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Cardiomyocyte Ca^{2+} homeostasis as a therapeutic target in heart failure with reduced and preserved ejection fraction

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Abstract

Heart failure is a highly prevalent syndrome of multiple etiologies and associated comorbidities, and aberrant intracellular Ca^{2+} homeostasis is a hallmark finding in heart failure patients. The cyclical changes in Ca^{2+} concentration within cardiomyocytes control cycles of cardiac contraction and relaxation, and dysregulation of Ca^{2+} handling processes leads to systolic dysfunction, diastolic dysfunction, and adverse remodeling. For this reason, greater understanding of Ca^{2+} handling mechanisms in heart failure is critical for selection of appropriate treatment strategies. In this review, we summarize the mechanisms of altered Ca^{2+} handling in two subsets of heart failure, heart failure with reduced ejection fraction and heart failure with preserved ejection fraction, and outline current and experimental treatments that target cardiomyocyte Ca^{2+} handling processes.

Introduction

Heart Failure (HF) remains an urgent public health crisis. By the year 2030 an estimated 8 million people in the US will be diagnosed with HF with associated yearly medical costs > \$50 billion [1]. The diagnosis of HF is primarily determined by ejection fraction and clinical presentation, with the cardinal manifestations of dyspnea, fatigue, exercise intolerance, and fluid retention. Heart failure is subclassified into two primary forms, the first HF with reduced ejection fraction (HFrEF, with ejection fraction < 50%) and the second HF with preserved ejection fraction (HFpEF) where ejection fraction is maintained. Regardless of HF subclass, the 5-year survival rate for all HF patients is less than 50%. The reader is referred to the following reviews for a comprehensive description of the clinical manifestations of HF syndromes [2–4]. The focus of this review is to examine the processes underlying aberrant cardiomyocyte Ca^{2+} homeostasis in HFrEF and HFpEF, with discussion of current and emerging treatment paradigms for this complex disease.

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Cardiomyocyte Ca^{2+} handling mechanisms

The process whereby the cardiomyocyte transduces electric signals of the action potential into mechanical force production is well-established, and is referred to as excitation-contraction coupling (ECC) [5]. In the healthy heart, ECC occurs via an exquisitely orchestrated series of events (Figure 1) initiated by opening of voltage-gated Na^+ ($\text{Na}_V1.5$) and Ca^{2+} ($\text{Ca}_V1.2$) channels with resulting cation influx and depolarization. $\text{Ca}_V1.2$ channels within the t-tubule network localize near Ryanodine Receptors (RyR_2) of the Sarcoplasmic Reticulum (SR), forming a specialized microdomain termed the dyadic cleft. Within this signaling microdomain Ca^{2+} influx via $\text{Ca}_V1.2$ opens RyR_2 , resulting in massive release of Ca^{2+} from the SR Ca^{2+} store. In sum, the Ca^{2+} -induced Ca^{2+} release (CICR) process results in a cytosolic Ca^{2+} elevation sufficient to initiate myofilament force production, cardiomyocyte contraction, and cardiac systole. Cardiomyocyte relaxation ensues when cytosolic Ca^{2+} is removed, which occurs predominantly by two mechanisms: 1) Ca^{2+} sequestration into the SR compartment via the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA, contributing 60–90% of Ca^{2+} removal), and 2) via the electrogenic $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) which exchanges 1 Ca^{2+} ion for 3 extracellular Na^+ ions. Under normal conditions, the amount of Ca^{2+} that enters the cardiomyocyte via $\text{Ca}_V1.2$ is extruded by NCX, and the amount released via RyR_2 is resequenced by SERCA, which allows both the cardiomyocyte and the SR to remain in “ Ca^{2+} flux balance”. Cellular processes that shift Ca^{2+} fluxes toward cardiomyocyte and SR Ca^{2+} accumulation enhance ECC, exemplified by cardiomyocyte β -adrenergic signaling following sympathetic stimulation. Binding of catecholamines to β_1 -adrenoreceptors induces $\text{G}\alpha_s$ -receptor-mediated production of cAMP and subsequent Protein Kinase A (PKA) phosphorylation of multiple Ca^{2+} handling proteins, most notably $\text{Ca}_V1.2$ and the SERCA inhibitory protein phospholamban (PLN). Phosphorylation of $\text{Ca}_V1.2$ leads to enhanced Ca^{2+} influx (favoring cardiomyocyte Ca^{2+} accumulation), while phosphorylation of PLN at the Serine16 PKA site leads to its dissociation from SERCA, removal of SERCA inhibition, and enhanced SERCA pumping ability (favoring SR Ca^{2+} accumulation). In addition, elevated Ca^{2+} during each contractile cycle activates Ca^{2+} /Calmodulin-dependent Protein Kinase II (CaMKII), which phosphorylates PLN at a distinct site (Thr17) and further dis-inhibits SERCA. A large body of literature points to enhanced RyR_2 activity following β_1 -adrenergic stimulation, although the mechanisms remain controversial [6,7]. Prominent possibilities include phosphorylation via PKA (Ser2808 or Ser2030), phosphorylation via CaMKII (Ser2814), oxidative/nitrosative modification, or complex combinations of these respective processes. During β_1 -receptor activation the increased trigger for CICR, enhanced activity of the RyR_2 release mechanism, and additional SR Ca^{2+} content collectively contribute to a considerable increase in the amplitude of the action potential-induced cytosolic Ca^{2+} transient. Indeed, the increase in peak systolic Ca^{2+} is more than sufficient to overcome the PKA-induced decrease in myofilament Ca^{2+} sensitivity that accompanies β_1 -receptor activation, and leads to enhanced contractile strength. Advantageously, the combination of decreased myofilament Ca^{2+} sensitivity and enhanced SR Ca^{2+} sequestration via SERCA also facilitates cardiomyocyte relaxation. In the healthy heart with optimal expression, localization, and function of Ca^{2+} handling proteins, the cardiomyocyte is able to utilize β -adrenergic signaling to increase inotropy and lusitropy, with enhanced cardiac performance.

Cardiomyocyte Ca^{2+} handling mechanisms in HFrEF

Cardiac dysfunction is intrinsic to the cardiomyocyte in HFrEF, in part due to abnormalities in cardiomyocyte Ca^{2+} handling. The systolic Ca^{2+} transient amplitude is reduced with a slower rate-of-rise, which associates with decreased cardiomyocyte shortening, slower contractile kinetics, and delayed onset of relaxation. A slower decay of the Ca^{2+} transient further impairs cardiomyocyte relaxation kinetics during diastole. At the molecular level, these phenotypic findings are due to complex changes in expression, localization, and function of key Ca^{2+} handling proteins within the cardiomyocyte, namely SERCA, PLN, NCX, and RyR_2 (Figure 2). A frequent finding in HFrEF is a decrease in SERCA expression or a decrease in PLN phosphorylation, both of which reduce SR Ca^{2+} reuptake, delay relaxation, and decrease SR Ca^{2+} content [8]. Interestingly, PLN phosphorylation status in HFrEF is typically not driven by depressed kinase function, but by enhanced function of protein phosphatases [9]. Additionally, expression of NCX is increased which allows NCX to outcompete SERCA for Ca^{2+} . Ca^{2+} fluxes therefore shift toward Ca^{2+} extrusion, leading to lower SR Ca^{2+} content and a subsequent decrease in SR Ca^{2+} release during ECC [10]. Cardiomyocytes in HFrEF also exhibit excessive RyR_2 channel activity, in combination with increased expression of the inositol 1,4,5-trisphosphate Receptor (IP_3R_2) release channel [11]. Excess RyR_2 and IP_3R_2 activity results in diastolic SR Ca^{2+} leak which further decreases SR Ca^{2+} content. These alterations in Ca^{2+} handling protein expression and function are compounded by ultrastructural derangements in the t-tubule and SR network due to elevated wall stress in HFrEF. Loss and disorganization of t-tubules [12] uncouples $\text{Ca}_v1.2$ from RyR_2 , rendering RyR_2 release clusters unable to fire immediately after $\text{Ca}_v1.2$ as part of ECC [13]. In severe HFrEF, “orphaned” RyR_2 channels may be foci for diastolic Ca^{2+} release events [14], with subsequent diastolic Ca^{2+} extrusion via NCX predisposing cardiomyocytes to delayed-afterdepolarizations (DADs) and arrhythmias.

Cardiomyocyte Ca^{2+} handling mechanisms in HFpEF

In contrast with the considerable literature on HFrEF, the alterations in Ca^{2+} handling associated with HFpEF are poorly defined. A major limitation to investigation of cardiomyocyte Ca^{2+} homeostasis in HFpEF is limited availability of cardiac tissue from HFpEF patients, as this HF population rarely undergoes cardiac transplantation. Complicating the lack of studies from human tissue is the paucity of animal models that recapitulate the complex clinical progression of HFpEF. Nevertheless, animal models with HFpEF-associated comorbidities exist including advancing age, obesity, type-2 diabetes, renal dysfunction, hypertension, and hypertrophy. As with HFpEF patients, these conditions associate with chronic inflammation and oxidative stress. Consistent with an extramyocardial origin of HFpEF, the amplitude of cardiomyocyte Ca^{2+} transients in many HFpEF-related models is normal [15–18] or even enhanced [19–21], suggesting an adaptive phase where Ca^{2+} flux shifts toward cardiomyocyte Ca^{2+} accumulation [22]. Indeed, excessive Ca^{2+} entry through $\text{Ca}_v1.2$ [19] and non-selective cation channels of the transient receptor potential (TRP) family [23–25] has been observed with hypertrophic remodeling. SR Ca^{2+} release through RyR_2 [15,16,21,26] and IP_3R_2 [20] is also increased in HFpEF-related models which further enhances Ca^{2+} cycling and contraction. In addition to effects on ECC, augmented Ca^{2+} entry through TRPC channels [24,27,28] or release via IP_3R_2 [29]

are established upstream regulators of hypertrophic remodeling processes. Cardiomyocyte Ca^{2+} accumulation in the absence of concomitant enhancement of SERCA activity leads to elevated diastolic Ca^{2+} , Ca^{2+} transients with preserved or enhanced amplitude, and slower Ca^{2+} reuptake kinetics with impaired relaxation. The effect on Ca^{2+} reuptake is particularly pronounced if SERCA expression decreases, as has been observed with advancing age, hypertrophy, obesity, diabetes, and renal disease. Ca^{2+} sequestration rate may be further reduced by glycation [30] or oxidative [31] modification of SERCA, depending on etiology. The inability of SERCA to expeditiously re-sequester Ca^{2+} becomes particularly evident at elevated stimulation frequencies, which may in part explain the chronotropic intolerance of the myocardium and reduced exercise capacity of HFpEF patients. In many animal models of HFpEF, the aforementioned adaptive phase transitions into cardiac decompensation/cardiomyopathy and a HFrEF-like phenotype with depressed cardiomyocyte Ca^{2+} transients and contractility [22]. However, the clinical relevance of this transition remains unclear, as clinical investigations suggest HFpEF and HFrEF are independent patient populations originating from distinct disease processes [4].

Therapeutic interventions to normalize cardiomyocyte Ca^{2+} handling

Cardiac glycosides

Digitalis compounds (cardiac glycosides) have been used since the 18th century to treat HF. By partially inhibiting the Na^+/K^+ -ATPase, glycosides elevate intracellular Na^+ , which extends the brief period of the action-potential when NCX “reverses” and contributes to Ca^{2+} influx (Figure 3, Table 1). Glycosides may also directly [32] or indirectly [33] activate RyR_2 and facilitate SR Ca^{2+} release. While such actions may further augment contractility they also likely contribute to the arrhythmogenic side-effects of cardiac glycosides. Low-dose digoxin has been evaluated in placebo trials [34] and remains in clinical use in HFrEF.

Istaroxime

Istaroxime is a dual-action steroid derivative that increases contractility in a manner similar to cardiac glycosides. However, istaroxime also enhances SERCA Ca^{2+} reuptake and relaxation, and is less arrhythmogenic than digoxin [35]. While initial clinical trials suggest istaroxime is effective in HFrEF due to its singular ability to enhance both contraction and relaxation [36], it has yet to progress into clinical use.

From β -agonists to β -blockers

Inotropic approaches which utilize the endogenous β_1 -adrenergic signal transduction machinery (e.g., β_1 -receptor agonists, phosphodiesterase (PDE) 3 inhibitors) are highly effective in enhancing contractility. However, such agents produce negative long-term outcomes in HFrEF, in part due to increased myocardial oxygen demand [37,38]. The augmented Ca^{2+} influx following β_1 -receptor activation, if sustained, also contributes to Ca^{2+} -dependent cardiomyocyte necrosis [39] and acceleration of adverse remodeling [40]. Accumulation of cardiomyocyte and SR Ca^{2+} is also detrimental in HFrEF due to leaky RyR_2 channels, which predisposes the cardiomyocyte to diastolic SR Ca^{2+} release, DADs, and arrhythmia. Thus, while inotropic strategies are still used clinically to restore cardiac output in acute cardiogenic shock, long-term use in HFrEF is contraindicated. The failure of

inotropic therapies in clinical trials led to the reciprocal approach of *preventing* β_1 -adrenergic signaling, and β -blockers are currently a standard first-line treatment approach in HFrEF. Carvedilol and its structural analogs have also been shown to prevent diastolic SR Ca^{2+} release by stabilizing RyR_2 [41], which serves as an additive anti-arrhythmic effect of this particular β -blocker.

Ranolazine

Intracellular Na^+ overload is prevalent in HF, in part due to increased activity of slowly-inactivating Na^+ channels (late Na^+ current). Ranolazine is an inhibitor of the late Na^+ current, which reduces Na^+ accumulation and shifts the electrochemical gradient of NCX towards greater Ca^{2+} extrusion and a reduction in diastolic Ca^{2+} [42].

PDE5/9 inhibitors

Levels of cGMP and downstream Protein Kinase G (PKG) activity are reduced in HFpEF patients [43], making inhibitors of PDEs that selectively degrade cGMP appealing treatments in HFpEF [44]. By phosphorylating Ca^{2+} channels (Cav1.2 and TRPC6) PKG inhibits Ca^{2+} influx and decreases hypertrophic gene transcription. PKG phosphorylation also reduces cardiomyocyte stiffness and produces lusitropic effects on relaxation. Despite highly encouraging preclinical results, the PDE5 inhibitor sildenafil did not show benefit in HFpEF patients [45]. Recent data indicate that PDE9 is upregulated in cardiomyocytes in HFpEF [46] and may represent an additional pharmacological target to increase cGMP-PKG signaling.

Preload/afterload reduction

Hemodynamic alterations leading to elevated preload and/or afterload induce cardiomyocyte mechanical stress, disruption in t-tubules, and production of reactive oxygen/nitrogen species with subsequent pro-arrhythmic SR Ca^{2+} release [47,48]. Therefore, approaches that reduce mechanical load likely exert beneficial secondary effects by improving $\text{Ca}_{\text{v}1.2}$ to RyR_2 coupling [49] and reducing diastolic SR Ca^{2+} leak. Angiotensin Converting Enzyme (ACE) inhibitors and Angiotensin Receptor Blocker (ARB) pharmacotherapies prevent Angiotensin II-mediated hypertrophic Ca^{2+} signaling through IP_3R_2 [29] and TRPC3/6 [50]. Excessive Ca^{2+} flux through these respective channels also associates with diastolic SR Ca^{2+} leak and arrhythmogenesis [20,51]. Thus, inhibition of Angiotensin II signaling may also exert anti-arrhythmic effects on ECC.

Adenoviral-associated virus (AAV) modulation of Ca^{2+} handling proteins

Recent advances in AAV vector design and delivery have made AAV-mediated expression of Ca^{2+} handling proteins a feasible therapy in HFrEF [52,53]. Most notably, AAV-mediated SERCA overexpression counteracts the decrease in SERCA pumping ability found in animal models of HFrEF, and Phase 2 trials of AAV-1.SERCA2a (Mydicar) were encouraging [54,55]. Unfortunately, Mydicar did not provide benefit in HFrEF patients in a recent, larger Phase 2b clinical trial [56]. Additional AAV gene therapy approaches that target cardiomyocyte Ca^{2+} handling include overexpression of the Ca^{2+} sensor protein S100A1, small ubiquitin-like modifier 1 (SUMO-1), a peptide inhibitor to prevent downregulation of

β -adrenoreceptors (β ARKct), protein phosphatase 1 inhibitor-1, and adenylyl cyclase 6. AAV-mediated transfer of micro-RNAs to alter gene transcription pathways in the setting of HF represent alternative approaches with therapeutic potential [57,58].

Conclusion

Therapies which target cardiomyocyte Ca^{2+} handling mechanisms are effective in HFrEF, where cardiac dysfunction is intrinsic to the cardiomyocyte. Cardiac dysfunction in HFpEF is complex and in many cases extrinsic to the cardiomyocyte. Nevertheless, cardiomyocyte Ca^{2+} handling processes are altered in HFpEF, and current phenotype-specific therapies may exert secondary beneficial effects on cardiomyocyte Ca^{2+} handling processes and disease progression.

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Highlights

- Heart failure is subclassified based on reduced or preserved ejection fraction.
- Treatments targeting cardiomyocyte Ca^{2+} homeostasis are effective in HFrEF.
- Half of all heart failure patients are diagnosed with HFpEF.
- Pharmacological treatment of HFpEF is largely ineffective.

Figure 1. Cardiomyocyte Ca^{2+} homeostasis

Action-potential induced depolarization via voltage-gated Na^+ (Na_v) and Ca^{2+} channels (Ca_v) is followed by repolarization via voltage-gated K^+ channels (K_v). During depolarization, Ca^{2+} influx via Ca_v triggers additional Ca^{2+} release from RyR_2 channels (RyR) of the sarcoplasmic reticulum (SR). This transiently elevates cytosolic Ca^{2+} and activates myofilament contraction. At the peak of the action potential, the electrochemical gradient for the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) briefly favors “reverse mode” exchange, contributing to additional Ca^{2+} influx. Cardiomyocyte relaxation occurs with repolarization. Cytosolic Ca^{2+} levels drop as Ca^{2+} is resequenced back into the SR via the SR Ca^{2+} ATPase (SERCA) and extruded from the cell via “forward mode” NCX. Meanwhile, background activity of the Na^+/K^+ -ATPase (NKA) maintains ionic gradients. With β -adrenergic stimulation (β_1 receptor) PKA-induced phosphorylation of Ca_v and RyR_2 increases Ca^{2+} influx and SR Ca^{2+} release, respectively. Phosphorylation of PLN reduces inhibition of SERCA which enhances SR Ca^{2+} reuptake and increases SR Ca^{2+} . In this way, the sympathetic response enhances the amplitude of cardiomyocyte Ca^{2+} transients and myocardial contractility.

Figure 2. Cardiomyocyte Ca^{2+} homeostasis in Heart Failure

In both HFrEF and HFpEF, diminished SERCA function leads to impaired SR Ca^{2+} sequestration and lower SR Ca^{2+} . Alterations in t-tubule structure lead to uncoupled Ca_v and RyR_2 channels. The “orphaned” RyR_2 cannot appropriately release Ca^{2+} , resulting in SR Ca^{2+} leak which is exacerbated by increased IP_3R -mediated Ca^{2+} leak. In HFrEF, increased NCX expression and “forward mode” diastolic Ca^{2+} extrusion further decreases SR Ca^{2+} . In HFpEF, alternative Ca^{2+} and Na^+ entry via TRP channels or the late Na^+ current exists. Elevated Na^+ favors “reverse mode” Ca^{2+} entry during the peak of the action-potential, predisposing cardiomyocytes to cellular Ca^{2+} overload.

Figure 3. Therapies targeting cardiomyocyte Ca²⁺ homeostasis in Heart Failure

β_1 -agonists and PDE3 inhibitors lead to enhanced cAMP-PKA signaling and increased Ca²⁺ transients, but have adverse long-term effects in heart failure. Cardiac glycosides and Istaroxime inhibit NKA, leading to a secondary elevation in Na⁺, NCX-mediated Ca²⁺ entry, and increased amplitude of Ca²⁺ transients. Istaroxime also enhances SERCA Ca²⁺ reuptake leading to beneficial effects on relaxation. By increasing SERCA expression, AAV-SERCA gene therapy enhances SR Ca²⁺ reuptake and increases SR Ca²⁺. Therapies which reduce cardiomyocyte Ca²⁺ stress include PDE5/9 Inhibitors and Ranolazine. In HFpEF, PDE5 and PDE9 expression is increased, leading to decreased cGMP levels, lower PKG activity, and excessive TRP and Ca_v channel activity. These effects are counteracted by PDE5/9 inhibitors. Ranolazine inhibits Na⁺ entry via the late Na⁺ current and prevents NCX-mediated Ca²⁺ entry.

Table 1

Heart Failure therapies with direct or indirect actions on cardiomyocyte Ca^{2+} homeostasis

Therapy	Cardiomyocyte Mechanism of Action	Effect on Cardiomyocyte Ca^{2+}	Effect on Heart	Clinical Trials and Use in HFpEF	Clinical Trials and Use in HFpEF
Cardiac Glycosides	Inhibits $\text{Na}^+/\text{K}^+/\text{ATPase}$, with NCX-mediated secondary elevation in Ca^{2+}	Increases Ca^{2+} transient amplitude	Positive inotropy	DIG-REF study, digoxin reduced hospitalizations in HFpEF. <i>ACCF/AHA recommendation for stage C patients.</i>	DIG-PEF study, digoxin had no effect on hospitalization rate or mortality in HFpEF
Istaroxime	Inhibits $\text{Na}^+/\text{K}^+/\text{ATPase}$, increases SERCA activity	Increases Ca^{2+} transient amplitude and Ca^{2+} sequestration	Positive inotropy and lusitropy	HORIZON-HF Phase 2 trial demonstrated improved hemodynamics and decreased diastolic stiffness with istaroxime in HFpEF.	Phase 0 trial investigating response to istaroxime in HFpEF currently in progress.
β -blockers	Inhibits β -adrenergic signaling Stabilizes RyR (carvedilol)	Decreases PKA-mediated phosphorylation of Ca^{2+} handling proteins Prevents pro-arrhythmic SR Ca^{2+} release	Inhibits inotropic catecholamine response Decreases myocardial oxygen demand Anti-arrhythmic effects	Multiple clinical trials have demonstrated marked improvement in outcomes and QOL. <i>ACCF/AHA recommendation in Stage C patients and Stage D patients who can tolerate therapeutic doses.</i>	ELANDD trial found no increase in exercise capacity with nebivolol in HFpEF SENIORS phase 4 trial of nebivolol (β_1 -selective) in HFpEF is currently in progress. Previous phases demonstrated mild reduction in mortality and pulmonary HTN. J-DHF trial demonstrated no improvement in outcomes with carvedilol in HFpEF carvedilol vs. nebivolol study currently in progress (NCT02619526)
Ca^{2+} channel blockers	Inhibits L-type Ca^{2+} channel	Reduces Ca^{2+} influx	Negative inotropy	Shown to increase mortality. <i>ACCF/AHA contraindication for any stage of HFpEF.</i>	DEMAND trial of nifedipine in HFpEF currently in progress (NCT0157481). Preliminary data shows improvement in LV peak filling rate.
Ranolazine	Inhibits late Na^+ current and prevents NCX-mediated secondary elevation in Ca^{2+}	Reduces cellular Ca^{2+} stress	Improves diastolic relaxation	No clinical trials to date	RALI-DHF phase 2 trial found that ranolazine improved pulmonary capillary wedge pressure, but no change in relaxation parameters. RAZE trial currently in progress (NCT01505179)
PDE5/9 inhibitors, Nitrates, and sGC stimulators	Increases intracellular cGMP and PKG phosphorylation	Reduces activity of L-Type Ca^{2+} channel and TRPC channels	Prevents Ca^{2+} -dependent cardiac remodeling Improves diastolic relaxation Significant beneficial effects on the heart independent of cardiomyocyte Ca^{2+}	VICTORIA phase 3 trial of vericiguat currently in progress (NCT02861534)	RELAX trial demonstrated no benefit of sildenafil in HFpEF Phase 2 trial investigating inorganic nitrate (KNO_3) demonstrated improved exercise capacity with HFpEF in preliminary results. DYNAMIC phase 2 trial investigating riociguat in HFpEF currently in progress. (NCT02744339) SOCRATES-PRESERVED phase 2 trial investigating vericiguat in HFpEF currently in progress (NCT01951638)

Therapy	Cardiomyocyte Mechanism of Action	Effect on Cardiomyocyte Ca^{2+}	Effect on Heart	Clinical Trials and Use in HFpEF	Clinical Trials and Use in HFpEF
					NEAT-HFpEF phase 2 trial isosorbide mononitrate reduced exercise capacity and had no change on QOL in HFpEF Current phase 2 trial, investigation of isosorbide dinitrate $\pm$ hydralazine in HFpEF (NCT01516346) No clinical trials to date of PDE9A inhibitors, anticipated in future.
Gene Therapies	AAV1.SERC A2a (Overexpression of SERCA)	Improves Ca^{2+} sequestration and contractility	Positive Inotropy and Lusitropy	CUPID2 phase 2 trial found no evidence of improved outcomes with AAV1.SERC A2a gene therapy in HFpEF	No clinical trials to date
ACE-I or ARB	Inhibits effects of Angiotensin II	Decreases IP R and 3 TRPC Ca^{2+} signaling	Prevents Ca^{2+} - dependent adverse cardiac remodeling Significant beneficial effects on the heart independent of cardiomyocyte Ca^{2+}	Multiple clinical trials have demonstrated marked improvement in outcomes and QOL. <i>ACCf/AHA first line indication for stage B patients</i>	I-PRESERVE phase 3 trial demonstrated no change in outcomes with irbesartan in HFpEF. PEP-CHF study demonstrated moderately improved outcomes with perindopril in HFpEF. However, the study was underpowered due to patient withdrawal, so uncertainty about its efficacy remains.
					CHARM-PRESERVE phase 3 trial demonstrated a moderate reduction in hospitalizations, but no impact on mortality with candesartan in HFpEF <i>ACCf/AHA indication for HFpEF patients with HTN, symptomatic atherosclerotic disease or diabetes mellitus, and one additional risk factor</i>
Preload/Afterload reduction Therapies including: ACE or ARB β -blockers Diuretics Aldosterone-antagonists Neprilysin inhibitors	Reduces mechanical stress on cardiomyocytes	Improves t-tubule organization, $Ca_v1.2$ - RyR_2 coupling Reduces ROS/RNS signaling	Prevents adverse cardiac remodeling Significant beneficial effects on the heart independent of cardiomyocyte Ca^{2+}	<i>ACCf/AHA recommendation for volume overloaded Stage C and D HFpEF patients.</i> <i>ACCf/AHA recommendation for add-on spironolactone in stage C patients with adequate renal function.</i> PARASAIL phase 4 trial of Sacubitril/Valsartan combination currently in progress. With marked improvement in outcomes from previous phases Sacubitril/valsartan was FDA approved use for HFpEF in 2015.	ROPA-DOP phase 4 trial of furosemide $\pm$ dopamine in HFpEF currently in progress. TOPCAT phase 3 trial demonstrated improved QOL but no change in diastolic indices with spironolactone in HFpEF. ALDO-DF study demonstrated improved diastolic function, but maximal exercise capacity, clinical symptoms, and QOL unchanged with long-term spironolactone in HFpEF RAAM-PEF phase 4 trial demonstrated no improvement in exercise capacity with epplerenone in HFpEF PARAGON-HF phase 3 trial investigating the efficacy of Sacubitril/Valsartan currently in progress.