Effect of Mavacamten in Women Compared With Men With Obstructive Hypertrophic Cardiomyopathy: Insights From EXPLORER-HCM	Women (n=102): older (62 y vs 56 years;), lower peak oxygen consumption (16.7 mL·kg⁻¹·min⁻¹ versus 21.3 mL·kg⁻¹·min⁻¹), more frequently NYHA III (42% versus 17), worse health status (KCCQ-CSS 64 versus 75), higher NT-proBNP (1704 ng/L Vs 990 ng/L; $P=0.004$) than men (n=149) Similar improvements in primary composite endpoint, peak LVOT gradient, p VO₂, and % ≥ 1 NYHA class improvement. Greater improvement in KCCQ-CSS (14.8 versus 6.1) and NTproBNP (-1322 ng/L versus -649 ng/L)
Mavacamten for Obstructive Hypertrophic Cardiomyopathy With or Without Hypertension: Post-Hoc Analysis of the EXPLORER-HCM Trial 119/251 had hypertension	Patients with hypertension: older (63.4 vs 54.0 y), lower pVO₂ (19 ± 3 vs 20 ± 4 mL/kg/min) Mavacamten-treated patients had improvement in all primary, secondary, and exploratory endpoints regardless of HTN status or mean SBP
Response to Mavacamten by Sarcomere Gene Mutation Status in EXPLORER-HCM 190/251 patients sequenced	73 SARC+ (M, n = 33; P, n = 40), 117 SARC- (M, n = 57; P, n = 60), 61 had no panel sequencing (M, n = 33; P, n = 28) Favourable response for the primary endpoint: SARC+ (odds ratio [OR], 4.43 [95% CI, 1.56-12.58]), SARC- (OR, 2.52 [95% CI, 0.99-6.42])

Table 10. EXPLORER-HCM secondary analyses

Special warnings reported by EMA and FDA (1) heart failure due to systolic dysfunction, and in case of serious intercurrent illness (infection, arrhythmia, major cardiac surgery) treatment interruption may be necessary to ensure that LVEF remains $\geq 50\%$,
(2) drug interactions leading to heart failure or loss of response: Mavacamten is metabolized, primarily through CYP2C19 (74%), CYP3A4 (18%), and CYP2C9 (8%), and mostly by CYP3A4 in poor metabolizers. Therefore, concomitant treatment with a strong CYP2C19 inhibitor (e.g., ticlopidine, fluconazole, fluvoxamine) and a strong CYP3A4 inhibitor (e.g., clarithromycin, itraconazole, ketoconazole, voriconazole, ritonavir, cobicistat, ceritinib, idelalisib, tucatinib) and concomitant treatment with strong CYP3A4 inhibitors in patients with CYP2C19 poor metaboliser are contraindicated. Co-administration of mavacamten with a strong inducer of both CYP2C19 and CYP3A4 (e.g., rifampicin) may lead to reduced efficacy and therefore co-administration with strong inducers of both CYP2C19 and CYP3A4 (e.g., rifampicin, apalutamide, enzalutamide, mitotane, phenytoin, carbamazepine, efavirenz, St. John's wort) is not recommended.
(3) Embryo-foetal toxicity: mavacamten may cause human embryo-foetal toxicity based on animal studies, therefore mavacamten is contraindicated during pregnancy and in women of childbearing potential not using effective contraception. Since hormonal contraception is a CYP3A4 substrate, concomitant use of mavacamten (CYP3A4 inducer) may cause contraceptive failure.

Table 11. Special warnings for Mavacamten administration.

	Mavacamten	Aficamten
Mechanism	Inhibits myosin ATPase activity Populate more myosins in the super-relaxed state shifting the DRX-SRX equilibrium more towards the SRX state	Inhibits myosin ATPase activity Slows the ATP turnover rate of myosins already in the SRX state, leading to a super-high relaxed state with a 10-fold lower ATPase turnover rate than those in the conventional SRX state. It does not affect the normal myosin DRX-SRX equilibrium
Binding-site	pocket located between the N-terminal and the Converter subdomains of the motor domain	Blebbistatin-ATP binding pocket of myosin
Dose	5-15mg	5-20 mg
Half life	7-9 days	3-4 days
Steady state	6 weeks	2 weeks
Drug interactions	Induction of CYP3A4, CYP2C9, CYP2C19, CYP2B6	No significant interactions

Table 12. Main differences between myosin inhibitors.

SEQUOIA	EXPLORER
Inclusion and exclusion criteria:	
resting LVOT-G ≥ 30 mm Hg and post-Valsalva LVOT-G ≥ 50 mm Hg during screening as determined by the echocardiography core laboratory	LVOT peak gradient ≥ 50 mm Hg during screening as assessed by echocardiography at rest, after Valsalva, or post-exercise (confirmed by echocardiography core laboratory interpretation)

	LVOT gradient with Valsalva maneuver at Screening TTE of ≥ 30 mmHg, determined by echocardiography core laboratory
LVEF $\geq 60\%$ at screening as determined by the echocardiography core laboratory	Has documented LVEF $\geq 55\%$ by echocardiography core laboratory read of Screening TTE at rest
RER ≥ 1.05 and pVO ₂ $< 90\%$ predicted on the screening CPET per the core laboratory	RER > 1.0 at screening per central reading; if RER is between 0.91 and 1.0, the patient may be enrolled only if it is determined by the central CPET laboratory that peak exercise has been achieved in the patient
Patients on beta-blockers, verapamil, diltiazem, or disopyramide should have been on a stable regimen for > 6 wks prior to randomization and anticipate remaining on the same medication regimen during the trial. Patients treated with disopyramide must also be concomitantly treated with a beta-blocker and/or calcium-channel blocker	Exclusion: Current treatment (within 14 days prior to Screening) or planned treatment during the study with disopyramide or ranolazine Current treatment (within 14 days prior to Screening) or planned treatment during the study with a combination of β blockers and verapamil or a combination of β -blockers and diltiazem
Exclusion: Significant valvular heart disease (per investigator judgment) a. Moderate-severe valvular aortic stenosis and/or regurgitation b. Moderate-severe mitral regurgitation not caused by systolic anterior motion of the mitral valve	Exclusion: known moderate or severe (as per investigator's judgment) aortic valve stenosis at Screening
Exclusion: History of LV systolic dysfunction (LVEF $< 45\%$) or stress cardiomyopathy at any time during the clinical course of the disease	
Study design	
Individualized doses of 2.5, 5, 10, or 15 mg were ultimately administered orally to achieve target reduction in LVOT gradient less than 30 mm Hg and a mavacamten plasma concentration between 350 ng/mL and 700 ng/mL Uptitration at week 8 and 14	uptitration will occur only if the peak Valsalva LVOT-G is ≥ 30 mm Hg and the biplane LVEF is $\geq 55\%$. Uptitration at week 2,4 and 6 (5,10,15,20mg)
Temporary discontinuation criteria: EF $< 40\%$	Temporary discontinuation criteria: EF $< 50\%$, QTc Fridericia > 520 ms
Baseline characteristics of the trials' populations	
76% white	93% white
60% on beta blockers 31% on CCBs 11% on Disopyramide	76% on beta blockers 20% on CCBs No Disopyramide
Results	
PRIMARY ENDPOINT: The placebo-corrected least-squares mean difference between the groups in the peak oxygen uptake was 1.7 ml per kilogram per minute (95% CI, 1.0 to 2.4; $P < 0.001$)	PRIMARY ENDPOINT: 37% vs 17% Either ≥ 1.5 mL/kg per min increase in pVO ₂ with ≥ 1 NYHA class improvement or ≥ 3.0 mL/kg per min increase in pVO ₂ with no worsening of NYHA class at week 30
Change from baseline KCCQ 7 (5 to 10)	Change from baseline KCCQ 9.1 (5.5 to 12.7)
Pvo ₂ change from baseline 1.7 (1.0 to 2.4)	Pvo ₂ change from baseline 1.4 (0.6-2.1)
≥ 1 NYHA class improvement 34% (23 to 45)	≥ 1 NYHA class improvement 34% (22 to 45)
LVOT < 30 after Valsalva manouver 46.4 (37.3-55.5)	Post exercise LVOT peak gradient < 30 mmHg 49.6 (39.3-59.9)

EF mean difference -4.0%, 95% CI -5.5 to -2.5	least-squares mean difference EF, -4.8 percentage points; 95% CI, -6.3 to -3.2
NTproBNP proportion of geometric mean ratio between the two groups 0.202, 95% CI 0.169 to 0.241	the geometric mean proportional change from baseline to week 24 in NT-proBNP between the two groups 0.20 (0.17 to 0.23)
Effect on primary endpoint by subgroups	
No differences on the primary endpoint by subgroups	Reduced effect on primary endpoint for patients on BBs
Safety	
Serious adverse events were reported in 8 patients (5.6%) in the aficamten group and in 13 patients (9.3%) in the placebo group. On both Aficamten and placebo 1 AF episode, on Afi 1 supraventricular arrhythmia, 0 on placebo. No stress cardiomyopathy reported.	serious adverse events were reported by 10 (8%) patients on mavacamten versus 20 events reported by 11 (9%) on placebo. Serious cardiac adverse events occurred in four patients in the mavacamten group (two atrial fibrillation, two stress cardiomyopathy); one of these presented during a study visit and simultaneously triggered a temporary discontinuation for LVEF less than 50%
7 patients on Aficamten transient reduction < 50% (4.9%)	7 patients on Mavacamten transient reduction < 50% (5.6%) 6 (3 on M and 3 on placebo) patients had a temporary discontinuation due to QTc prolongation

Table 13: Similarities and differences between SEQUOIA and EXPLORER HCM trials.

STUDY NAME AND STATUS	STUDY DESIGN	POPULATION	MAIN FINDINGS	SECONDARY ENDPOINTS
The Impact of Late Sodium Current Inhibition on Exercise Capacity in Subjects with Symptomatic Hypertrophic Cardiomyopathy (LIBERTY-HCM) Trial	Phase 2/3, Randomized, Double-Blind, Placebo-Controlled Study	Hcm NYHA class ≥ II peak VO ₂ < 80% of predicted	Prematurely terminated	Prematurely terminated
Functional effects of losartan in hypertrophic cardiomyopathy—a randomised clinical trial	randomised, placebo-controlled study 100 mg losartan daily or matching placebo for 12 months.	Adult patients with overt HCM	no effect on systolic function , global longitudinal strain, Doppler measures of diastolic function, left atrial volume , exercise	further progression of disease, with the most prominent impairment being an increase in left atrial volume of 6 mL/m ² (95% CI 3 to 9 mL/ m ² , p<0.0001) in both groups combined

			capacity compared with placebo	
Efficacy of Ranolazine in Patients With Symptomatic Hypertrophic Cardiomyopathy	multicenter, double-blind, phase 2 study	diagnosis of HCM, NYHA class II / III; peak oxygen consumption (VO ₂) at screening evaluation <75% of that predicted based on age and sex, sinus rhythm and absence of resting LVOT obstruction	no significant difference in VO ₂ peak change at 5 months in the ranolazine versus placebo group	reduction in 24-hour burden of premature ventricular complexes compared with placebo No changes in NTproBNP, E/e' and quality of life
Effect of Spironolactone on Myocardial Fibrosis and Other Clinical Variables in Patients with Hypertrophic Cardiomyopathy	Multicenter, double blind, placebo controlled trial	Patients with HCM with or without LVOT obstruction	No differences in serum markers of collagen synthesis or degradation, fibrosis by late gadolinium enhancement on CMR	No differences in Pvo ₂ , NYHA class, LV wall thickness, LA size, diastolic function
Effect of Trimetazidine Dihydrochloride Therapy on Exercise Capacity in Patients With Nonobstructive Hypertrophic Cardiomyopathy Trimetazidine: inhibitor of β -oxidation favouring glucose oxidation	randomized, placebo-controlled, double-blind clinical trial trimetazidine, 20 mg (n = 27), or placebo (n = 24) 3 times daily	nonobstructive HCM receiving stable medical therapy.	No difference in walking distance	peak oxygen consumption was 1.35 mL/kg per minute lower in the intervention group after 3 months
Metabolic modulator perhexiline corrects energy deficiency and improves exercise capacity in symptomatic hypertrophic cardiomyopathy	multi-centre randomised double blind placebo controlled trial	Symptomatic HCM Abnormal Peak VO ₂ No significant LVOT obstruction at rest (gradient < 30mmHg) Sinus rhythm	improvement in primary end point (peak $\dot{V}O_2$) (22.2 $\pm$ 0.2 to 24.3 $\pm$ 0.2 versus 23.6 $\pm$ 0.3 to 22.3 $\pm$ 0.2 mL $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹ ; <i>P</i> =0.003) and New York	improved myocardial ratios of phosphocreatine to adenosine triphosphate (from 1.27 $\pm$ 0.02 to 1.73 $\pm$ 0.02 versus 1.29 $\pm$ 0.01 to 1.23 $\pm$ 0.01; <i>P</i> =0.003) and normalized the

			Heart Association class ($P<0.001$)	abnormal prolongation of heart rate normalized time to peak filling between rest and exercise (0.11 ± 0.008 to -0.01 ± 0.005 versus 0.15 ± 0.007 to 0.11 ± 0.008 second; $P=0.03$)
Randomized controlled trial of perhexiline on regression of left ventricular hypertrophy in patients with symptomatic hypertrophic cardiomyopathy (RESOLVE-HCM trial) Perhexiline: potent carnitine palmitoyl transferase-1 (CPT-1) inhibitor-> shifts myocardial metabolism toward more energy-efficient glucose utilization	prospective, multicenter double-blind placebo-controlled randomized trial	HCM LVEF>55% NYHA class≥II NTproBNP>125 pg/ml	Change in Left Ventricular Hypertrophy (LVH) Not updated since 2021	Change in Left Ventricular (LV) mass Change in oxygen-sensitive Cardiac Magnetic Resonance Change in left ventricular diastolic function Change in NYHA class Change in CCS Not updated since 2021
Safety and Efficacy of Metabolic Modulation With Nineraxstat in Patients With Nonobstructive Hypertrophic Cardiomyopathy Nineraxstat: partial inhibition of fatty acid oxidation via direct competitive inhibition of 3-ketoacyl-CoA thiolase (3-KAT), shifts cardiac energy	Randomized double blind placebo controlled trial	HCM and left ventricular (LV) outflow gradient <30 mmHg, ejection fraction ≥50% and peak VO2 <80% predicted	better ventilatory efficiency (VE/VCO2 slope) compared to placebo with a least square (LS) mean difference between the groups of -2.1 (95% CI, -3.4, -0.6; $p=0.006$),	no significant difference in pVO2 , KCCQ-CS

metabolism from free fatty acid oxidation to glucose oxidation				
A randomized, double blind placebo controlled, parallel group, multicenter study to evaluate the efficacy and safety of sotagliflozin in symptomatic obstructive and non obstructive HCM	Randomized, double blind, placebo controlled trial	HCM KCCS-CSS<85 NYHA II/III LVEF>50%	Change from baseline in KCCQ	

Table 14. Completed and ongoing trials in non-obstructive HCM.

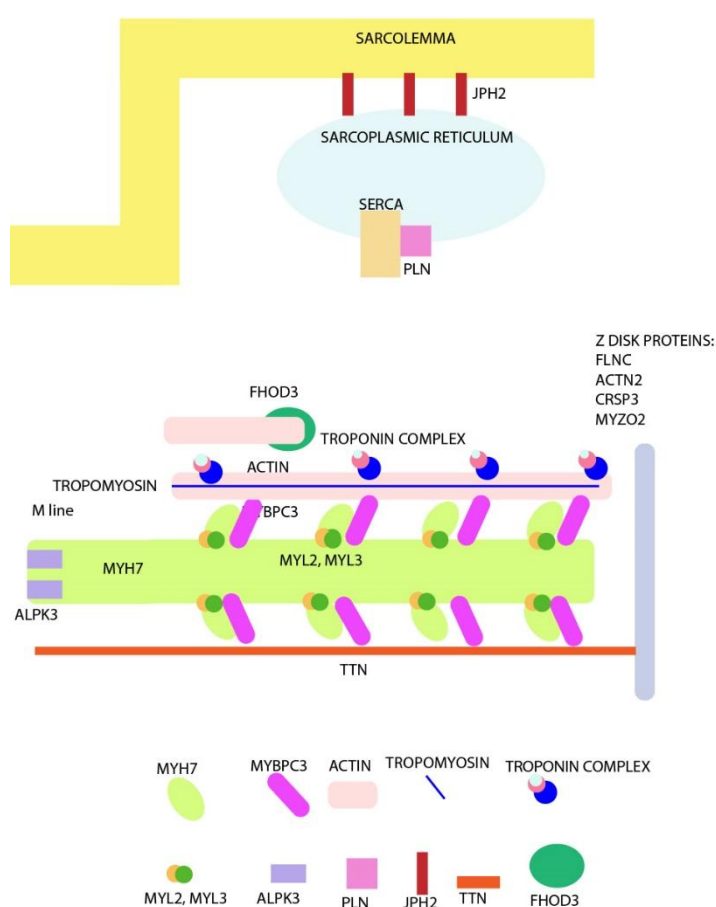


Figure 1. Genes involved in HCM pathophysiology

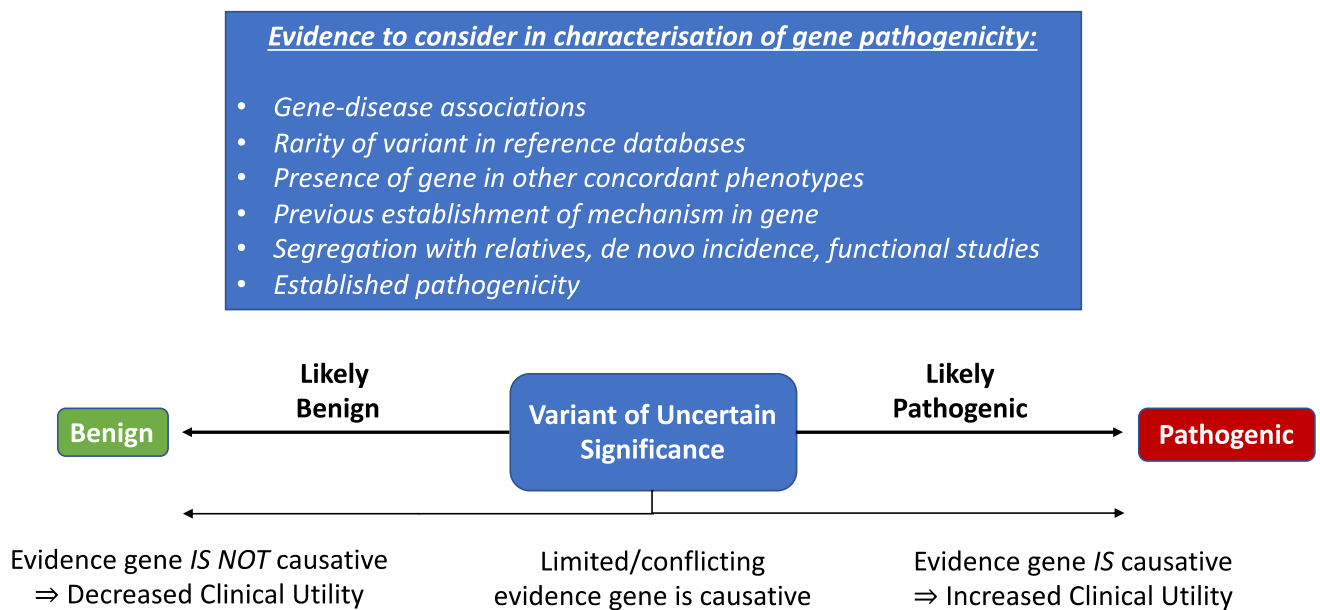


Figure 2. Evidence and variant classification to classify variants across a spectrum from benign to pathogenic (modified from Semsarian et al).¹⁵

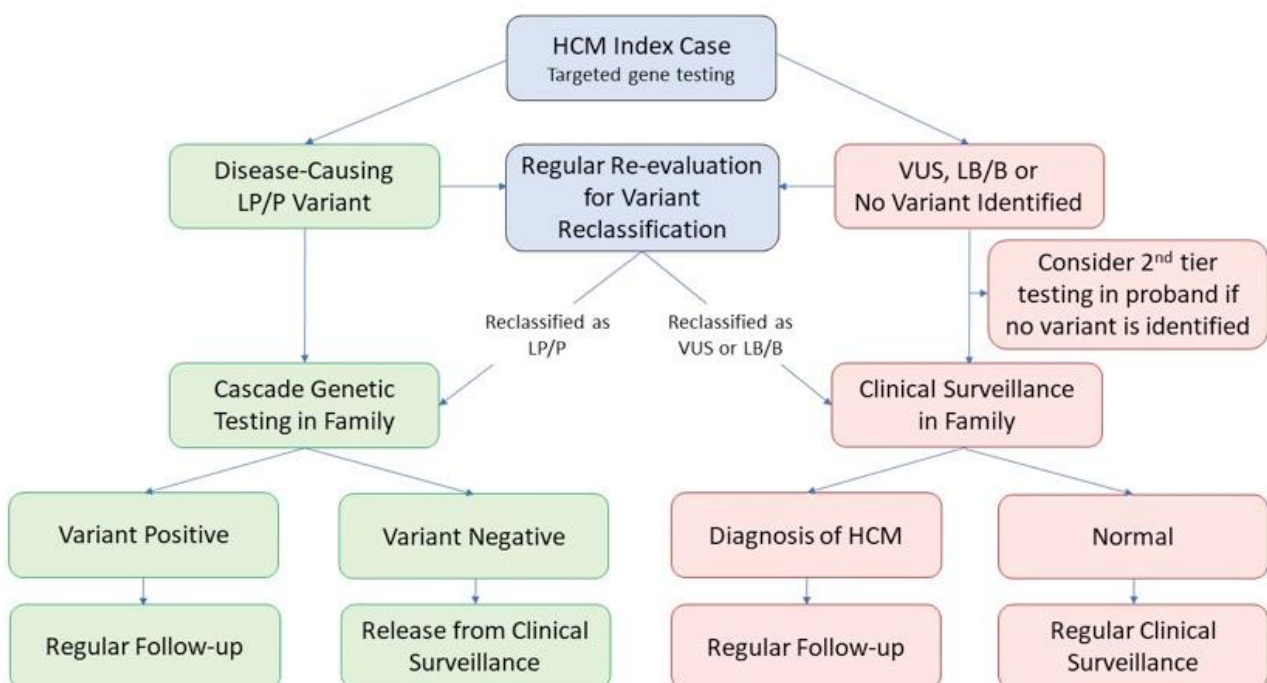


Figure 3. Clinical pathway for genetic testing in HCM. P=pathogenic, LP=likely pathogenic, VUS=variant of uncertain significance, LB=likely benign, B=benign. (modified from Ommen et al).²

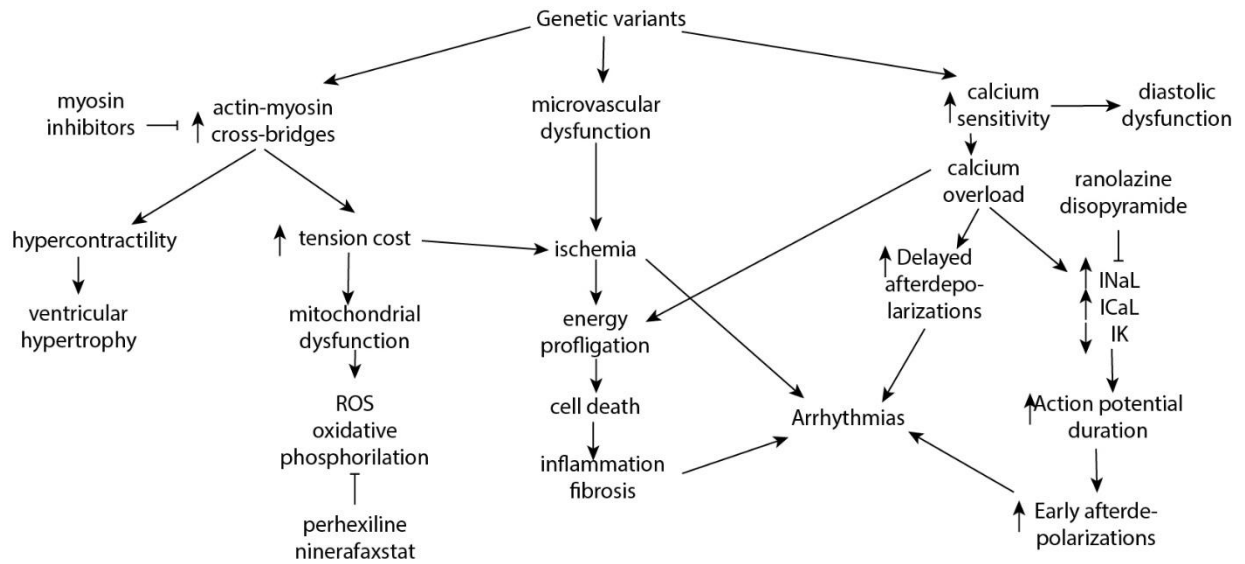


Figure 4. Pathophysiology of hypertrophic cardiomyopathy.

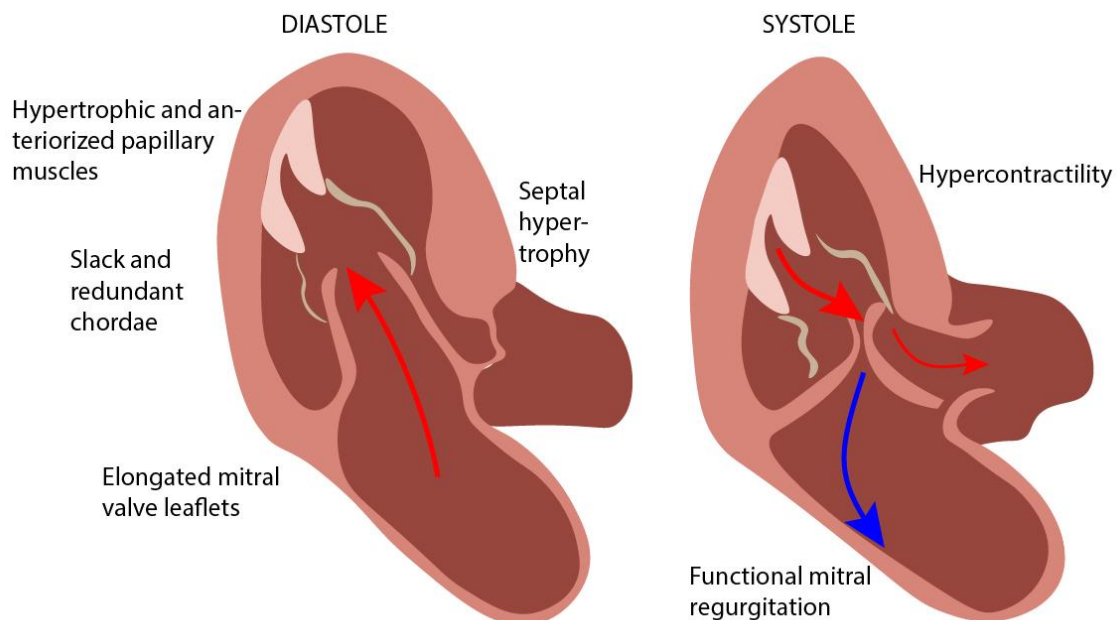


Figure 5. Left ventricular outflow tract obstruction.

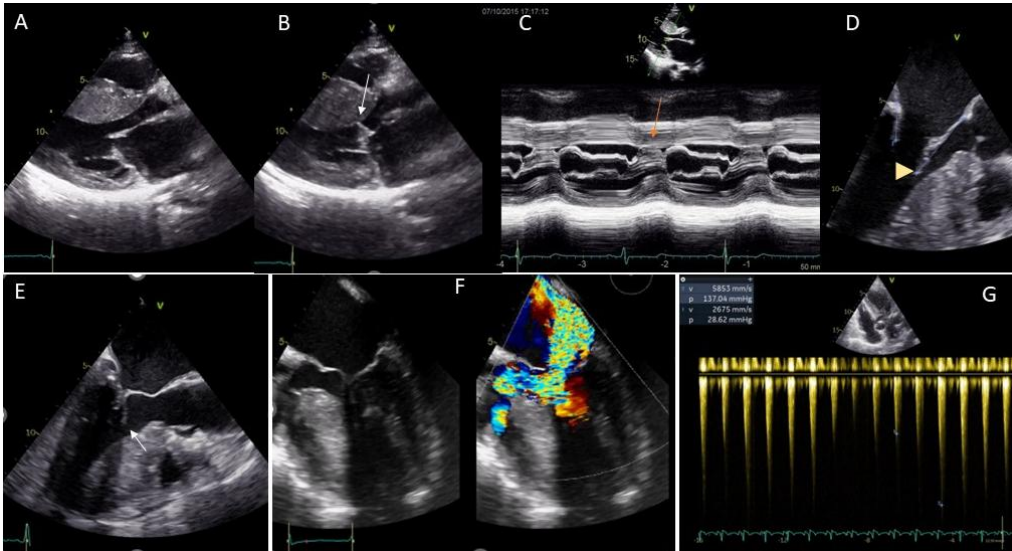


Figure 6. Echocardiographic features of a 32 years old HOCM patient. A. Parasternal long axis view showing the asymmetric hypertrophy with the interventricular septum of 21 mm and posterior wall of 13 mm. B. Systolic frame from parasternal long axis view illustrates systolic anterior motion (SAM) of the anterior mitral leaflet with septal contact (white arrow) leading to LVOT obstruction. C. M-mode from parasternal long axis view illustrating the contact interval between the anterior mitral leaflet and the interventricular septum (orange arrow). D-E. Mid-esophageal view at 120° allows diastolic measurement of mitral leaflet lengths (21 mm for posterior leaflet, 34 mm for anterior leaflet) and illustrates the systolic anterior motion of the anterior mitral leaflet (orange arrow at contact point of the mitral valve with the septum). F. Mid-esophageal view at 0° with simultaneous color Doppler showing severe eccentric mitral regurgitation and systolic turbulence in the LV outflow tract. G. Doppler continuous wave aligned in the left ventricular outflow tract finds a gradient at rest of 27 mmHg increasing to 138 mmHg at Valsalva maneuver.

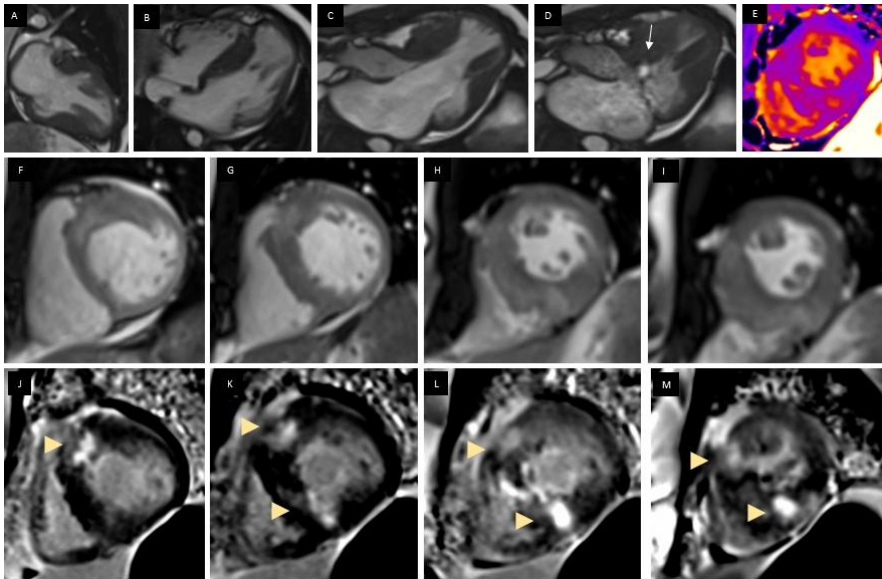


Figure 7. Contrast enhanced cardiac MRI in a 26 years-old carrier of a MYBPC3 mutation. Balanced-SSFP sequences diastolic frames from his 2 chambers (A), 4 chamber (B) and 3 chamber (C) views, showing LV asymmetric hypertrophy as well as papillary muscles apical insertion. D. Systolic frame from 3 chamber view showing systolic anterior motion of the mitral leaflet with septal contact and subsequent systolic flow acceleration in the left ventricle outflow tract (white arrow). E. Native T1 mapping in short axis view showing increased T1 values in the hypertrophied myocardium but normal T1 values in the thin walls. F-I. Balanced-SSFP sequences diastolic frames in short axis views at several ventricular levels illustrating the spiral distribution of hypertrophy from base (hypertrophy of anterior and anteroseptal walls) to apex (hypertrophy of inferoseptal and inferior walls) – important for myectomy planning. J-M. Late Gadolinium Enhancement imaging in short axis views showing patchy areas of hyperenhancement, representing extensive myocardial fibrosis of the hypertrophied left ventricular walls.

Figure 1: Aetiology of 301 children with infant-onset HCM

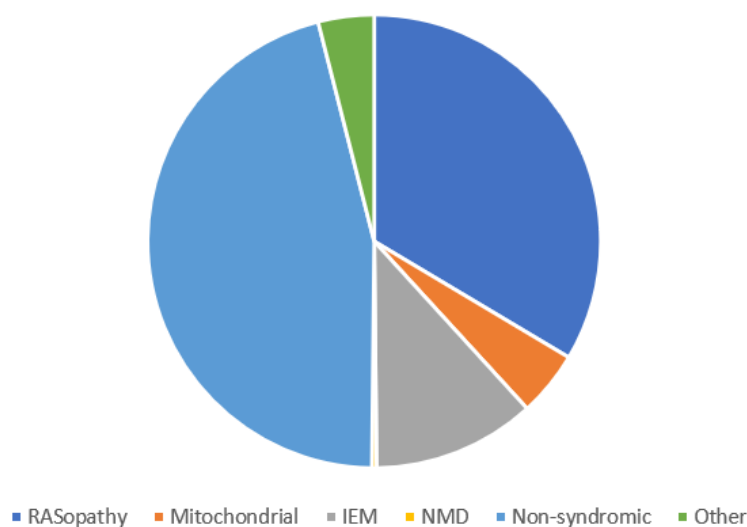


Figure 8. Aetiology of 301 children with infant-onset HCM

Key: IEM – inborn error of metabolism; NMD – neuromuscular disease

Adapted from Norrish et al. (PMID 34486247)

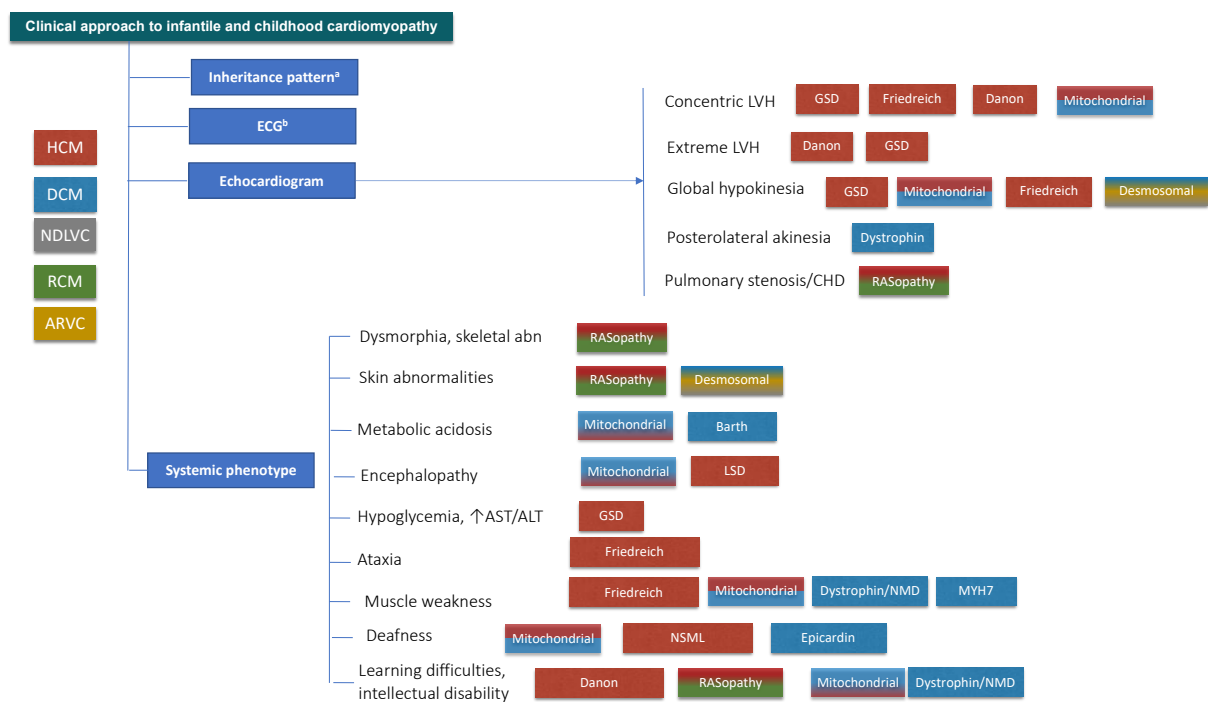


Figure 9. Diagnostic workflow for infant-onset hypertrophic cardiomyopathy.

Adapted from 2023 ESC Cardiomyopathy GL

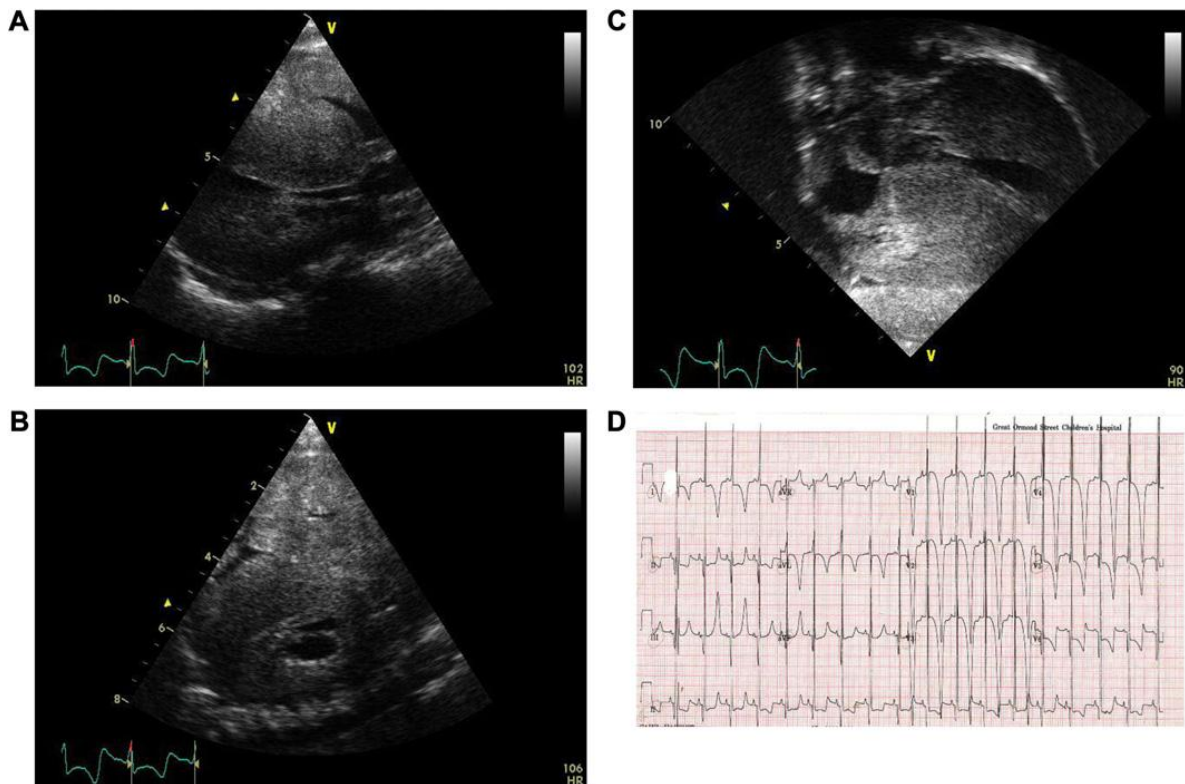


Figure 2 Echocardiographic (ECG) and electrocardiographic features of Pompe disease. (A) Parasternal long-axis view. (B) Parasternal short-axis view. (C) Subcostal 4-chamber view showing severe, concentric biventricular hypertrophy with small left and right ventricular cavity dimensions. (D) 12-lead ECG showing severe biventricular hypertrophy, widespread repolarisation abnormalities and a short PR interval.

Figure 10. ECG and echo of a child with Pompe Disease From Moak and Kaski [PMID 22591735].

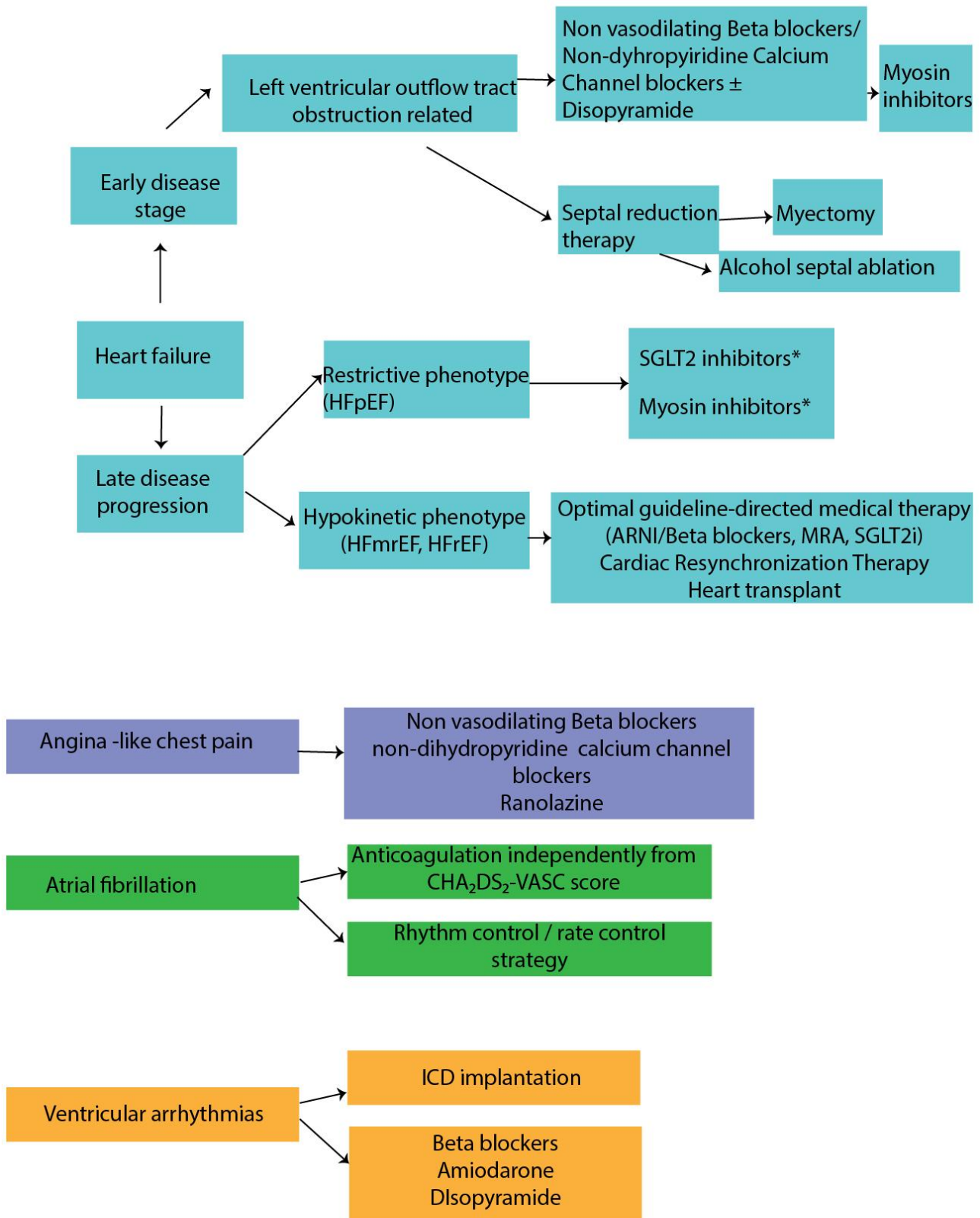


Figure 11. Summary of HCM therapeutic options. *Ongoing phase 3 clinical trials

PART 2:

Real-world experience using myosin inhibitors for the treatment of hypertrophic cardiomyopathy

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited myocardial disease defined by left ventricular (LV) hypertrophy unexplained by abnormal loading conditions. [2] Patients with HCM often present with impaired functional capacity and quality of life, and for decades the therapeutic arsenal for this condition included only unspecific and partially effective therapies for heart failure.[140] Myosin inhibitors are the first molecules developed to specifically target the pathophysiological abnormalities characteristic of this disease and potentially modify its natural history. These molecules reduce myosin's ATPase activity and the number of actin-myosin cross-bridges counteracting hypercontractility, responsible for left ventricular outflow tract obstruction and diastolic dysfunction. In the pivotal trials EXPLORER-HCM and SEQUOIA-HCM myosin inhibitors improved functional capacity and symptoms of patients with obstructive HCM.[119,125] However, limited data exists on the real-world use of myosin inhibitors and the effect of these drugs on echocardiography and electrocardiography parameters.

Methods

Study population

This prospective observational study evaluated patients with hypertrophic cardiomyopathy (HCM) treated with myosin inhibitors at the Cardiomyopathy Referral Center of the University of Florence.

All patients signed informed consent forms to participate in the study.

The diagnosis of HCM was established in presence of otherwise unexplained left ventricular hypertrophy greater or equal to 15mm, in familial cases the threshold was 13mm.

HCM associated with left ventricular outflow tract (LVOT) obstruction greater or equal to 50mmHg at rest or under provocation was defined as obstructive, else it was described as non-obstructive.

Exclusion criteria included: ejection fraction $\leq 55\%$, known infiltrative or storage disorder causing cardiac hypertrophy (Fabry disease, amyloidosis or Noonan syndrome with LV hypertrophy), known moderate or severe aortic stenosis.

Electronic medical records were reviewed for baseline clinical and genetic data.

Electrocardiography and echocardiography

Patients underwent electrocardiograms and echocardiograms at baseline and weeks 4, 8, 12, 24, 36, 48, and 60 following the first drug administration. All echocardiograms were acquired by expert cardiologists according to a prespecified imaging protocol. The ECG and echocardiography images were reviewed and interpreted by the same cardiologist.

The amplitude of the QRS complex and the peak of the T wave (expressed as a negative number if below the isoelectric line) were evaluated in all ECG leads. Electrocardiography was considered non-interpretable

in the case of paced ventricular rhythm. None of the patients evaluated had pathological Q waves, LBBB or RBBB.

Left ventricular (LV) thickness was measured at the basal interventricular septum in parasternal long-axis view. Maximal LV thickness was evaluated in parasternal long or short axis view, taking care of excluding para-septal structures and always measuring the same site for each patient. Simpson's biplane technique was used to measure left ventricular ejection fraction (LVEF), and resting and post Valsalva LVOT gradients were carefully identified avoiding contamination of LVOT waveform by mitral regurgitation.

Statistical analysis

Continuous variables are reported as median (IQR), while categorical variables are reported as raw numbers (percentage). The chi-square test and Wilcoxon rank sum test were used to compare frequencies and medians, respectively. A linear mixed effects model was fit to evaluate the change over time of echocardiography and electrocardiography parameters, considering as fixed effects the evaluation time and the baseline parameter and as random effects the single individual. Correlations between the change in echocardiography and electrocardiography parameters (defined as parameter at week 36-baseline) were evaluated by cubic spline models[141], while multivariate analyses were performed by generalized additive models. Firth's penalized logistic regression was used to evaluate univariate predictors of left ventricular dysfunction and non-response to therapy.[142]

Results

Thirty-one patients were enrolled, of whom 10 (32%) were women (Table 1). The median age was 55 years (IQR 50.5–59.9), and the median follow-up time was 11 months (IQR 2.8–14.4).

Nineteen patients (61%) were on mavacamten, while 12 (39%) were on Aficamten. Twelve patients (39%) were enrolled in the FOREST-HCM study, 6 patients (19%) in the MAVA-LTE study, 2 (6.5%) in the ODYSSEY-HCM trial, and 11 (35%) were included in the mavacamten early access program.

Twenty patients (65%) had a pathogenic/likely pathogenic variant identified, with the most frequent variants in *MYH7* (n=10) and *MYBPC3* (n=7) genes. One patient underwent a myectomy, and four patients (13%) had an ICD implanted. Twenty-five patients (81%) were in NYHA class II, while six (19%) were in NYHA class III. Four patients (31%) had angina symptoms.

Background medical therapy included beta-blockers for all patients with 24 (77%) on nadolol and 15 (48%) patients on disopyramide.

Baseline echocardiography showed a median maximal LV wall thickness of 18 mm (IQR 17-21); 15 (52%) patients had a neutral morphology, 10 (34%) had a reverse curve, and 4 (14%) had a sigmoid morphology. Diastolic dysfunction was evident through reduced septal (4 cm/s, IQR 3.5-4) and lateral (5 cm/s, IQR 4-6) e' velocities, elevated E/e' (17.2, IQR 13.5-23.6) and left atrial dilation with a median left atrial volume of 112ml (IQR 95-121). Twenty-eight patients (90%) had mitral regurgitation, of which 9 (32%) had at least moderate regurgitation. Ejection fraction was hyperdynamic with a median value of 80% (IQR 70-80) and measures of RV function were within the normal range. Twenty-eight patients (90%) had obstructive HCM, median LVOT gradient was 44mmHg (IQR 21.5-68), and median LVOT gradient under Valsalva maneuver was 80mmHg (IQR 59-95.5).

On electrocardiography, QRS amplitude was increased across all leads, with the highest values observed in lead V3, where the median amplitude was 20 mm (IQR 14–27 mm). Negative T waves were present in lead V6 (median -0.5 mm, IQR -2.0 to 1.6 mm), lead DI (median -0.5 mm, IQR -2.0 to 2.0 mm), and lead aVL (median -0.5 mm, IQR -2.0 to -0.6 mm).

Variable	Whole population N = 31	Women N = 10	Men N = 21	p- value
Age (years)	54.7 (50.5, 59.9)	57.9 (54.0, 62.6)	54.6 (48.9, 59.7)	0.3
Follow up (months)	11.0 (2.8, 14.4)	9.4 (3.6, 13.1)	13.6 (2.7, 14.6)	0.5
Height (cm)	176.0 (166.0, 179.5)	163.0 (160.0, 169.0)	178.0 (170.0, 180.0)	0.004
Weight (kg)	79.0 (73.0, 88.0)	74.5 (68.3, 85.5)	79.0 (76.0, 88.0)	0.3
Genetic Testing				0.2
Negative	5 / 31 (16%)	0 / 10 (0%)	5 / 21 (24%)	
Not performed	6 / 31 (19%)	3 / 10 (30%)	3 / 21 (14%)	
Positive	20 / 31 (65%)	7 / 10 (70%)	13 / 21 (62%)	
Genotype				0.4
ANKRD1	1 / 20 (5.0%)	0 / 7 (0%)	1 / 13 (7.7%)	
MYBPC3	6 / 20 (30%)	1 / 7 (14%)	5 / 13 (38%)	
MYH7	10 / 20 (50%)	4 / 7 (57%)	6 / 13 (46%)	
PRKG2	1 / 20 (5.0%)	1 / 7 (14%)	0 / 13 (0%)	
TNNI3	1 / 20 (5.0%)	0 / 7 (0%)	1 / 13 (7.7%)	
TNNT2	1 / 20 (5.0%)	1 / 7 (14%)	0 / 13 (0%)	
Interpretation				0.3
Likely pathogenic	3 / 20 (15%)	2 / 7 (29%)	1 / 13 (7.7%)	
Pathogenic	11 / 20 (55%)	4 / 7 (57%)	7 / 13 (54%)	
VUS	6 / 20 (30%)	1 / 7 (14%)	5 / 13 (38%)	
Maximum IVS (mm)	18.0 (17.0, 21.0)	17.0 (17.0, 18.0)	20.0 (18.0, 23.0)	0.032
IVS (mm)	18.0 (17.0, 19.0)	17.0 (16.0, 17.8)	18.0 (17.0, 19.0)	0.038
EDD (mm)	41.0 (37.5, 44.0)	39.5 (36.3, 42.3)	41.0 (38.0, 44.0)	0.2
PW (mm)	12.0 (11.0, 13.0)	11.5 (11.0, 12.0)	12.0 (11.0, 13.0)	0.7
AML (mm)	30.0 (24.0, 33.0)	24.0 (22.3, 26.8)	32.0 (26.0, 34.0)	0.010
E wave (cm/s)	81.0 (64.5, 107.0)	93.0 (67.5, 127.8)	76.0 (65.0, 99.0)	0.4
A wave (cm/s)	76.0 (51.0, 89.0)	77.0 (60.8, 92.8)	76.0 (47.5, 86.0)	0.4
Septal e' (cm/s)	4.0 (3.5, 4.0)	4.0 (4.0, 4.0)	4.0 (3.0, 4.0)	0.7
Lateral e' (cm/s)	5.0 (4.0, 6.0)	5.5 (4.3, 6.0)	5.0 (4.0, 6.0)	>0.9
E/e'	17.2 (13.5, 23.6)	18.6 (15.9, 22.3)	15.2 (13.3, 23.7)	0.4
Left atrial volume (ml)	112.0 (95.0, 121.5)	112.0 (92.5, 120.0)	112.0 (98.0, 122.0)	0.7
Mitral regurgitation				0.9
None	3 / 31 (9.7%)	0 / 10 (0%)	3 / 21 (14%)	
Mild	19 / 31 (61%)	7 / 10 (70%)	12 / 21 (57%)	
Moderate	6 / 31 (19%)	2 / 10 (20%)	4 / 21 (19%)	
Moderate/severe	3 / 31 (9.7%)	1 / 10 (10%)	2 / 21 (9.5%)	
Rest LVOT gradient (mmHg)	44.0 (21.5, 68.0)	52.0 (38.3, 66.0)	37.0 (21.0, 68.0)	0.6
Valsalva LVOT gradient (mmHg)	80.0 (59.0, 95.5)	93.0 (58.0, 99.0)	76.0 (66.0, 92.0)	0.5
EDV (ml)	98.0 (86.5, 131.0)	88.0 (83.3, 98.3)	119.0 (92.0, 143.0)	0.052
ESV (ml)	23.0 (20.0, 34.5)	21.5 (20.0, 23.8)	24.0 (20.0, 36.0)	0.7
EF	0.8 (0.7, 0.8)	0.8 (0.7, 0.8)	0.8 (0.7, 0.8)	0.4
TAPSE (mm)	24.0 (21.8, 27.0)	25.0 (24.0, 27.0)	23.0 (21.0, 24.8)	0.077

Reason for enrollment				0.8
Early access program	11 / 31 (35%)	4 / 10 (40%)	7 / 21 (33%)	
Forest-HCM trial	12 / 31 (39%)	4 / 10 (40%)	8 / 21 (38%)	
MAVA LTE trial	6 / 31 (19%)	1 / 10 (10%)	5 / 21 (24%)	
ODISSEY-HCM trial	2 / 31 (6.5%)	1 / 10 (10%)	1 / 21 (4.8%)	
Family history of hcm	7 / 31 (23%)	3 / 10 (30%)	4 / 21 (19%)	0.7
Hypertension	10 / 31 (32%)	5 / 10 (50%)	5 / 21 (24%)	0.2
Atrial fibrillation	5 / 31 (16%)	2 / 10 (20%)	3 / 21 (14%)	>0.9
Diabetes	2 / 31 (6.5%)	0 / 10 (0%)	2 / 21 (9.5%)	>0.9
Coronary artery disease	3 / 31 (9.7%)	1 / 10 (10%)	2 / 21 (9.5%)	>0.9
ICD implantation	4 / 31 (13%)	0 / 10 (0%)	4 / 21 (19%)	0.3
Prior myectomy	1 / 31 (3.2%)	0 / 10 (0%)	1 / 21 (4.8%)	>0.9
Beta Blockers	31 / 31 (100%)	10 / 10 (100%)	21 / 21 (100%)	
				>0.9
Bisoprolol	2 / 31 (6.5%)	1 / 10 (10%)	1 / 21 (4.8%)	
Metoprolol	3 / 31 (9.7%)	1 / 10 (10%)	2 / 21 (9.5%)	
Nadolol	24 / 31 (77%)	8 / 10 (80%)	16 / 21 (76%)	
Propranolol	2 / 31 (6.5%)	0 / 10 (0%)	2 / 21 (9.5%)	
Disopyramide	15 / 31 (48%)	5 / 10 (50%)	10 / 21 (48%)	>0.9
dose				0.7
250 (mg/die)	8 / 16 (50%)	4 / 5 (80%)	4 / 11 (36%)	
375 (mg/die)	1 / 16 (6.3%)	0 / 5 (0%)	1 / 11 (9.1%)	
500 (mg/die)	6 / 16 (38%)	1 / 5 (20%)	5 / 11 (45%)	
Missing	15	5	10	
NYHA class				0.007
2	25 / 31 (81%)	5 / 10 (50%)	20 / 21 (95%)	
3	6 / 31 (19%)	5 / 10 (50%)	1 / 21 (4.8%)	
Palpitations	0 / 31 (0%)	0 / 10 (0%)	0 / 21 (0%)	
Syncope	1 / 31 (3.2%)	0 / 10 (0%)	1 / 21 (4.8%)	>0.9
CCS angina class				0.7
0	27 / 31 (87%)	10 / 10 (100%)	17 / 21 (81%)	
1	1 / 31 (3.2%)	0 / 10 (0%)	1 / 21 (4.8%)	
2	3 / 31 (9.7%)	0 / 10 (0%)	3 / 21 (14%)	
Morphology				0.7
neutral	15 / 31 (48%)	6 / 10 (60%)	9 / 21 (43%)	
reverse curve	11 / 31 (35%)	3 / 10 (30%)	8 / 21 (38%)	
sigmoid	5 / 31 (16%)	1 / 10 (10%)	4 / 21 (19%)	
SAM	28 / 31 (90%)	9 / 10 (90%)	19 / 21 (90%)	>0.9
QRS amplitude V1 (mm)	16.0 (13.0, 21.3)	15.5 (15.0, 23.3)	16.0 (12.3, 19.5)	0.5
QRS amplitude V2 (mm)	19.5 (13.8, 28.3)	20.0 (16.0, 26.8)	19.5 (13.0, 30.8)	0.8
QRS amplitude V3 (mm)	20.0 (14.0, 27.0)	20.0 (14.3, 24.3)	20.0 (14.8, 28.5)	0.5
QRS amplitude V4 (mm)	20.0 (16.0, 23.5)	17.5 (14.5, 23.8)	20.5 (18.3, 23.0)	0.2
QRS amplitude V5 (mm)	15.5 (12.8, 22.5)	15.5 (13.3, 18.8)	15.5 (12.3, 27.0)	0.7

QRS amplitude V6 (mm)	14.0 (10.0, 17.3)	13.5 (10.5, 15.8)	14.5 (8.5, 24.5)	0.5
QRS amplitude I (mm)	12.0 (9.8, 15.3)	12.5 (10.0, 15.3)	11.5 (9.3, 14.5)	0.8
QRS amplitude II (mm)	10.0 (9.0, 15.3)	12.5 (10.0, 15.8)	9.0 (8.3, 14.8)	0.3
QRS amplitude III (mm)	10.0 (7.8, 15.3)	10.0 (6.0, 13.0)	10.0 (8.3, 15.8)	0.5
QRS amplitude aVR (mm)	10.0 (8.8, 12.5)	11.0 (10.0, 13.5)	10.0 (8.3, 12.0)	0.5
QRS amplitude aVL (mm)	10.0 (6.8, 13.3)	9.5 (5.5, 13.8)	10.0 (8.3, 13.0)	0.8
QRS amplitude aVF (mm)	8.5 (6.0, 15.0)	7.5 (6.3, 10.0)	9.5 (5.3, 15.0)	>0.9
Peak T wave inversion V1 (mm)	3.0 (1.0, 4.1)	1.5 (-0.5, 3.9)	3.3 (2.1, 4.0)	0.15
Peak T wave inversion V2 (mm)	6.0 (3.9, 8.3)	5.5 (4.0, 6.8)	6.0 (3.6, 8.8)	0.8
Peak T wave inversion V3 (mm)	5.0 (2.8, 6.3)	5.0 (4.3, 5.8)	4.0 (2.3, 6.8)	0.8
Peak T wave inversion V4 (mm)	2.5 (0.9, 4.3)	3.0 (2.1, 3.4)	2.0 (0.6, 4.8)	>0.9
Peak T wave inversion V5 (mm)	1.5 (-2.0, 2.5)	1.8 (-0.1, 2.5)	1.3 (-2.4, 2.0)	0.5
Peak T wave inversion V6 (mm)	-0.5 (-2.0, 1.6)	0.8 (-1.0, 1.9)	-1.0 (-2.4, 1.4)	0.3
Peak T wave inversion I (mm)	-0.5 (-2.0, 2.0)	-0.8 (-1.8, 2.9)	-0.3 (-1.8, 1.8)	0.5
Peak T wave inversion II (mm)	1.5 (-1.0, 3.0)	2.3 (-0.5, 4.0)	1.3 (-1.0, 2.0)	0.2
Peak T wave inversion III (mm)	2.0 (0.0, 2.0)	1.5 (1.0, 2.0)	2.0 (0.0, 2.0)	0.8
Peak T wave inversion aVR (mm)	-1.0 (-2.0, 0.6)	-0.8 (-3.0, 0.8)	-1.0 (-2.0, 0.1)	0.8
Peak T wave inversion aVL (mm)	-0.5 (-2.0, 0.6)	-0.5 (-1.8, -0.1)	-0.8 (-1.8, 0.9)	>0.9
Peak T wave inversion aVF (mm)	1.3 (-0.1, 2.5)	2.0 (1.3, 2.9)	0.8 (-0.4, 2.0)	0.12

Table 4. *VUS: variant of uncertain significance, IVS: interventricular septum, EDD: end-diastolic diameter, PW: posterior wall, AML: anterior mitral leaflet, LVOT: left ventricular outflow tract, EDV: end-diastolic volume, ESV: end-systolic volume, EF: ejection fraction, TAPSE: tricuspid annular plane systolic excursion, ICD: implantable cardioverter defibrillator, NYHA class: New York Heart Association class, CCS: Canadian Cardiovascular society, SAM: systolic anterior motion of the mitral valve.*

Myosin inhibitors effect on echocardiography and ECG parameters over time

Treatment with myosin inhibitors was associated with a reduction in maximum LV wall thickness and PW with a mean decrease by week 60 of -2.8 mm (95% CI -3.5 to -2.2) and -1.8 mm (95% CI -2.4 to -1.3), respectively (Table 2). Diastolic function improved, as shown by increases in septal and lateral e' velocities over time, with mean increment compared to baseline of 0.9 cm/s (95% CI 0.3 to 0.9) and 1.3 cm/s (95% CI 0.3 to 2.3). E/e' showed a mean reduction compared to baseline of 4.8 (95% CI -7.12 to -2.56) and left atrial volume decreased by -29 ml (95% CI -38 to -19) by week 60 compared to baseline. Ejection fraction was slightly reduced, with a mean decrease compared to baseline of -0.07% (95% CI -0.03 to -3.5). Baseline LVOT

gradient at rest and after Valsalva maneuver showed an early and significant reduction, with mean decreases from baseline of -36 mmHg (95% CI -44 to -27) and -57 mmHg (95% CI -69 to -45) by week 60, respectively.

On electrocardiography, QRS amplitude decreased in all leads with the greatest and earliest reduction in those with the highest baseline amplitudes such as V1 (-4.6 mm, 95% CI -6.3 to -3.0), V2 (-8.5 mm, 95% CI -11.6 to -5.5) and V3 (-6.1 mm, 95% CI -9.3 to -3.8). Pathological T wave inversion normalized, with an early and significant increase in T wave peaks in V5 and V6, showing mean increases of 3.01 mm (95% CI 1.7 to 4.6) and 2.9 mm (95% CI 1.8 to 3.9), respectively. A similar pattern was seen in I (2.03 mm, 95% CI 2.8 to 4.3) and aVL (1.0, 95% CI 1.6 to 2.9). A graphical representation of the models is reported in Supplementary Figures 13-18.

Time	estimate	95% CI lower	95% CI upper	statistic	P value
Maximum septal wall thickness					
(Intercept)	1.200	-0.402	2.802	1.520	0.137
Week 4	-0.161	-0.690	0.367	-0.603	0.547
Week 8	-0.772	-1.312	-0.232	-2.824	0.005
Week 12	-1.378	-1.959	-0.797	-4.685	0.000
Week 24	-1.642	-2.232	-1.053	-5.500	0.000
Week 36	-2.065	-2.684	-1.447	-6.595	0.000
Week 48	-2.320	-2.962	-1.678	-7.135	0.000
Week 60	-2.879	-3.549	-2.208	-8.477	0.000
Baseline maximum septal wall thickness	0.939	0.860	1.017	24.375	0.000
Interventricular septal thickness					
(Intercept)	-0.279	-2.740	2.181	-0.230	0.820
Week 4	-0.387	-0.830	0.056	-1.725	0.087
Week 8	-0.828	-1.281	-0.374	-3.607	0.000
Week 12	-0.814	-1.296	-0.332	-3.333	0.001
Week 24	-1.256	-1.752	-0.760	-4.999	0.000
Week 36	-1.889	-2.409	-1.368	-7.168	0.000
Week 48	-2.071	-2.611	-1.530	-7.568	0.000
Week 60	-1.885	-2.449	-1.320	-6.595	0.000
Baseline Interventricular septal thickness	1.015	0.881	1.150	15.284	0.000
End-diastolic diameter					
(Intercept)	15.567	7.681	23.453	4.008	0.000
Week 4	-0.032	-1.697	1.632	-0.038	0.970
Week 8	-0.733	-2.432	0.965	-0.853	0.395
Week 12	-1.669	-3.469	0.131	-1.831	0.069
Week 24	0.165	-1.686	2.015	0.176	0.861
Week 36	-0.296	-2.237	1.646	-0.301	0.764
Week 48	-0.911	-2.926	1.105	-0.892	0.374
Week 60	-0.246	-2.351	1.859	-0.231	0.818

Baseline end diastolic diameter	0.623	0.434	0.811	6.717	0.000
Posterior wall					
(Intercept)	2.530	0.345	4.715	2.347	0.024
Week 4	-0.258	-0.894	0.378	-0.802	0.424
Week 8	-0.540	-1.189	0.108	-1.646	0.102
Week 12	-0.108	-0.795	0.579	-0.311	0.756
Week 24	-0.757	-1.463	-0.051	-2.117	0.036
Week 36	-1.447	-2.187	-0.706	-3.857	0.000
Week 48	-0.905	-1.674	-0.136	-2.324	0.021
Week 60	-1.190	-1.992	-0.387	-2.927	0.004
Baseline posterior wall	0.787	0.609	0.966	8.949	0.000
E wave					
(Intercept)	27.216	12.753	41.680	3.812	0.001
Week 4	2.065	-5.185	9.314	0.563	0.575
Week 8	-7.185	-14.587	0.218	-1.918	0.057
Week 12	-4.783	-12.641	3.075	-1.202	0.231
Week 24	-4.061	-12.142	4.021	-0.992	0.323
Week 36	-7.769	-16.411	0.872	-1.776	0.078
Week 48	1.221	-7.591	10.033	0.274	0.785
Week 60	-10.443	-19.878	-1.008	-2.186	0.030
Baseline E wave	0.684	0.531	0.836	9.162	0.000
A wave					
(Intercept)	22.774	6.981	38.567	2.904	0.006
Week 4	-1.276	-13.200	10.648	-0.212	0.833
Week 8	6.655	-5.512	18.822	1.081	0.281
Week 12	-0.437	-13.367	12.493	-0.067	0.947
Week 24	2.688	-10.633	16.009	0.399	0.691
Week 36	-1.701	-16.037	12.634	-0.235	0.815
Week 48	3.884	-10.764	18.532	0.524	0.601
Week 60	-0.107	-15.968	15.753	-0.013	0.989
Baseline A wave	0.692	0.515	0.869	8.038	0.000
Septal e'					
(Intercept)	1.497	0.259	2.735	2.452	0.019
Week 4	0.516	0.049	0.984	2.182	0.031
Week 8	0.357	-0.121	0.834	1.476	0.142
Week 12	0.710	0.202	1.217	2.763	0.006
Week 24	0.760	0.237	1.282	2.874	0.005
Week 36	0.586	0.027	1.145	2.071	0.040
Week 48	0.963	0.393	1.533	3.339	0.001
Week 60	0.918	0.308	1.528	2.971	0.003

Baseline Septal e'	0.635	0.350	0.919	4.544	0.000
Lateral e'					
(Intercept)	2.928	1.247	4.610	3.537	0.001
Week 4	0.806	0.001	1.611	1.979	0.050
Week 8	1.219	0.397	2.041	2.929	0.004
Week 12	0.469	-0.404	1.342	1.061	0.290
Week 24	1.314	0.416	2.212	2.890	0.004
Week 36	1.163	0.202	2.124	2.391	0.018
Week 48	1.795	0.815	2.775	3.619	0.000
Week 60	1.341	0.292	2.390	2.525	0.013
Baseline Lateral e'	0.466	0.187	0.745	3.417	0.002
E/e'					
(Intercept)	5.890	2.511	9.269	3.543	0.001
Week 4	-1.445	-3.193	0.303	-1.634	0.104
Week 8	-3.032	-4.817	-1.247	-3.356	0.001
Week 12	-2.967	-4.863	-1.071	-3.091	0.002
Week 24	-3.751	-5.701	-1.800	-3.799	0.000
Week 36	-4.206	-6.292	-2.120	-3.983	0.000
Week 48	-3.566	-5.693	-1.439	-3.312	0.001
Week 60	-4.847	-7.124	-2.569	-4.204	0.000
Baseline E/e'	0.685	0.523	0.847	8.694	0.000
Deceleration time					
(Intercept)	58.990	6.660	111.320	2.284	0.028
Week 4	5.484	-18.703	29.670	0.448	0.655
Week 8	-0.169	-24.852	24.513	-0.014	0.989
Week 12	3.247	-22.942	29.436	0.245	0.807
Week 24	13.211	-13.717	40.140	0.969	0.334
Week 36	-3.649	-32.426	25.129	-0.250	0.803
Week 48	-22.789	-52.139	6.561	-1.534	0.127
Week 60	11.218	-20.212	42.649	0.705	0.482
Baseline Deceleration time	0.724	0.498	0.951	6.522	0.000
Left atrial volume					
(Intercept)	28.918	8.871	48.965	2.925	0.006
Week 4	-10.290	-17.841	-2.740	-2.693	0.008
Week 8	-15.983	-23.700	-8.266	-4.092	0.000
Week 12	-18.793	-27.001	-10.585	-4.523	0.000
Week 24	-18.029	-26.478	-9.580	-4.215	0.000
Week 36	-25.198	-34.074	-16.322	-5.608	0.000
Week 48	-24.718	-33.939	-15.498	-5.295	0.000
Week 60	-28.882	-38.512	-19.252	-5.924	0.000

Baseline Left atrial volume	0.742	0.575	0.910	9.017	0.000
End-diastolic volume					
(Intercept)	29.182	13.798	44.566	3.849	0.000
Week 4	-0.161	-7.342	7.020	-0.044	0.965
Week 8	1.602	-5.730	8.934	0.432	0.667
Week 12	2.001	-5.782	9.784	0.508	0.612
Week 24	-4.157	-12.163	3.848	-1.026	0.307
Week 36	-0.261	-8.667	8.145	-0.061	0.951
Week 48	-0.134	-8.861	8.593	-0.030	0.976
Week 60	-7.712	-16.829	1.405	-1.671	0.097
Baseline End diastolic volume	0.738	0.611	0.865	11.891	0.000
End-systolic volume					
(Intercept)	0.139	-7.766	8.044	0.036	0.972
Week 4	1.516	-2.927	5.959	0.674	0.501
Week 8	3.807	-0.724	8.337	1.660	0.099
Week 12	4.405	-0.393	9.203	1.813	0.072
Week 24	1.378	-3.554	6.310	0.552	0.582
Week 36	2.351	-2.824	7.526	0.897	0.371
Week 48	5.679	0.309	11.049	2.088	0.038
Week 60	4.684	-0.925	10.293	1.649	0.101
Baseline end systolic volume	0.249	0.185	0.313	7.971	0.000
TAPSE					
(Intercept)	2.993	0.175	5.810	2.174	0.038
Week 4	-0.333	-1.128	0.461	-0.830	0.408
Week 8	-0.088	-0.890	0.715	-0.216	0.829
Week 12	-0.665	-1.487	0.156	-1.600	0.112
Week 24	-0.376	-1.231	0.480	-0.868	0.387
Week 36	-0.461	-1.343	0.422	-1.031	0.304
Week 48	-0.934	-1.850	-0.018	-2.016	0.046
Week 60	-0.263	-1.267	0.741	-0.517	0.606
Baseline TAPSE	0.875	0.760	0.990	15.613	0.000
Ejection fraction					
(Intercept)	0.457	0.275	0.638	5.122	0.000
Week 4	-0.018	-0.049	0.014	-1.116	0.266
Week 8	-0.030	-0.062	0.002	-1.845	0.067
Week 12	-0.037	-0.071	-0.004	-2.179	0.031
Week 24	-0.032	-0.067	0.003	-1.830	0.069
Week 36	-0.026	-0.062	0.011	-1.377	0.170
Week 48	-0.052	-0.090	-0.014	-2.706	0.008

Week 60	-0.072	-0.111	-0.032	-3.566	0.000
Baseline Ejection fraction	0.394	0.156	0.633	3.367	0.002
Resting LVOT gradient					
(Intercept)	33.444	25.478	41.411	8.404	0.000
Week 4	-26.387	-33.322	-19.452	-7.520	0.000
Week 8	-33.043	-40.116	-25.970	-9.232	0.000
Week 12	-35.026	-42.522	-27.529	-9.231	0.000
Week 24	-35.562	-43.277	-27.848	-9.107	0.000
Week 36	-35.071	-43.302	-26.841	-8.417	0.000
Week 48	-38.550	-46.948	-30.153	-9.068	0.000
Week 60	-36.073	-44.841	-27.304	-8.126	0.000
Baseline resting LVOT gradient	0.333	0.217	0.449	5.894	0.000
Valsalva LVOT gradient					
(Intercept)	45.923	29.915	61.930	5.806	0.000
Week 4	-35.935	-45.371	-26.500	-7.525	0.000
Week 8	-41.493	-51.125	-31.861	-8.511	0.000
Week 12	-49.401	-59.622	-39.181	-9.548	0.000
Week 24	-48.594	-59.107	-38.081	-9.131	0.000
Week 36	-53.132	-64.368	-41.896	-9.341	0.000
Week 48	-59.974	-71.431	-48.517	-10.340	0.000
Week 60	-57.423	-69.390	-45.456	-9.478	0.000
Baseline Valsalva LVOT gradient	0.395	0.211	0.580	4.400	0.000
QRS amplitude V1					
(Intercept)	5.218	1.697	8.740	3.015	0.005
Week 4	-0.786	-2.149	0.578	-1.139	0.257
Week 8	-2.036	-3.399	-0.672	-2.952	0.004
Week 12	-1.676	-3.174	-0.177	-2.211	0.029
Week 24	-2.089	-3.637	-0.541	-2.667	0.009
Week 36	-3.170	-4.746	-1.593	-3.974	0.000
Week 48	-3.242	-4.849	-1.635	-3.986	0.000
Week 60	-4.689	-6.329	-3.049	-5.650	0.000
Baseline QRS amplitude V1	0.692	0.499	0.885	7.338	0.000
QRS amplitude V2					
(Intercept)	8.524	4.785	12.262	4.604	0.000
Week 4	-4.286	-6.847	-1.725	-3.309	0.001
Week 8	-4.607	-7.168	-2.046	-3.557	0.001

Week 12	-4.723	-7.532	-1.914	-3.324	0.001
Week 24	-6.013	-8.916	-3.111	-4.095	0.000
Week 36	-6.620	-9.576	-3.665	-4.428	0.000
Week 48	-7.696	-10.709	-4.684	-5.050	0.000
Week 60	-8.567	-11.641	-5.492	-5.508	0.000
Baseline QRS amplitude V2	0.632	0.504	0.760	10.137	0.000
QRS amplitude V3					
(Intercept)	6.201	2.737	9.665	3.621	0.001
Week 4	-2.821	-5.169	-0.474	-2.377	0.019
Week 8	-3.571	-5.919	-1.224	-3.008	0.003
Week 12	-4.825	-7.401	-2.250	-3.704	0.000
Week 24	-6.219	-8.880	-3.558	-4.621	0.000
Week 36	-5.730	-8.440	-3.020	-4.181	0.000
Week 48	-6.554	-9.316	-3.792	-4.692	0.000
Week 60	-6.194	-9.013	-3.375	-4.344	0.000
Baseline QRS amplitude V3	0.736	0.618	0.854	12.815	0.000
QRS amplitude V4					
(Intercept)	6.642	3.740	9.543	4.586	0.000
Week 4	-0.071	-2.329	2.186	-0.063	0.950
Week 8	-2.893	-5.150	-0.635	-2.534	0.012
Week 12	-3.185	-5.655	-0.715	-2.548	0.012
Week 24	-4.956	-7.506	-2.406	-3.841	0.000
Week 36	-4.376	-6.973	-1.780	-3.331	0.001
Week 48	-4.693	-7.339	-2.047	-3.506	0.001
Week 60	-5.667	-8.367	-2.967	-4.149	0.000
Baseline QRS amplitude V4	0.697	0.595	0.798	13.915	0.000
QRS amplitude V5					
(Intercept)	4.215	2.353	6.077	4.499	0.000
Week 4	-0.143	-2.137	1.851	-0.142	0.888
Week 8	-2.179	-4.172	-0.185	-2.160	0.032
Week 12	-2.161	-4.315	-0.008	-1.984	0.049
Week 24	-5.407	-7.624	-3.190	-4.819	0.000
Week 36	-3.586	-5.839	-1.333	-3.145	0.002
Week 48	-3.405	-5.698	-1.111	-2.933	0.004
Week 60	-4.014	-6.352	-1.677	-3.392	0.001
Baseline QRS amplitude V5	0.779	0.713	0.844	24.431	0.000

QRS amplitude V6						
	(Intercept)	4.280	2.400	6.160	4.572	0.000
	Week 4	-0.964	-2.362	0.433	-1.364	0.175
	Week 8	-2.143	-3.541	-0.745	-3.030	0.003
	Week 12	-2.520	-4.048	-0.992	-3.260	0.001
	Week 24	-3.229	-4.807	-1.651	-4.043	0.000
	Week 36	-3.599	-5.205	-1.993	-4.428	0.000
	Week 48	-2.411	-4.048	-0.774	-2.910	0.004
	Week 60	-3.473	-5.144	-1.803	-4.108	0.000
	Baseline QRS amplitude V6	0.723	0.627	0.820	15.256	0.000
QRS amplitude I						
	(Intercept)	3.307	1.317	5.298	3.343	0.002
	Week 4	-0.000	-1.369	1.369	-0.000	1.000
	Week 8	-0.571	-1.940	0.798	-0.825	0.411
	Week 12	-0.031	-1.530	1.468	-0.041	0.967
	Week 24	-1.199	-2.747	0.350	-1.530	0.128
	Week 36	-1.409	-2.985	0.168	-1.766	0.079
	Week 48	-2.498	-4.105	-0.892	-3.073	0.003
	Week 60	-3.236	-4.876	-1.597	-3.901	0.000
	Baseline QRS amplitude I	0.743	0.616	0.869	11.998	0.000
QRS amplitude II						
	(Intercept)	3.897	1.812	5.983	3.802	0.001
	Week 4	-0.500	-1.653	0.653	-0.857	0.393
	Week 8	-0.893	-2.046	0.260	-1.531	0.128
	Week 12	-1.683	-2.948	-0.417	-2.628	0.010
	Week 24	-2.081	-3.389	-0.774	-3.146	0.002
	Week 36	-1.979	-3.311	-0.648	-2.938	0.004
	Week 48	-2.434	-3.791	-1.076	-3.543	0.001
	Week 60	-2.716	-4.101	-1.330	-3.874	0.000
	Baseline QRS amplitude II	0.693	0.551	0.834	10.094	0.000
QRS amplitude III						
	(Intercept)	2.205	0.003	4.407	2.027	0.050
	Week 4	-0.821	-2.117	0.474	-1.253	0.212
	Week 8	-0.893	-2.189	0.403	-1.362	0.175
	Week 12	-2.455	-3.879	-1.031	-3.408	0.001
	Week 24	-2.149	-3.620	-0.678	-2.887	0.004
	Week 36	-2.283	-3.781	-0.785	-3.012	0.003
	Week 48	-2.732	-4.260	-1.205	-3.536	0.001
	Week 60	-2.836	-4.395	-1.277	-3.596	0.000

	Baseline QRS amplitude III	0.816	0.665	0.967	11.067	0.000
QRS amplitude aVR						
	(Intercept)	4.195	2.542	5.848	5.161	0.000
	Week 4	-0.607	-1.561	0.347	-1.258	0.210
	Week 8	-1.321	-2.275	-0.367	-2.739	0.007
	Week 12	-1.350	-2.397	-0.303	-2.550	0.012
	Week 24	-2.088	-3.170	-1.007	-3.816	0.000
	Week 36	-1.879	-2.980	-0.778	-3.372	0.001
	Week 48	-2.198	-3.320	-1.075	-3.868	0.000
	Week 60	-2.400	-3.546	-1.254	-4.140	0.000
	Baseline QRS amplitude aVR	0.635	0.513	0.757	10.740	0.000
QRS amplitude aVL						
	(Intercept)	1.841	-0.606	4.287	1.526	0.136
	Week 4	-0.571	-1.832	0.689	-0.896	0.372
	Week 8	-1.250	-2.511	0.011	-1.960	0.052
	Week 12	-1.881	-3.268	-0.495	-2.682	0.008
	Week 24	-1.781	-3.214	-0.347	-2.455	0.015
	Week 36	-2.783	-4.243	-1.323	-3.768	0.000
	Week 48	-2.841	-4.330	-1.353	-3.773	0.000
	Week 60	-2.905	-4.424	-1.386	-3.779	0.000
	Baseline QRS amplitude aVL	0.830	0.641	1.020	8.959	0.000
QRS amplitude aVF						
	(Intercept)	2.915	1.425	4.405	3.937	0.000
	Week 4	-0.786	-1.922	0.350	-1.367	0.174
	Week 8	-1.107	-2.243	0.029	-1.926	0.056
	Week 12	-2.230	-3.475	-0.985	-3.540	0.001
	Week 24	-1.623	-2.909	-0.337	-2.495	0.014
	Week 36	-1.744	-3.053	-0.435	-2.632	0.009
	Week 48	-1.764	-3.098	-0.429	-2.612	0.010
	Week 60	-1.426	-2.788	-0.065	-2.070	0.040
	Baseline QRS amplitude aVF	0.715	0.604	0.825	13.293	0.000
T wave peak V1						
	(Intercept)	1.092	0.430	1.754	3.289	0.002
	Week 4	-0.714	-1.409	-0.019	-2.033	0.044
	Week 8	-0.750	-1.445	-0.055	-2.134	0.035
	Week 12	-0.973	-1.732	-0.215	-2.536	0.012

	Week 24	-1.257	-2.040	-0.474	-3.171	0.002
	Week 36	-1.212	-2.009	-0.414	-3.003	0.003
	Week 48	-1.502	-2.314	-0.689	-3.653	0.000
	Week 60	-1.545	-2.374	-0.716	-3.682	0.000
	Baseline T wave peak V1	0.613	0.484	0.742	9.842	0.000
T wave peak V2						
	(Intercept)	1.421	0.393	2.449	2.782	0.008
	Week 4	-0.768	-1.638	0.103	-1.744	0.083
	Week 8	-0.893	-1.763	-0.022	-2.028	0.044
	Week 12	-0.637	-1.585	0.312	-1.326	0.187
	Week 24	-1.184	-2.164	-0.205	-2.390	0.018
	Week 36	-0.657	-1.654	0.340	-1.303	0.195
	Week 48	-1.337	-2.353	-0.321	-2.601	0.010
	Week 60	-1.704	-2.741	-0.668	-3.249	0.001
	Baseline T wave peak V2	0.772	0.647	0.898	12.756	0.000
T wave peak V3						
	(Intercept)	1.144	-0.240	2.528	1.658	0.103
	Week 4	0.643	-0.541	1.827	1.074	0.285
	Week 8	0.411	-0.773	1.594	0.686	0.494
	Week 12	0.003	-1.294	1.299	0.004	0.997
	Week 24	-0.103	-1.442	1.237	-0.152	0.880
	Week 36	0.647	-0.717	2.011	0.938	0.350
	Week 48	0.854	-0.536	2.245	1.214	0.227
	Week 60	-0.013	-1.432	1.406	-0.018	0.986
	Baseline T wave peak V3	0.760	0.565	0.955	8.011	0.000
T wave peak V4						
	(Intercept)	0.938	-0.271	2.148	1.543	0.127
	Week 4	0.643	-0.615	1.901	1.010	0.314
	Week 8	0.750	-0.508	2.008	1.179	0.240
	Week 12	0.894	-0.485	2.273	1.281	0.202
	Week 24	1.610	0.186	3.035	2.234	0.027
	Week 36	2.132	0.681	3.583	2.904	0.004
	Week 48	2.433	0.955	3.912	3.252	0.001
	Week 60	2.368	0.858	3.877	3.100	0.002
	Baseline T wave peak V4	0.580	0.376	0.784	5.838	0.000
T wave peak V5						
	(Intercept)	0.178	-0.927	1.284	0.321	0.749
	Week 4	1.464	0.237	2.692	2.358	0.020
	Week 8	1.071	-0.156	2.299	1.726	0.087

	Week 12	2.043	0.697	3.389	3.000	0.003
	Week 24	2.622	1.231	4.013	3.725	0.000
	Week 36	3.388	1.971	4.805	4.726	0.000
	Week 48	2.857	1.412	4.301	3.908	0.000
	Week 60	3.183	1.709	4.658	4.267	0.000
	baseline_T_V5	0.412	0.173	0.652	3.536	0.002
T wave peak V6						
	(Intercept)	-0.213	-0.977	0.551	-0.554	0.581
	Week 4	1.161	0.288	2.033	2.629	0.009
	Week 8	1.036	0.163	1.908	2.346	0.020
	Week 12	1.942	0.986	2.899	4.014	0.000
	Week 24	2.178	1.190	3.167	4.355	0.000
	Week 36	2.414	1.408	3.421	4.740	0.000
	Week 48	3.154	2.128	4.181	6.073	0.000
	Week 60	2.920	1.872	3.968	5.507	0.000
	Baseline T wave peak V6	0.558	0.371	0.744	6.133	0.000
T wave peak I						
	(Intercept)	-0.059	-0.641	0.524	-0.200	0.842
	Week 4	0.732	0.037	1.427	2.083	0.039
	Week 8	0.821	0.126	1.516	2.337	0.021
	Week 12	1.167	0.407	1.928	3.034	0.003
	Week 24	1.582	0.797	2.368	3.982	0.000
	Week 36	1.422	0.623	2.222	3.515	0.001
	Week 48	1.764	0.949	2.579	4.278	0.000
	Week 60	2.032	1.201	2.864	4.830	0.000
	Baseline T wave peak I	0.589	0.442	0.736	8.260	0.000
T wave peak II						
	(Intercept)	0.615	0.007	1.223	2.011	0.047
	Week 4	0.589	-0.072	1.251	1.760	0.080
	Week 8	0.964	0.303	1.626	2.881	0.005
	Week 12	1.019	0.294	1.744	2.776	0.006
	Week 24	1.314	0.565	2.064	3.465	0.001
	Week 36	1.109	0.346	1.872	2.872	0.005
	Week 48	1.382	0.605	2.160	3.512	0.001
	Week 60	1.638	0.844	2.432	4.078	0.000
	Baseline T wave peak II	0.435	0.278	0.593	5.690	0.000
T wave peak III						
	(Intercept)	0.517	-0.081	1.115	1.729	0.089
	Week 4	-0.268	-0.819	0.284	-0.960	0.339
	Week 8	-0.018	-0.569	0.534	-0.064	0.949
	Week 12	-0.429	-1.035	0.177	-1.398	0.164

	Week 24	-0.537	-1.164	0.089	-1.695	0.092
	Week 36	-0.545	-1.183	0.093	-1.688	0.093
	Week 48	-0.457	-1.107	0.194	-1.388	0.167
	Week 60	-0.449	-1.113	0.215	-1.336	0.184
	Baseline T wave peak III	0.609	0.400	0.818	5.986	0.000
T wave peak aVR						
	(Intercept)	-0.444	-1.020	0.132	-1.527	0.130
	Week 4	-0.357	-1.032	0.318	-1.046	0.297
	Week 8	-0.464	-1.139	0.210	-1.360	0.176
	Week 12	-0.833	-1.571	-0.095	-2.232	0.027
	Week 24	-1.018	-1.780	-0.256	-2.639	0.009
	Week 36	-0.886	-1.662	-0.110	-2.257	0.025
	Week 48	-1.482	-2.273	-0.692	-3.704	0.000
	Week 60	-1.386	-2.193	-0.579	-3.394	0.001
	baseline_T_aVR	0.460	0.305	0.615	6.102	0.000
T wave peak aVL						
	(Intercept)	-0.232	-0.734	0.270	-0.918	0.361
	Week 4	0.339	-0.221	0.899	1.197	0.233
	Week 8	0.589	0.029	1.149	2.080	0.039
	Week 12	1.193	0.579	1.807	3.841	0.000
	Week 24	1.200	0.566	1.834	3.740	0.000
	Week 36	1.187	0.542	1.833	3.634	0.000
	Week 48	1.266	0.608	1.924	3.802	0.000
	Week 60	1.007	0.335	1.678	2.963	0.004
	Baseline T wave peak aVL	0.667	0.485	0.849	7.553	0.000
T wave peak aVF						
	(Intercept)	0.488	0.014	0.962	2.051	0.044
	Week 4	0.393	-0.093	0.879	1.598	0.112
	Week 8	0.518	0.032	1.004	2.106	0.037
	Week 12	0.225	-0.308	0.758	0.833	0.406
	Week 24	0.352	-0.199	0.903	1.263	0.209
	Week 36	0.679	0.118	1.240	2.393	0.018
	Week 48	0.700	0.128	1.272	2.418	0.017
	Week 60	0.854	0.270	1.438	2.892	0.004
	Baseline T wave peak aVF	0.537	0.364	0.709	6.385	0.000

Table 5. Mean Changes Over Time of Echocardiographic and Electrocardiographic Parameters Through Mixed-Effects Model Analysis considering as fixed effects evaluation time and the baseline parameter and as random effects the single individual. CI: confidence interval.

Correlation analysis of echocardiography and electrocardiography parameters

Cubic spline graphs demonstrated significant correlations between changes in QRS amplitude in leads V1 and V2 and changes in maximum LV thickness, septal e', both rest and Valsalva LVOT gradients (Table 3, Figure 12). In multivariate models that included changes in maximal LV wall thickness, septal e' or change in rest LVOT gradient, only change in maximal LV wall thickness was significantly correlated with changes in QRS amplitude in V1 and V2 (Table 4). Change in QRS amplitude in aVL was correlated with change in PW thickness. Additionally, changes in T wave peaks in V2 and V5 were associated with changes in lateral e' with a trend for V6.

Response	F statistic	P value
maximum IVS		
IVS	29.139	0.000
PW	0.663	0.589
septal e'	8.011	0.003
lateral e'	0.585	0.635
resting LVOT gradient	2.012	0.162
Valsalva LVOT gradient	1.625	0.232
QRS amplitude V1	11.353	0.001
T wave peak V1	4.808	0.020
QRS amplitude V2	5.676	0.012
T wave peak V2	0.533	0.668
QRS amplitude V3	2.667	0.095
T wave peak V3	0.865	0.486
QRS amplitude V4	0.681	0.581
T wave peak V4	2.387	0.120
QRS amplitude V5	2.185	0.143
T wave peak V5	0.716	0.561
QRS amplitude V6	1.896	0.184
T wave peak V6	1.226	0.343
QRS amplitude I	0.324	0.808
T wave peak I	5.568	0.013
QRS amplitude II	0.399	0.756
T wave peak II	5.131	0.016
QRS amplitude III	0.451	0.721
T wave peak III	1.655	0.229
QRS amplitude aVR	0.871	0.483
T wave peak aVR	2.232	0.137
QRS amplitude aVL	2.150	0.147
T wave peak aVL	1.568	0.248
QRS amplitude aVF	0.249	0.861
T wave peak aVF	1.701	0.220
Interventricular septal thickness		
delta_PW	0.457	0.717
septal e'	1.892	0.181
lateral e'	0.210	0.888
resting LVOT gradient	0.287	0.834
Valsalva LVOT gradient	0.146	0.930

QRS amplitude V1	2.006	0.167
T wave peak V1	2.510	0.108
QRS amplitude V2	0.237	0.869
T wave peak V2	0.585	0.637
QRS amplitude V3	0.858	0.489
T wave peak V3	1.483	0.269
QRS amplitude V4	0.869	0.484
T wave peak V4	1.267	0.330
QRS amplitude V5	1.330	0.311
T wave peak V5	1.804	0.200
QRS amplitude V6	0.047	0.986
T wave peak V6	2.253	0.135
QRS amplitude I	1.795	0.202
T wave peak I	0.361	0.782
QRS amplitude II	0.559	0.652
T wave peak II	0.683	0.579
QRS amplitude III	0.900	0.470
T wave peak III	4.973	0.018
QRS amplitude aVR	1.087	0.392
T wave peak aVR	0.169	0.915
QRS amplitude aVL	2.206	0.140
T wave peak aVL	1.021	0.418
QRS amplitude aVF	1.226	0.343
T wave peak aVF	1.266	0.330
Posterior wall		
septal e'	3.491	0.047
lateral e'	1.312	0.312
resting LVOT gradient	2.005	0.163
Valsalva LVOT gradient	0.770	0.531
QRS amplitude V1	0.450	0.722
T wave peak V1	0.845	0.495
QRS amplitude V2	0.738	0.549
T wave peak V2	0.762	0.537
QRS amplitude V3	1.926	0.179
T wave peak V3	0.041	0.989
QRS amplitude V4	0.213	0.886
T wave peak V4	1.349	0.305
QRS amplitude V5	0.656	0.594
T wave peak V5	1.615	0.238
QRS amplitude V6	0.753	0.542
T wave peak V6	1.205	0.350
QRS amplitude I	1.207	0.349
T wave peak I	0.848	0.494
QRS amplitude II	0.258	0.854
T wave peak II	0.054	0.983
QRS amplitude III	1.322	0.313
T wave peak III	0.699	0.571

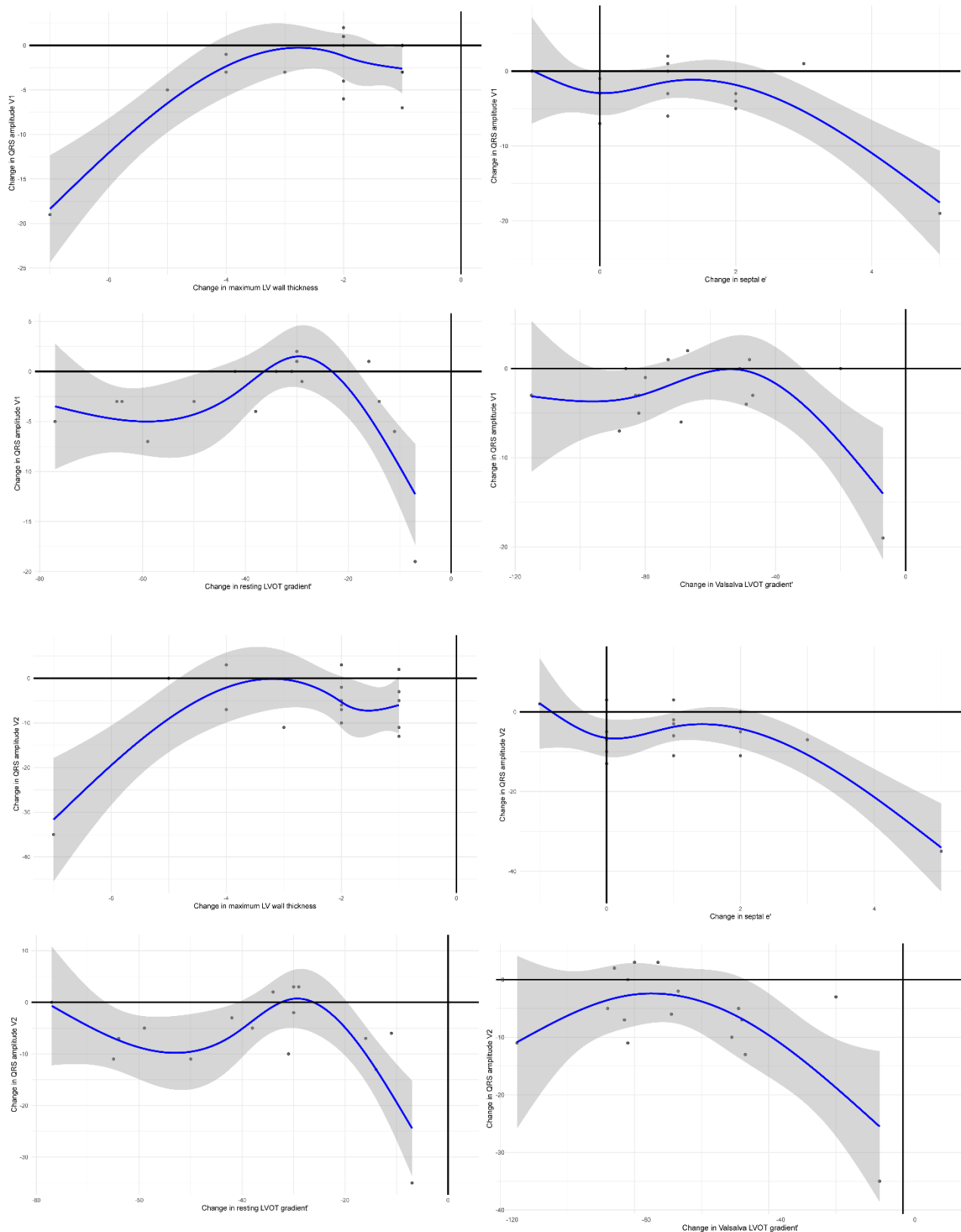
QRS amplitude aVR	1.517	0.260
T wave peak aVR	0.694	0.573
QRS amplitude aVL	4.422	0.026
T wave peak aVL	0.355	0.786
QRS amplitude aVF	0.055	0.982
T wave peak aVF	0.588	0.634
septal e'		
lateral e'	1.215	0.344
resting LVOT gradient	1.405	0.286
Valsalva LVOT gradient	1.842	0.189
QRS amplitude V1	7.459	0.004
T wave peak V1	0.349	0.790
QRS amplitude V2	10.859	0.001
T wave peak V2	1.355	0.303
QRS amplitude V3	5.213	0.016
T wave peak V3	0.366	0.779
QRS amplitude V4	1.237	0.339
T wave peak V4	0.058	0.981
QRS amplitude V5	1.539	0.255
T wave peak V5	0.273	0.844
QRS amplitude V6	2.166	0.145
T wave peak V6	0.602	0.626
QRS amplitude I	0.062	0.979
T wave peak I	1.038	0.411
QRS amplitude II	0.117	0.949
T wave peak II	1.691	0.222
QRS amplitude III	0.683	0.579
T wave peak III	0.496	0.692
QRS amplitude aVR	0.269	0.846
T wave peak aVR	0.556	0.654
QRS amplitude aVL	0.541	0.663
T wave peak aVL	0.661	0.591
QRS amplitude aVF	0.015	0.997
T wave peak aVF	0.558	0.653
lateral e'		
resting LVOT gradient	0.224	0.878
Valsalva LVOT gradient	0.030	0.993
QRS amplitude V1	2.121	0.151
T wave peak V1	0.329	0.804
QRS amplitude V2	0.580	0.639
T wave peak V2	10.171	0.001
QRS amplitude V3	0.771	0.532
T wave peak V3	0.613	0.620
QRS amplitude V4	0.313	0.815
T wave peak V4	1.750	0.210
QRS amplitude V5	0.681	0.581
T wave peak V5	3.731	0.042

QRS amplitude V6	0.822	0.506
T wave peak V6	3.239	0.060
QRS amplitude I	1.180	0.358
T wave peak I	0.378	0.771
QRS amplitude II	0.831	0.502
T wave peak II	0.374	0.773
QRS amplitude III	2.228	0.137
T wave peak III	0.264	0.850
QRS amplitude aVR	3.125	0.066
T wave peak aVR	1.081	0.394
QRS amplitude aVL	2.305	0.129
T wave peak aVL	0.361	0.782
QRS amplitude aVF	0.649	0.599
T wave peak aVF	2.584	0.102
resting LVOT gradient		
Valsalva LVOT gradient	7.091	0.005
QRS amplitude V1	7.349	0.005
T wave peak V1	1.326	0.312
QRS amplitude V2	7.172	0.005
T wave peak V2	2.011	0.166
QRS amplitude V3	1.405	0.289
T wave peak V3	1.765	0.207
QRS amplitude V4	1.089	0.391
T wave peak V4	0.689	0.576
QRS amplitude V5	0.643	0.602
T wave peak V5	0.032	0.992
QRS amplitude V6	10.061	0.001
T wave peak V6	0.219	0.881
QRS amplitude I	0.374	0.773
T wave peak I	1.390	0.293
QRS amplitude II	3.579	0.047
T wave peak II	0.964	0.441
QRS amplitude III	0.748	0.544
T wave peak III	0.501	0.689
QRS amplitude aVR	1.257	0.333
T wave peak aVR	0.379	0.770
QRS amplitude aVL	0.086	0.967
T wave peak aVL	0.733	0.552
QRS amplitude aVF	1.165	0.364
T wave peak aVF	0.077	0.971
Valsalva LVOT gradient		
QRS amplitude V1	3.994	0.035
T wave peak V1	1.307	0.317
QRS amplitude V2	4.443	0.026
T wave peak V2	0.571	0.645
QRS amplitude V3	3.748	0.041
T wave peak V3	0.757	0.539

QRS amplitude V4	1.947	0.176
T wave peak V4	0.435	0.732
QRS amplitude V5	2.381	0.121
T wave peak V5	0.171	0.914
QRS amplitude V6	0.761	0.537
T wave peak V6	0.212	0.886
QRS amplitude I	0.138	0.936
T wave peak I	0.821	0.507
QRS amplitude II	0.477	0.704
T wave peak II	1.548	0.253
QRS amplitude III	0.598	0.628
T wave peak III	1.632	0.234
QRS amplitude aVR	0.748	0.544
T wave peak aVR	1.554	0.251
QRS amplitude aVL	0.495	0.692
T wave peak aVL	0.134	0.938
QRS amplitude aVF	0.313	0.815
T wave peak aVF	0.806	0.514
Ejection fraction		
delta_max	3.412	0.050
IVS	3.703	0.040
delta_PW	0.534	0.667
septal e'	1.586	0.240
lateral e'	0.826	0.503
resting LVOT gradient	0.588	0.633
Valsalva LVOT gradient	2.495	0.106
QRS amplitude V1	0.823	0.506
T wave peak V1	2.820	0.084
QRS amplitude V2	0.282	0.837
T wave peak V2	0.750	0.543
QRS amplitude V3	2.589	0.101
T wave peak V3	0.021	0.996
QRS amplitude V4	0.138	0.936
T wave peak V4	0.536	0.666
QRS amplitude V5	0.498	0.690
T wave peak V5	0.420	0.742
QRS amplitude V6	1.160	0.365
T wave peak V6	1.114	0.382
QRS amplitude I	2.402	0.119
T wave peak I	0.846	0.495
QRS amplitude II	0.440	0.728
T wave peak II	0.078	0.971
QRS amplitude III	1.286	0.324
T wave peak III	1.037	0.411
QRS amplitude aVR	1.942	0.177
T wave peak aVR	0.722	0.558
QRS amplitude aVL	2.043	0.162

T wave peak aVL	0.369	0.777
QRS amplitude aVF	0.937	0.453
T wave peak aVF	0.263	0.851

Table 6. Table of F-statistics and p-values for cubic spline models evaluating the relationship between the change from baseline to week 36 of electrocardiography and echocardiography parameter. The cubic spline models were fitted with 3 degrees of freedom, and the F-statistic indicates the overall significance of the relationship between the predictor and response variables.



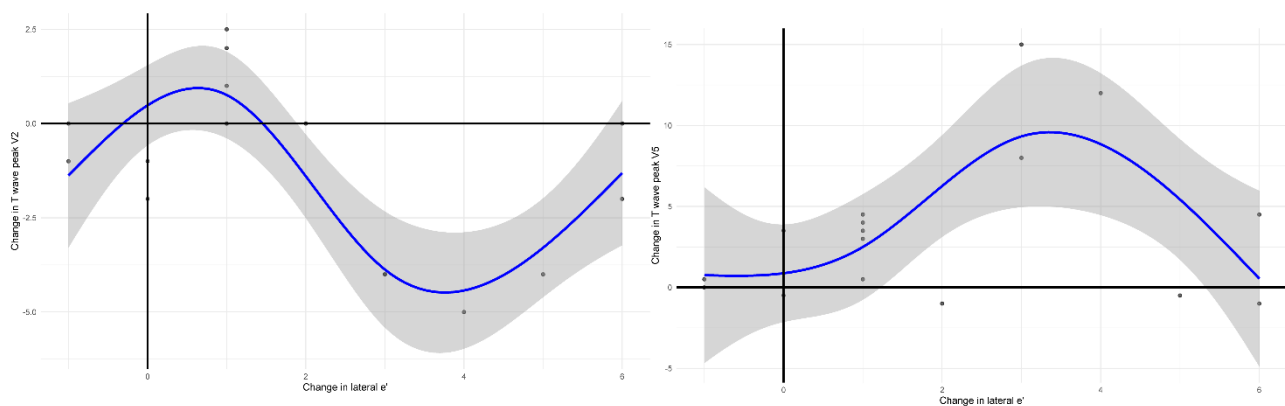


Figure 12. Scatter plot depicting the relationship between changes in electrocardiography and echocardiography parameters with a cubic spline fit ($df = 3$). The blue curve represents the cubic spline regression model with confidence intervals, while the black lines indicate the reference axes at zero for both variables.

term	estimate	edf	P value
Model 1: Δ QRS V1~ Δ max LV wall thickness + Δ LVOT gradient			
Intercept	-3.125		0.001
maximum LV wall thickness		1.920	0.000
resting LVOT gradient		1.148	0.907
Model 2: Δ QRS V1~ Δ max LV wall thickness + Δ sep e'			
Intercept	-3.125		0.001
maximum LV wall thickness		1.920	0.004
septal e'		1.000	0.682
Model 3: Δ QRS V2~ Δ max LV wall thickness + Δ LVOT gradient			
Intercept	-6.688		0.001
maximum LV wall thickness		1.885	0.009
resting LVOT gradient		1.000	0.356
Model 4: Δ QRS V2~ Δ max LV wall thickness + Δ sep e'			
Intercept	-6.688		0.001
maximum LV wall thickness		1.376	0.668
septal e'		1.627	0.062

Table 7. Summary of generalized additive models (GAMs) evaluating the relationship between changes in QRS amplitudes in lead V1 (Δ QRS V1) and lead V2 (Δ QRS V2) with predictors: changes in maximum LV wall thickness (Δ max LV wall thickness), changes in LVOT gradient (Δ LVOT), and changes in septal e' (Δ e sep). Spline smoothing functions with 3 degrees of freedom ($k = 3$) were applied to model the non-linear relationships. LV: left ventricular. Max: maximum. Sep: septal. LVOT: left ventricular outflow tract. Edf: Effective Degrees of Freedom.

Adverse events and response to therapy

One patient on Aficamten and 2 patients on Mavacamten developed hypertension after 2, 35, and 3 months of therapy, respectively. Hypertension was well controlled after up-titration of antihypertensive medications.

One patient on Aficamten and 2 patients on mavacamten developed an LVEF drop to below 50% after 2, 13, and 1 month of therapy, respectively. The patient on aficamten discontinued treatment due to the simultaneous occurrence of atrial fibrillation and heart failure. The patients on mavacamten temporarily interrupted the treatment with complete recovery of LV function and then restarted therapy at a lower dose. In univariate logistic regression analysis patients with obstructive HCM had a lower risk of developing LV dysfunction (OR 0.003, 95% CI 0.000-0.065, $p < 0.01$) (Table 5).

At week 36, 3 patients (total number of patients with obstructive HCM and week 36 follow-up $n=17$, 17%) didn't show a reduction of LVOT gradient after Valsalva maneuver to below 50mmHg, thus not achieving a hemodynamic response. Univariate predictors of non-hemodynamic response included increased length of the anterior mitral leaflet (OR 2.0, 95% CI 1.01-44, $p=0.03$) and a reduced ejection fraction (OR 0.01, 95% CI 0.01;0.47, $p=0.01$) (Table 6).

At week 36, one patient (total number of patients with week 36 follow-up $n=19$, 5%) didn't show a reduction in NYHA class ≥ 1 , despite achieving a hemodynamic response. By week 36, all four patients who had reported angina symptoms were completely asymptomatic. Moreover, at week 36, 7 patients ($n=19$, 36%) showed a reduction of left atrial volume of at least 25%, no predictors of atrial response were found in univariate analysis.

Predictor	OR	CI Lower	CI Upper	p value
obstructive HCM	0.003	0.000	0.065	0.000
Age	0.944	0.869	1.023	0.146
Male Sex	0.812	0.094	9.789	0.852
Mavacamten vs Aficamten	1.095	0.128	13.114	0.934
Maximum septal wall thickness	1.095	0.843	1.359	0.433
Ejection fraction	0.494	0.000	7.546	0.935
septal e'	0.780	0.169	2.009	0.658
lateral e'	1.011	0.475	1.777	0.972

Table 8. Logistic regression results assessing the association between left ventricular (LV) dysfunction and various predictors, including patient demographics (age, sex), baseline LV function parameters (maximum LV wall thickness, e' septal, e' lateral velocities, and ejection fraction), and treatment with myosin inhibitors. The table displays odds ratios (OR) with 95% confidence intervals (CI) and associated p-values for each predictor.

Predictor	OR	CI Lower	CI Upper	P value
Age	0.963	0.863	1.104	0.505
Sex	2.391	0.156	352.181	0.567
Mavacamten vs Aficamten	0.253	0.002	3.771	0.347
Anterior mitral leaflet length	2.014	1.021	44.465	0.037
Maximum left ventricular wall thickness	0.985	0.495	1.317	0.930
Ejection fraction	0.018	0.000	0.470	0.011
septal e'	0.661	0.050	3.104	0.648
lateral e'	1.032	0.403	1.900	0.927

Table 9. Logistic regression results evaluating predictors of hemodynamic non-response to treatment defined as Valsalva LVOT gradient at week 36 $\geq$ 50 mmHg. The table shows odds ratios (OR), 95% confidence intervals (CI), and p-values for each predictor.

Limitations

The study's sample size is limited due to the recent introduction of this class of drugs into clinical practice. We don't have information on biomarkers, functional capacity, and cardiac magnetic resonance modifications. These data are hypothesis-generating and should be confirmed in large registry studies.

Discussion

In this study, treatment with myosin inhibitors demonstrated significant improvements across structural, functional, and electrical parameters in patients with HCM. As the first targeted therapy for sarcomeric HCM, myosin inhibitors reduce hypercontractility by decreasing myosin's ATPase activity, normalizing the number of actin-myosin cross-bridges. This leads to substantial clinical benefits, positioning myosin inhibitors as a promising therapeutic option for HCM management.

Myocardial structural remodeling associated with myosin inhibitor therapy

Myosin inhibitors are associated with significant reduction in LVOT gradients, leading to improved functional capacity and symptom relief in patients with obstructive HCM[119,125]. However, their effect expands further, leading to a pharmacologically induced LV remodeling which in this cohort occurred early and was significant as soon as the 8th week of treatment.

Several mechanisms could contribute to this structural effect. LVOT obstruction causes an afterload increase that could trigger secondary LV hypertrophy. Probably by the same mechanism, SRT is associated with a regression of LV hypertrophy in sites other than the septum.[143,144]

However, myosin inhibitors could also have a direct effect on myocytes. In HCM mice expressing α -cardiac myosin heavy chain missense variants R403Q, R719W, or R453C without LVOT obstruction, decreasing sarcomere power was associated with a reduction in LV wall thickness in overt disease and it prevented the hypertrophic phenotype appearance in the pre-clinic phase. Notably, these effects were associated with hypertrophic gene programs suppression.[117]

These drugs could also improve the energetic balance. Hypercontractility in HCM leads to energetic decompensation and mitochondrial dysfunction, which are implicated in LV hypertrophy. By normalizing the energetic demands of the sarcomere, myosin inhibitors may help to mitigate the hypertrophic response.

Myosin inhibitors improved diastolic function as shown by the reduction in LA size and the increment of e' velocities. Diastolic dysfunction is driven by increased calcium sensitivity, hypercontractility, microvascular dysfunction, and LVOT obstruction. Thus, again, myosin inhibitors may act through LVOT obstruction relief, but also by a direct mechanism. Indeed, in cellular models, mavacamten could partially rescue the increased calcium sensitivity associated with thin filament variants.[145] The diastolic function amelioration comes at the price of a slight reduction in LVEF, which could be interpreted as the normalization of the hyperdynamic contractility typical of HCM.

Electrical normalization associated with myosin inhibitors therapy

ECG abnormalities are present in 90% of patients with HCM and include signs of left ventricular hypertrophy, pathological Q waves, ST segment depression or elevation, and T wave inversion.

In this analysis, we showed that myosin inhibitors are associated with the normalization of ECG abnormalities, through a quantitative evaluation of the modification of QRS amplitude and the T wave peak over time.

Specifically, we observed a reduction in the QRS amplitude across all ECG leads with a notable decrease of 8mV (95% CI -11 to -5) in V2. QRS amplitude is correlated with myocardial hypertrophy, indeed an increased QRS amplitude is common in HCM phenocopies associated with severe LV hypertrophy.[146] In this study, the echocardiographic LV thickness reduction is mirrored by a decrease in QRS voltage. This phenomenon is similar to the therapeutic response of aortic stenosis when aortic valve replacement is associated with LV remodeling and reduction in QRS amplitude.[147,148] Furthermore, a similar electrical response to therapy is observed with enzyme replacement therapy for Pompe disease, where restoring acid α -glucosidase activity leads to regression of left ventricular hypertrophy on both echocardiography and ECG.[149]

Moreover, we observed a T-wave normalization in the inferior-lateral leads. A simulation study using multiscale whole-organ computer models based on cardiac magnetic resonance images showed that longer action potential duration due to HCM ionic remodeling led to T wave inversion in leads V4-V6. [150]. Therefore, the appearance of positive T waves mediated by myosin inhibitors could be due to the rescue of calcium handling abnormalities and normalization of ionic remodeling. This mechanism could justify the improvement in diastolic function and the correlation between changes in T wave peak and lateral e' .

The ECG improvements contrast with the conduction disturbances typically associated with SRT, which may lead to pacemaker implantation.

Adverse events

Ten percent of patients developed LV dysfunction, that was completely reversed by treatment interruption. Patients with non-obstructive HCM, presented a higher risk of developing LV dysfunction, probably because hypercontractility is usually less common compared to patients with obstructive HCM. In the obstructive cohort of the EXPLORER-HCM trial the mean (SD) LVEF was 74 (6)[119] while in the non-obstructive cohort of REDWOOD-HCM was 70 (6.1) (t-test, $p < 0.01$)[127]. Thus, the latter category of patients may be more susceptible to LV dysfunction since their EF starting point is lower. Furthermore, the reduced EF compared to the obstructive phenotype could be related to a more advanced phenotype of the disease, in which fibrotic replacement is already present, leaving less vital myocardium and having a higher risk of developing LV dysfunction.

Seventeen percent of patients with obstructive HCM didn't show a hemodynamic response, defined as a Valsalva LVOT gradient < 50 mmHg after 36 weeks of therapy. Predictors of non-response included an increased length of the anterior mitral leaflet (AML) and a reduced ejection fraction. LVOT obstruction is a multifactorial phenomenon, and besides hypercontractility, an excessive length of the anterior mitral leaflet,

hypertrophic and anteriorized papillary muscles, elongated and slack chordae, trigons fibrosis, and a small LVOT diameter all contribute to the development of the systolic anterior motion of the mitral valve. These anatomical factors, such as an elongated AML and associated mitral valve abnormalities, contribute to LVOT obstruction independently of hypercontractility, limiting the efficacy of myosin inhibitors in such cases. Similarly, if LVEF is not hypercontractile, myosin inhibition could be less effective in reducing the gradient.

Most patients experienced an improvement in symptoms, with 96% reporting an increase of at least 1 NYHA class. Angina was completely resolved in all affected patients. Angina in the absence of obstructive coronary artery disease is frequent in HCM and is associated with structural and functional abnormalities of the microcirculation. The functional abnormalities are related to small vessel compression due to hypercontractility and LVOT obstruction, both targeted by myosin inhibitors. Therefore, myosin inhibitors could be considered an option also for patients with angina symptoms.

Conclusions

Myosin inhibitors demonstrated a comprehensive therapeutic benefit in HCM, including reductions in LVOT gradients, LV hypertrophy, diastolic function amelioration, and normalization of ECG abnormalities. The structural improvements seen with echocardiography were mirrored by electrical changes on ECG, reinforcing the potential for ECG monitoring to assess treatment response. These findings underscore the value of myosin inhibitors as a disease-modifying therapy in both obstructive and non-obstructive HCM, offering promise for improving patient outcomes. Further studies are warranted to explore long-term effects and potential predictors of treatment response.

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Supplementary material

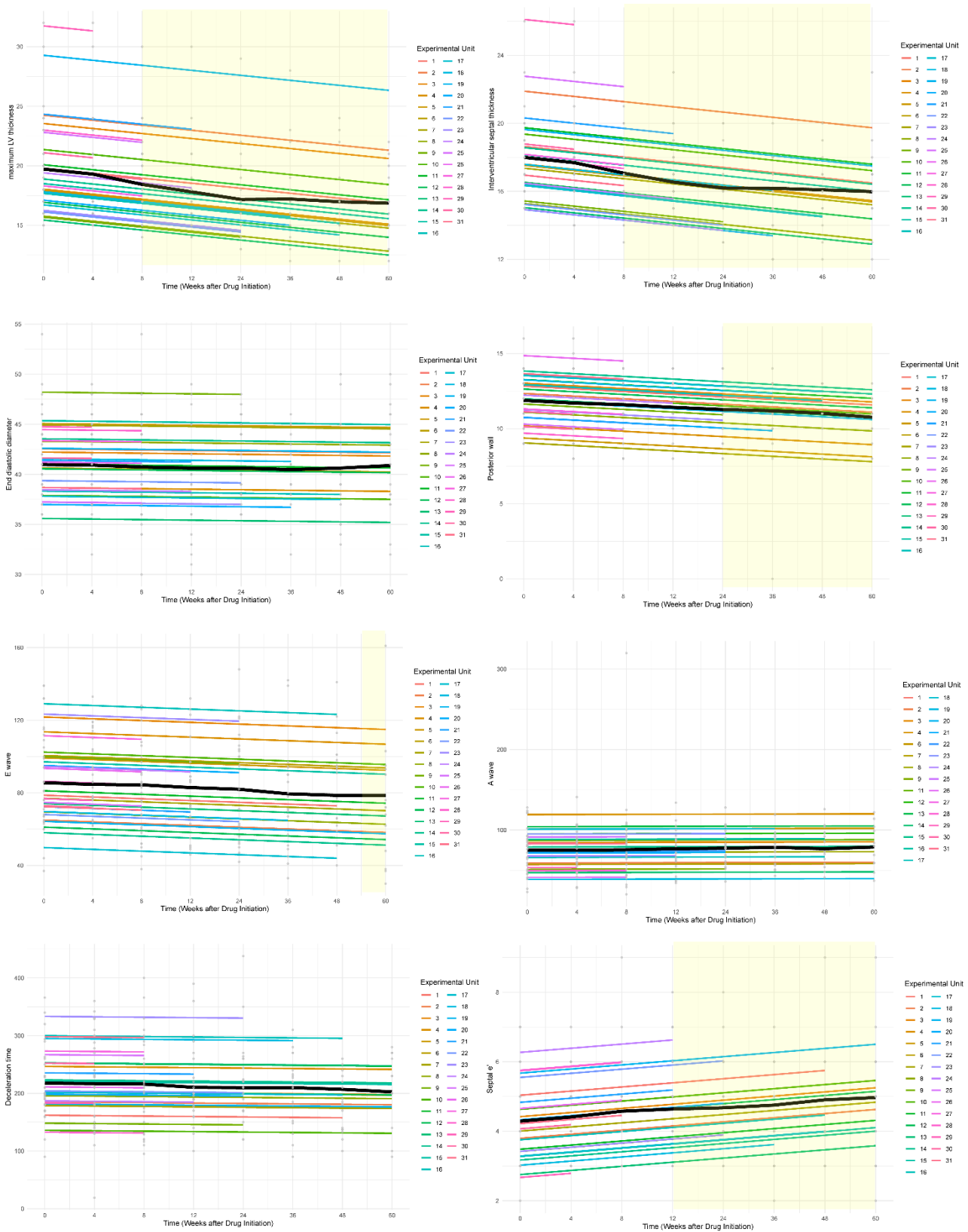


Figure 13. Model fit over time for echocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.

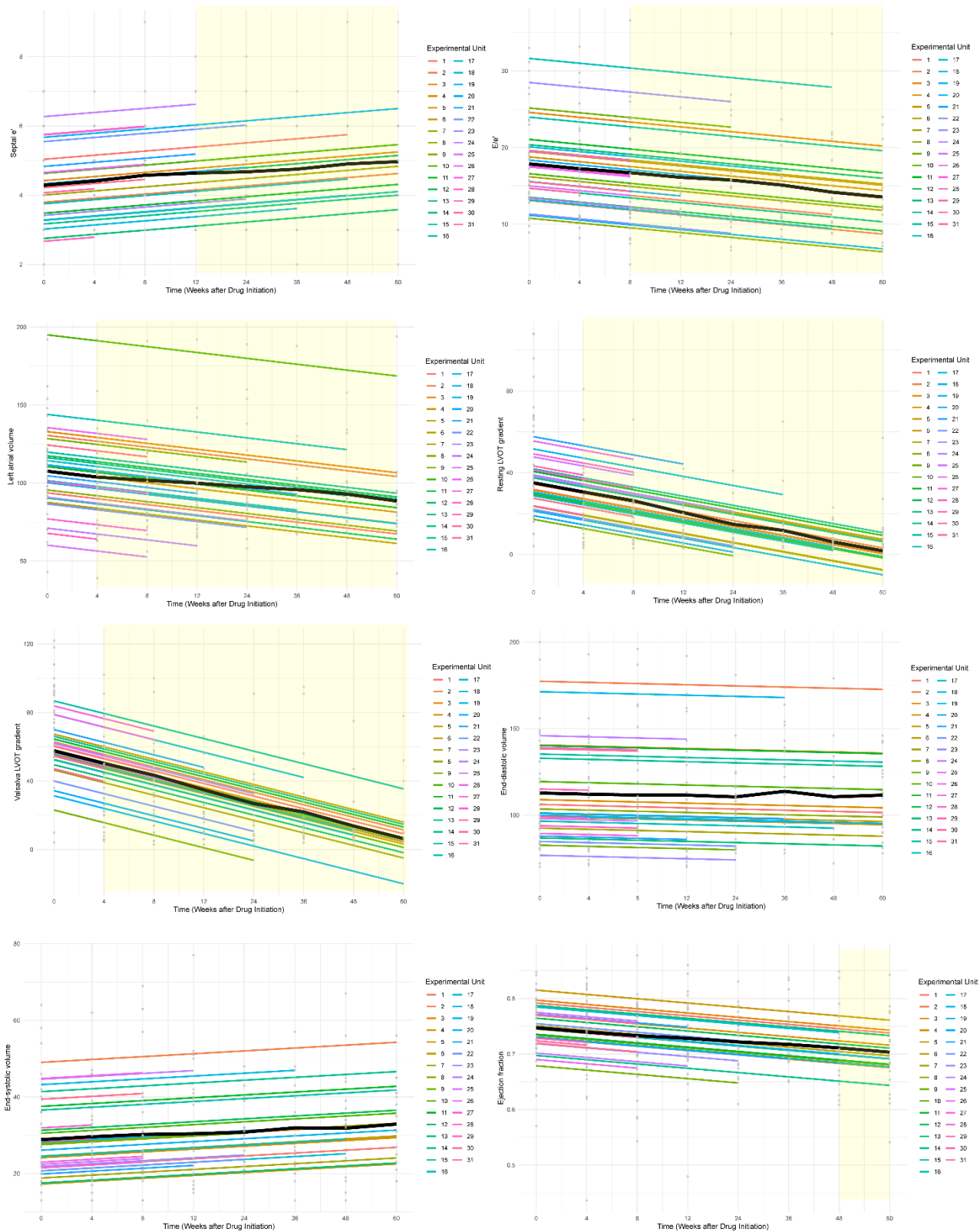


Figure 14. Model fit over time for echocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.

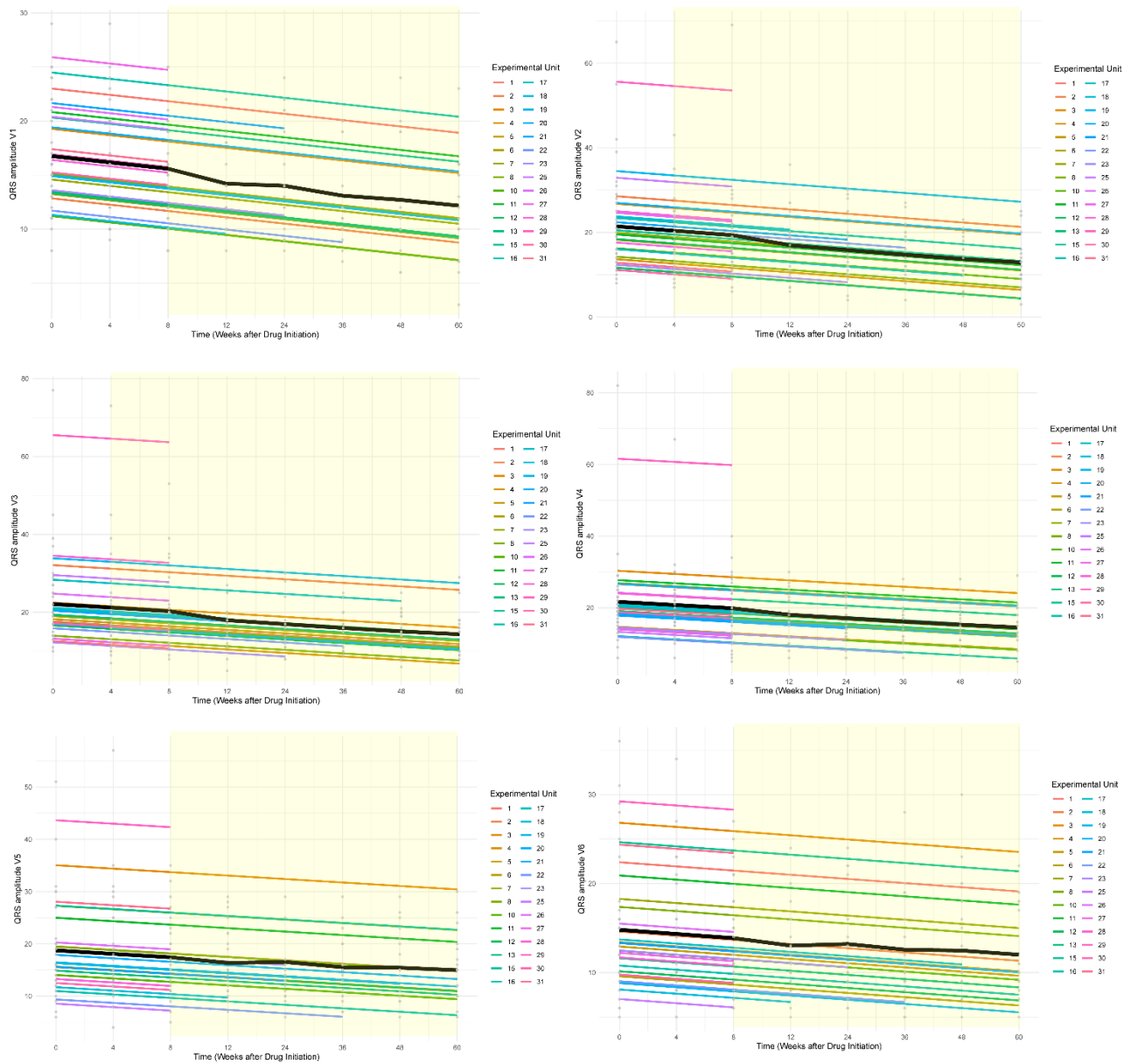


Figure 15. Model fit over time for electrocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.

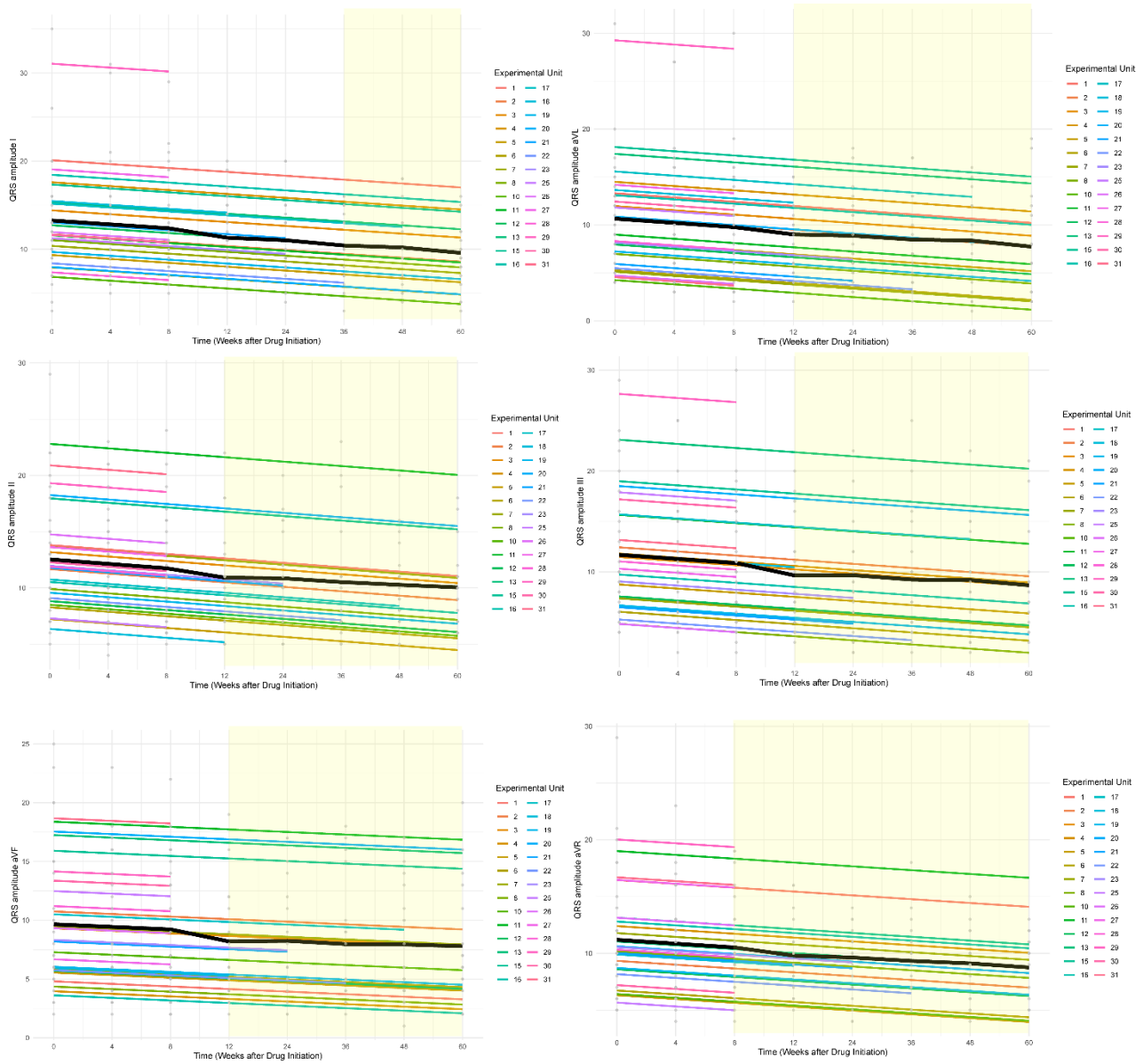


Figure 16. Model fit over time for electrocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.

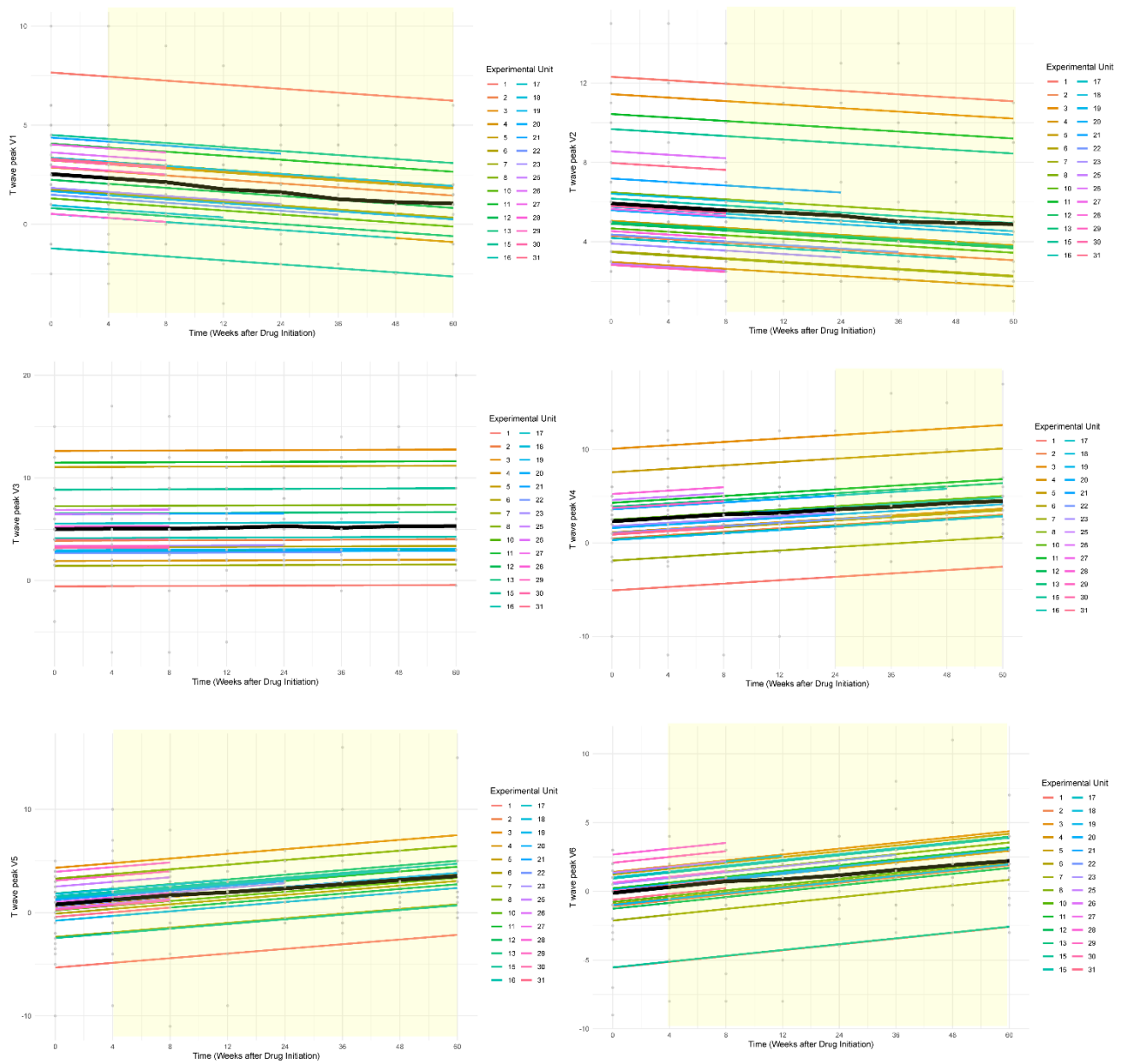


Figure 17. Model fit over time for electrocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.

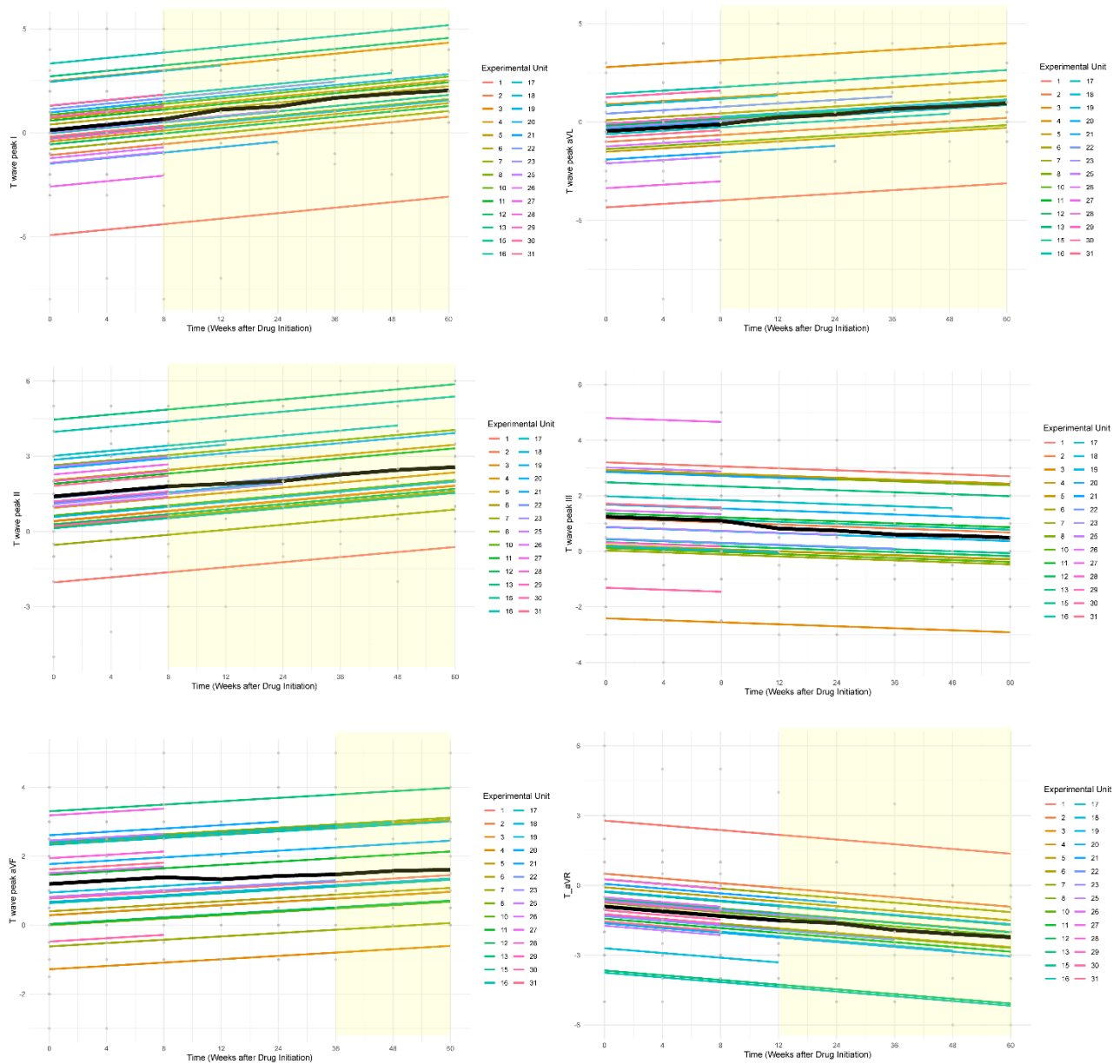


Figure 18. Model fit over time for electrocardiography parameters. The colored lines represent individual conditional fits, showing how each subject's predicted values change over time based on both fixed (evaluation week and baseline parameter) and random effects (single individual). The bold black line represents the marginal fit, which depicts the population-averaged effect of time on the dependent variable, derived from the fixed effects of the mixed-effects model. Residuals are calculated as the difference between observed values and marginal predictions.