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Leveraging Signaling Pathways to Treat Heart Failure with Reduced Ejection Fraction: *Past, Present and Future*

Miguel Pinilla-Vera[1,*], **Virginia S. Hahn**[1,*], and **David A. Kass**[1,2]

[1]Division of Cardiology, Department of Medicine,

[2]Department of Pharmacology and Molecular Sciences, The Johns Hopkins University, Baltimore Maryland, 21205

Abstract

Advances in the treatment of heart failure with a reduced ejection fraction (HFrEF) due to systolic dysfunction are engaging an ever-expanding compendium of molecular signaling targets. Well established approaches modifying hemodynamics and cell biology by neurohumoral receptor blockade are evolving, exploring the role and impact of modulating intracellular signaling pathways with more direct myocardial effects. Even well tread avenues are being reconsidered with new insights into the signaling engaged and thus opportunity to treat underlying myocardial disease. This review explores therapies that have proven successful, those that have not, those that are moving into the clinic but whose utility remains to be confirmed, and those that remain in the experimental realm. The emphasis is on signaling pathways that are tractable for therapeutic manipulation. Of the approaches yet to be tested in humans, we chose those with a well-established experimental history, where clinical translation may be around the corner. The breadth of opportunities bodes well for the next generation of heart failure therapeutics.

Keywords

heart failure; signaling pathways; therapy; pharmacology; epigenetics

Introduction

The past few decades have witnessed transformative progress in the treatment of heart failure (HF) with depressed systolic function, but persistent societal and economic burdens from heart failure cry out for even better therapeutic arrows in our quiver. Current therapies encompass small molecules, biologicals, implantable devices, and heart transplantation, as summarized in the clinical stage-specific practice guidelines[1, 2] and Supplemental Table 1. In this review, we focus on molecular therapeutic targets for heart failure – past, present, and future. A number of signaling pathways a number of which were. Along the way, we highlight the successes and failures, and discuss approaches making their way from animals to humans. We organized this discussion into four major sections – evidence-based therapies

Address correspondence: David A. Kass, M.D., Ross 858, Division of Cardiology, 720 Rutland Avenue, Baltimore, MD 21025, (410) 955-7153, (410) 502-2558 (FAX), dkass@jhmi.edu.

*The authors contributed equally to the work.

currently in use, new therapies undergoing clinical trials, approaches that have been largely abandoned due to disappointing clinical data, and new avenues being pursued in experimental studies that may launch to humans in the relatively near future. The scope of this effort is encapsulated in Figure 1–which highlights the various signaling pathways engaged, and identifies the therapeutic target in use or currently under study for treating HF. The reader is referred to a review issue from 2011 that covers additional pathways[3]. We hope the current update offers a succinct survey for both scientist and physician to appreciate what we have achieved and where we need to go.

Beta-adrenergic blockers

Heart failure is associated with hyper-activation of the sympathetic nervous system mediated largely by epinephrine and norepinephrine, in an effort to sustain cardiac output.[4] While appropriate for acute responses, sustained “hyperadrenergic” drive contributes to myocardial toxicity and HF. Identification of different adrenergic receptors dates back nearly 80 years when Raymond Ahlquist named β-adrenergic receptors as those mediating vasodilation, uterine relaxation, and “excitatory” effects of epinephrine in the heart.[5]

The β1-adrenergic receptor (β1AR) is the most common in the normal mammalian heart (80%), the remaining being mostly β2-receptors (β2AR). However, β1-receptor density declines in HF by 60% while β2-density is unchanged. β1-receptors primarily couple to the protein Gαs, activating adenylyl cyclase to generate cAMP and thus stimulate protein kinase A (PKA). PKA phosphorylation of L-type Ca channels, myosin binding protein C, phospholamban, troponin I, titin, and other proteins involved with excitation-contraction result in greater contractility, heart rate, diastolic distensibility, and relaxation.[6] Genetically induced excess β1AR expression (15 fold over baseline) causes spontaneous heart failure,[7] whereas its suppression impedes chronic myocardial toxicity. A widely used beta-blocker - carvedilol (a β1,β2, α1 AR blocker) confers additional properties, in that its binding to the β1AR induces a conformational change favoring coupling to inhibitory G-protein - Gαi rather than stimulatory Gαs. This in turn activates the β-arrestin pathway in a process known as biased agonism, transactivating extracellular growth factor receptor and promoting activation of mitogen activated kinase ERK1/2 as well as Akt kinase to confer cardioprotection.[6, 8] The biased agonism features of carvedilol have been demonstrated *in vitro* and *in vivo* in mice[9].

The β2- receptor primarily engages Gαs but also Gαi through a mechanism dependent on PKA. Gαi activation inhibits adenyl cyclase, counteracting Gαs. While β1AR stimulation results in more diffuse cytosolic cyclic adenosine monophosphate (cAMP) generation, β2AR stimulation induces plasma membrane-localized pools, though this compartmentation is reduced in HF leading to more diffuse β2AR-cAMP.[10] Engineered mice are able to tolerate cardiac overexpression of β2AR up to 60 fold (vs. less than 15 fold of β1AR)[7] without developing heart failure, with cardiomyopathy manifesting at 100 fold expression level.[11] β2AR also exhibits biased agonism, with β-arrestin modification and transactivation signaling.[6]

HF results in marked desensitization of βARs, particularly β1AR, decreasing myocardial responses to catecholamines, but not to the point of preventing the noxious effects of the

hyperadrenergic state. The cause is multifactorial, involving reduced receptor expression, uncoupling of β1AR and β2AR from cAMP-signaling by GRK2 and GRK5 phosphorylation, β-arrestin activation, receptor internalization, and enhanced Gαi coupling.[6] βAR blockers counteract the detrimental effects of the hyper-adrenergic state, in spite of the apparent paradox that the β-adrenergic signaling is already downregulated. Even though β1AR are downregulated by around 60% in the failing heart, cardiomyocytes are still under toxic hyper-adrenergic stimulation[12, 13]. Thus, further gentle inhibition of β1AR signaling with β1 blockers is protective, associated with enhanced downstream signaling, organ responsiveness to acute catecholamines, and increased expression of β1AR. Restoration of plasma membrane β1AR density and β1AR/β2AR ratio can confer additional effects.[14] This enhanced signaling means that on-demand catecholamine response can be more sensitively transduced while competing with the AR blocker at the receptor level. Chronic β-blockade also reverses a pathological gene expression program, increasing the expression of α-myosin heavy chain, decreasing β-myosin heavy chain, and increasing SERCA2a expression.[15] Moreover, βAR blockers have beneficial hemodynamic effects, decreasing cardiac work, afterload, and oxygen consumption.[16]

Multiple studies of βAR blockade in HFrEF patients have revealed reduced hospital admission, improved quality of life, slowed disease progression, and reduced mortality. Though initially, concerns were raised that βAR blockade would result in functional decompensation in patients with LV dysfunction,[17] studies pioneered by Waagstein and colleagues using the cardioselective β-blocker practolol showed the opposite.[18] These agents are now strongly recommended as first line therapy after a myocardial infarction to prevent HF development. Three βAR blockers have reduced mortality in heart failure in large, controlled clinical trials: bisoprolol,[19] metoprolol succinate (long acting),[20] and carvedilol.[21] While meta-analysis has tried to determine if one approach is superior to another, the issue remains unclear. Carvedilol has additional vasodilator effects that could be helpful but also are less well tolerated in patients with low blood pressure. Whether it provides added value from biased agonism is unknown.

Inhibitors of the renin-angiotensin-aldosterone system (RAAS)

Efforts to enhance cardiac performance by reducing systemic vascular resistance started in the 1980s, first using a combination of isosorbide dinitrate and hydralazine, and later angiotensin-converting enzyme-inhibitors (ACE inhibitors). Prior basic research had shown RAAS signaling is upregulated in HFrEF and key to maintaining blood pressure in acute HF and to plasma volume expansion in chronic HF.[22] Both plasma renin activity and aldosterone were shown to rise in chronic advanced HF patients.[23] Importantly, whereas various vasoactive agents existed to lower cardiac afterload, targeting the RAAS system proved most effective long term. Cardio-protection by RAAS antagonism suppresses this pathway in both general circulation and in the myocardium itself,[24] the latter also reducing pathological hypertrophy, fibrosis, and dysfunction[25] via effects on both cardiomyocytes and fibroblasts.[25] This is primarily linked to Gαq-coupled angiotensin type 1 receptor (AT1R) signaling.[26]

ACE inhibition is an example of a HF-therapy eureka moment, as the CONSENSUS trial testing enalapril found such a marked decline in absolute 6 month mortality (44% to 26% in severe HF patients) that it was terminated early, after enrolling just 253 patients.[27] It took nearly 30 years to move beyond this in a trial involving >8000 patients (discussed next). The ACE inhibitor Enalapril reduces mortality and hospitalizations in patients with symptomatic and asymptomatic LV dysfunction. Similar efficacy is obtained by blocking AT1R[28] and by aldosterone receptor[29] blockers. Two downstream cleavage products of angiotensin II, angiotensin 1–7 and angiotensin 1–9, have beneficial effects in the heart.[30, 31] While this suggested angiotensin receptor blockers (ARBs) might be superior to ACE inhibitors, the data does not support this. Generation of these cleavage products by non ACE-dependent mechanisms as well as lack of ACE2 suppression by current ACE inhibitors may explain this.

Aldosterone is a key component of the RAAS that is secreted from the adrenal cortex in response to angiotensin II. In addition to augmenting sodium reabsorption in the distal convoluted tubule, aldosterone promotes vascular and myocardial hypertrophy, fibrosis, inflammation, endothelial dysfunction, and oxidative stress. Its receptor is upregulated in cardiomyocytes from HFrEF patients,[32] and aldosterone antagonists improve ventricular remodeling, heart failure outcomes, and sudden cardiac death.[33] The mechanisms of this antiarrhythmic effect include reduced extracellular matrix deposition, reduced ventricular remodeling, and direct effects on intracellular calcium signaling in cardiomyocytes.[34]

Combining neprilysin with AT1R inhibition

Neutral endopeptidases (NEP) proteolytically modify and degrade proteins, including those secreted into the bloodstream. One target is the natriuretic peptides, and the concept that blocking NEP might enhance natriuretic activity took shape about 35 years ago.[35] Neprilysin is a membrane bound zinc-dependent endopeptidase with >50 substrates, that was first discovered in the proximal renal tubule, and later in the brain where it metabolizes endogenous opioids. It cleaves substrates in multiple organ systems, with cardiovascular effects potentially related to its degradation of natriuretic peptides (atrial natriuretic peptide, ANP>>brain natriuretic peptide, BNP), adrenomedullin, substance P, and bradykinin, which work together to cause vasodilation. Studies in animal models and humans demonstrated an increase in circulating and urinary cyclic guanosine monophosphate (a consequence of augmented natriuretic peptide signaling[36]) after neprilysin inhibitor treatment.[35, 37] Serum BNP levels have also been seen to rise in some trials, whereas NT-proBNP which is not targeted by NEP generally declines, likely reflecting reduced cardiac volumes.[38–40] Caution is needed interpreting the BNP change as the assay can also react with its cleavage products[41], and ANP which is anticipated to be more impacted by NEP inhibition, remains to be measured. NEP also cleaves angiotensin and bradykinin, and their rise upon NEP inhibition can cause adverse side effects, including angioedema,[38] and why AT1R blockers are concomitantly employed. Angiotensin is also cleaved into two subfragments, angiotensin 1–7 and angiotensin 1–9, which have beneficial effects in the heart,[30, 31] and NEP inhibition may also increase these to provide further benefit than just a reduction in natriuretic peptide clearance.[42] The pivotal trial reported in 2014 testing a combined AT1R blocker (valsartan) and neprilysin inhibitor (sacubitril) versus an ACE inhibitor was conducted in >8000 HF

patients with moderate disease. It found a 20% relative reduction and 4.7% absolute reduction in cardiovascular death or heart failure hospitalization.[38] Subsequent studies have noted a reduction in arrhythmia burden, though the exact mechanism beyond improved ventricular remodeling is unknown.[43] The drug is formed by attaching the component molecules together with an inert linker that is cleaved, resulting in 1:1 stoichiometry. Valsartan is present to suppress potential detrimental effects from elevated angiotensin II levels due to sacubitril. Subsequent studies have confirmed beneficial effects and this combination is rapidly becoming a standard of care.

Combination isosorbide dinitrate and hydralazine: Rationale in a sub-population

Combination therapy with nitrates and hydralazine in chronic heart failure was first developed to achieve the hemodynamic benefits of both preload and afterload reduction seen with sodium nitroprusside in acute heart failure.[44] Postulated mechanisms include stimulation of nitric oxide (NO) signaling and lowering oxidative stress by hydralazine; though these hypotheses have not been proven. The Vasodilator-Heart Failure Trial (V-HeFT I) was the first randomized, placebo-controlled study of hydralazine and isosorbide dinitrate (ISDN) compared to prazosin or placebo, and showed a mortality benefit in HF patients.[45] V-HeFT II compared enalapril to combination hydralazine-ISDN finding a borderline mortality benefit favoring enalapril (p=0.08).[46] However, subgroup analysis of both studies identified self-described black patients as having a greater mortality benefit with hydralazine-ISDN vs enalapril,[47] and this was subsequently confirmed in a trial of self-described black HF patients (A-HeFT).[48] Why this applies only to this self-described racial cohort remains uncertain. Theories include more reduced NO and greater oxidative/nitrosative stress in these patients[49] coupled to a higher prevalence of SNPs affecting endothelial nitric oxide synthase[50] and G-protein beta-3 subunit.[51] Despite being a class I recommendation in clinical guidelines, hydralazine-ISDN remains markedly underutilized in this population,[52] potentially due to poor tolerability, the inconvenience of 3× daily dosing, and even social caution over race-specific therapy.

Ivabradine: Controlling heart rate alone

Elevated heart rate is viewed as a biomarker of HF severity caused by hyper-sympathetic stimulation.[53] Thus, much like βAR blockade first did, purposely slowing heart rate would seem imprudent. Development of I_f (funny channel) blockers such as ivabradine allowed this hypothesis to be tested, and conventional wisdom was wrong - again. I_f current is composed of K^+ and Na^+ flux, and the channels are hyperpolarization-activated and cyclic nucleotide-gated (HCN4).[54] Ivabradine blocks I_f intracellularly, reducing diastolic depolarization rate of pacemaker cells to slow the heart rate.[54] As it blocks the channel in its open state, it is more potent at higher heart rates.[54] Since it has no impact on intrinsic contractility (beyond that regulated by heart rate), it has been considered in HF patients intolerant to βAR-blockers. In clinical trials, ivabradine improved clinical outcome in moderate-severe HFrEF patients with HR ≥70 bpm on standard therapy, reducing cardiovascular death or hospitalization by 18% after ~2 years[55]. However, only 26% of subjects had received appropriate βAR-blocker doses, so similar outcomes might arise with β-blocker up-titration. Current guidelines recommend ivabradine to reduce HF-related hospital admissions in patients with resting HR>70 bpm, with stable HFrEF on standard medical therapy, or who are intolerant to or

contraindicated for βAR-blockade. However, patients intolerant to βAR-blockers are often sicker than those studied in the ivabradine trials, so the precise sweet spot for this drug remains to be clarified.

Sodium-glucose cotransporter 2 (SGLT2) Inhibitors: Oral anti-diabetic drugs with serendipitous cardiovascular effects

SGLT2 belongs to the 6-member SLC5 family of sodium-dependent glucose cotransporters. The low-capacity, high-affinity Na-glucose co-transporter, SGLT1, is mainly expressed in the gastrointestinal tract, whereas the high-capacity, low-affinity transporter, SGLT2, is expressed primarily in the kidney in the brush border of the proximal tubule, where it controls 90% of glucose reabsorption from the urine.[56] Inhibition of SGLT2 induces glucosuria, associated with hemoglobin A1c reduction of 0.7–1.0% and weight loss of 2–3 Kg in patients with type-2 diabetes (T2D). Despite a seemingly strong scientific basis for benefits of anti-glycemic agents on the failing heart, SGLT2 inhibitors were the first that indeed also reduced HF hospitalization and cardiovascular death in patients with T2D.[57] This discovery followed a series of studies with other T2D therapies that yielded neutral results or posed risk concerns. In what started out as another standard FDA-mandated cardiovascular safety trial – inhibiting SGLT2 conferred very surprising beneficial effects. The EMPA-REG OUTCOME trial[58] of 7020 patients with T2D and a high risk of CV events, showed those randomized to empagliflozin versus placebo had a substantial decline in the composite outcome (cardiovascular death, myocardial infarction, or stroke), driven primarily by a decline in cardiovascular death. Emplagliflozin also lowered all-cause mortality and hospital admissions for heart failure, worsening of renal function and need for renal replacement therapy. Systolic blood pressure declined ~5 mmHg. There have been several follow-up trials, notably the 17160 subject DECLARE–TIMI 58[59] study of dapagliflozin, another SGLT2 inhibitor, also in T2D patients with increased CVD risk, that found active therapy also reduced the composite outcome of cardiovascular death or hospitalization for heart failure, being driven primarily by the latter.

These data are remarkable not only for the magnitude of the effects observed, but also for the lack of clarity regarding the mechanisms underlying them. SGLT2 inhibitors confer anti-inflammatory effects, reduce oxidative stress, improve endothelial function, and modulate neurohormonal pathways in experimental models.[60] They may also reduce plasma volume via natriuresis and lower blood pressure by this and/or direct vasodilation mechanisms. However, such hemodynamic effects should be achievable with existing diuretics and vasodilators. Other mechanisms include shifting cardiac energy substrate to favor fatty acids and ketone bodies. While SGLT2 is not expressed at the protein level in cardiomyocytes, its inhibition is associated with reduced myocyte shortening, L-type Ca^{2+} channel currents[61] and inhibition of the Na^{+}/H^{+} exchanger-1 in isolated cardiomyocytes.[62] These would depress myocyte function, so hard to reconcile with better HF survival. More work is clearly needed.

New approaches under active clinical investigation

Small molecule sarcomere activators to enhance contractility

Current clinically approved positive inotropes (e.g. dobutamine, milrinone) increase intracellular calcium, energy demand, and arrhythmia risk by raising cAMP levels in the myocyte. Levosimendan also elevates cAMP, but has additional effects, impacting troponin C to enhance sarcomere force-calcium responsiveness, and vasodilating by ATP-sensitive potassium channel agonism. For nearly 3 decades, efforts have been made to avoid calcium and cAMP pathways and instead enhance sarcomere function directly, but their story lines are twisty with many dead ends, most due to worsening diastole or poor pharmacological properties. Omecamtiv Mercarbil (OM, Cytokinetics, San Francisco, CA) represents the most recent and to date most promising sarcomere enhancer.[63] OM binds specifically to cardiac myosin at the ATP-ase catalytic domain, increasing ATPase activity, and favoring formation of force-generating crossbridges.[63] This increases contractility while simultaneously prolonging systole, but does not alter intracellular calcium, and demands less oxygen consumption than with βAR-agonists. Phase 2 studies report improved cardiac function,[64] and while some safety concerns have been raised regarding low level increases in circulating troponin-I, the overall safety profile was sufficient to move onto the current pivotal trial (GALACTIC-HF, NCT02929329, Phase 3, Recruiting). This trial should be complete in early 2021. Other myosin activators, e.g. MYK-491 (MyoKardia, Inc., San Francisco, CA) have recently entered Phase 1b/2a studies in stable HFrEF patients (NCT03447990).

Cyclic GMP-targeted Phosphodiesterase (PDE) inhibitors: PDE1 and PDE9

The phosphodiesterases (PDEs) are an 11-member superfamily of enzymes expressed as ~100 isoforms, that hydrolyze cAMP and/or cyclic guanosine monophosphate (cGMP) with varying selectivity.[65] Differential expression of each PDE in various cell types provides further specificity for their effects. PDE3 inhibitors were explored for HFrEF (e.g enoximone, milrinone), to elevate cAMP. These inhibitors confer positive inotropic, chronotropic, and vasodilatory effects, but they remain in limited clinical use due to worsened HF mortality with chronic therapy,[66] and arrhythmia concerns.

Attention has also turned to cGMP signaling which allosterically activates protein kinase G (PKG) by binding to regulatory domains in its N-terminus, disinhibiting the C-terminus catalytic domain. PKG's primarily role in CV disease is known as vasodilation, yet many studies have revealed potent intracellular signaling capable of blunting pathological hypertrophy, fibrosis, ischemic disease, and mitochondrial toxicity.[65] Signaling is transduced by the phosphorylation and inactivation of the non-selective transient receptor potential cation channel – TRPC6,[67] stimulation of regulator of G-protein signaling (RGS2 and RGS4)[68] to block Gαq-coupled signaling, enhancing proteosome activity,[69] and most recently, TSC2 (tuberin) to potently control activation of the mechanistic target of rapamycin – complex-1[70]. Sarcomere proteins modified by PKG include troponin I to enhance relaxation, myosin binding protein C that impacts adrenergic-stimulated contractile reserve, and titin to increase diastolic distensibility.[65]

Once generated, cGMP hydrolysis is selectively regulated by PDE5 and PDE9, and may be further impacted by several dual-substrate esterases - PDE1, PDE2, and PDE3 under the right conditions.[71] PDE5 has been the most studied cGMP-PDE so far, and its inhibitors (e.g. sildenafil, tadalafil) are used to treat pulmonary hypertension and erectile dysfunction. Despite substantial experimental data supporting PDE5 inhibition for HF indications,[71] clinical translation remains uncertain. Multiple small single-center trials in HFrEF patients reported improved exercise physiology, capacity, pulmonary pressures, and associated hemodynamics, from PDE5 inhibition.[72] However, there is no large-scale multicenter HFrEF trial, and a ~200 patient study of HFpEF did not support benefit, and has left its fate uncertain[73].

The other cGMP-selective phosphodiesterase – PDE9 – has recently moved forward as an inhibitor target in clinical HF trials. This PDE has the highest affinity for cGMP, and is expressed at the mRNA level mostly in the brain and gut, with some in kidney, and low levels in skeletal muscle, fat, and the heart.[74] Its potential involvement with HF was first revealed in a 2015 study where myocardial PDE9A expression was found to increase in HFrEF (and HFpEF), and its genetic or pharmacological inhibition improved heart function, reduced hypertrophy and fibrosis in pressure-overloaded mouse hearts.[75] This study also revealed PDE9 favors hydrolysis of cGMP generated by natriuretic peptide signaling, in contrast to PDE5 that controls cGMP coupled to nitric oxide signaling. PDE9 inhibition remains an effective means of countering hypertrophic heart disease even when NO synthase is blocked, whereas PDE5 inhibition is ineffective in this setting. While inhibiting either stimulates PKG, the phospho-kinome that is modified contains many different targets.[75] More strikingly, there are marked disparities in microRNA changes, with PDE5 inhibition reducing many miRNAs enhanced by pressure-overload, whereas PDE9 inhibition alters essentially none[76]. This is despite the fact that cardiac improvement is very similar with each treatment – reduced hypertrophy and fibrosis, and enhanced function. The reliance of PDE5 inhibitor efficacy on NO-cGMP signaling may have sex-specific therapy implications, as PDE5 inhibitors fail to counter heart disease in female mice following ovariectomy. Their benefit is restored by estrogen replacement, highlighting a key role of estrogen-dependent NO signaling to PDE5 regulation.[77] Clinical trials of a PDE9 selective antagonist (CRD-733) for HFrEF began in late 2018.

Yet another PDE whose inhibition recently entered clinical trials for HFrEF is PDE1. This is a dual-substrate Ca^{2+}-calmodulin activated esterase, expressed by three genes to yield isoforms with different substrate selectivity. The heart expresses PDE1A and PDE1C, the former being >20× more avid for cGMP over cAMP, whereas the latter has equal selectivity. This is likely important for human translation, as mice and rats mostly express PDE1A, whereas larger mammals such as rabbits, dogs, and humans express mostly PDE1C.[78] In a recent preclinical study, a highly selective PDE1 inhibitor (ITI-214, Intracellular Therapies, Inc. NY) was tested in conscious dogs and anesthetized rabbits. In both models, the compound produced positive inotropic, lusitropic, chronotropic effects, and arterial vasodilation.[78] Similar changes with much less heart rate rise were observed in dogs with HFrEF. PDE1 inhibition appears to impact a cAMP pool different to that coupled to βAR or PDE3 regulation. Unlike cilostamide (PDE3-inhibitor), ITI-214 did not enhance myocyte force or calcium transients in cells pre-stimulated with isoproterenol, but did impact

signaling linked to adenosine type 2b receptors. These adenosine receptors are important regulators of ischemia-reperfusion injury as well as linked to cAMP-regulated contraction.[78] A link to adenosine receptor signaling is supported by another study of ischemia-induced apoptosis in myocytes.[79] Much more work is needed to dissect where PDE1 regulated cAMP signaling occurs, what exactly lies downstream, and what other features differentiate it from other methods to enhance cAMP. As ITI-214 had already passed Phase I human safety studies, a clinical Phase Ib-IIa HFrEF study was started in the fall of 2018 (NCT03387215) to assess safety and cardiac/hemodynamic effects of a single dose.

Nitroxyl (HNO) – combined inotropy, lusitropy, and vasodilation.

Angello Angeli's salt, the first known nitroxyl (HNO) donor was described in 1896. Unlike Alfred Nobel, whose contemporaneous discovery of nitroglycerin quickly led to a highly remunerative business in explosives, Angeli's salt had to wait more than a century before it found translational utility. Although HNO is chemically similar to the more famous nitric oxide, its biological effects differ substantially. HNO is considered highly electrophilic and avidly reacts with negative charged thiol groups (thiolates) of proteins, a key feature of its biological effects. It reversibly converts thiol to disulfide residues and/or to sulfinamides (less reversibly). HNO also has antioxidant properties by reacting with oxidizing radicals to generate NO.[80] HNO-induced vasorelaxation requires modification of soluble guanylate cyclase[81] much like NO; however its augmentation of contractility is cGMP/sGC/PKG and cAMP/PKA independent, and not impacted by βAR blockade. Rather, HNO enhances contractility by increasing Ca^{2+} cycling through the sarcoplasmic reticulum by generating an intermolecular di-sulfide in phospholamban to disinhibit SERCA2a, increasing the open probability of the ryanodine receptor (RyR2), and enhancing myofilament calcium sensitivity coupled to thiol modification of tropomyosin, actin, and myosin light chain.[82–85] Angeli's salt is very unstable, and the search for room temperature-stable HNO donors that could serve as pharmaceuticals began in earnest in 2005. These molecules were tested in experimental animals and humans, confirming positive inotropy, lusitropy, and vasodilation effects.[86, 87] A third generation HNO donor BMS-986231 was tested in acute HF, where the compound rapidly and sustainably lowered pulmonary capillary pressure while improving cardiac index, without altering heart rate, or inducing arrhythmia, hypotension, or other significant adverse events.[88] Ongoing Phase 2B trials (NCT03357731, NCT03016325, NCT03730961) are further testing its clinical efficacy.

Soluble guanylate cyclase stimulators/activators

Activation of the NO-cGMP pathway using NO donors, organic or inorganic nitrates or nitrites are being studied for HFrEF and HFpEF. They have limitations due to tolerance or requiring enzymatic conversion for efficacy, as well as oxidant stressors that impair the capacity of NO to generate cGMP and activate PKG. An alternative approach is to stimulate soluble guanylyl cyclase (sGC) directly to generate cGMP. NO interacts with the reduced heme at the proteins central core to catalyze cGMP synthesis from GTP, and cGMP in turn activates PKG. While circumventing inadequate NO-stimulated cGMP, this approach does not mimic other NO effects from S-nitrosation of proteins and anti-oxidant chemistry.[89]

A class of sGC activators (ataciguat and cinaciguat) or stimulators (riociguat and vericiguat) have been developed, extensively tested in various experimental models, and in initial clinical trials. The activators are heme-independent but also lack synergistic stimulation by NO. The stimulators regulate the cyclase allosterically, allowing for synergistic effects upon NO binding to heme.[89] The activators have very potent vasodilator effects and trials of one of these, cinaciguat, in HFrEF patients were terminated early due hypotension and lack of benefit.[89, 90] However, the stimulator vericiguat was also tested in HFrEF patients, and while the study missed the primary end-point (reducing N-terminal pro-BNP levels), high doses suggested potential lowering of cardiovascular death and hospitalization rates.[91] This spawned the ongoing Phase III VICTORIA trial testing vericiguat in HFrEF (NCT02861534). The other stimulator, riociguat, is approved for chronic thromboembolic pulmonary hypertension.

Apelin

Apelin (APJ)a was first identified in 1993 as an orphan G-protein coupled receptor. Its amino acid sequence is similar to AT1R but does not bind angiotensin.[92] Its ligand apelin was discovered in 1998, and is an adipokine synthesized as a 77 amino acids pre-propeptide. Pyr1-apelin-13 has a high affinity for the APJ receptor and is the main isoform in the heart. [92] The APJ receptor works as a bifunctional transducer. On one hand, it behaves as a mechanosensor to stimulate hypertrophy, as APJ-null cardiomyocytes display less hypertrophy to stretch stimuli, and whole hearts less hypertrophy following transverse aortic constriction (TAC). The load-induced hypertrophic response is G-protein independent but β-arrestin dependent. On the other hand, apelin binding activates Gαi, blunting stretch-induced hypertrophy initiated by the same receptor. This response is bidirectional as stretch also reduces G-protein signaling triggered by apelin binding the APJ receptor.[93] Apelin also blunts hypertrophic responses to other GPCR agonists such as angiotensin II.[94] Thus, its regulatory role is rather complex. In addition, apelin stimulates contractility, and this can be blocked by inhibiting phospholipase C (PLC), protein kinase C, Na^+/H^+ exchanger, and Na^+/Ca^{2+} exchanger,[95] much like Gαq-coupled signaling via PLC. Apelin increases force more in isolated failing than normal cardiac muscle due to increased intracellular Ca^{2+} and not alterations in myofilament Ca^{2+} sensitivity.[96] Several apelin agonists have been used with some success *in vitro* and *in vivo*.[97] Novartis is currently undergoing a phase-2 trial of CLR325 for HFrEF (NCT02696967), and assessing hemodynamic effects in patients with obesity and diabetes (NCT03449251). Amgen also has developed a small molecule apelin analog AMG 986, and is conducting studies to assess acute and chronic hemodynamic effects (NCT03276728).

Anti-inflammatory targets: IL-1β

Anti-inflammatory therapy started in earnest with efforts to block tumor necrosis factor alpha (TNF-α) as its elevation in HFrEF[98] was thought to contribute to chamber dysfunction.[99] However, the chimeric TNF-α neutralizing antibody (infliximab) provided no clinical benefit despite a modest rise in EF, and at higher doses, increased death or hospitalization from HF.[100] Etanercept, a TNF-α receptor-decoy to bind circulating TNF-α also failed to show clinical benefit.[101] Blockade of interleukin-6, TGF-β, and interleukin-18, have yielded mixed results, and broad immunosuppression with corticosteroids, intravenous

immunoglobulin, cyclosporine, and methotrexate have been unsuccessful.[99, 102] Interleukin-1, whose main circulating form is IL-1β, is a major acute and chronic pro-inflammatory chemokine. Following acute myocardial infarction, injured cells release the precursor peptide that is activated by assembly of the inflammasome. IL-1β is directly implicated in impaired systolic function by uncoupling adenyl cyclase from the βAR, reducing L-type Ca^{2+}channel current, and by downregulating phospholamban and SERCA2a, among other mechanisms.[103] Multiple animal studies have shown improved outcome after myocardial infarction (acute and more chronic) using various antibody approaches to neutralize the IL-1β stimulus (reviewed in[103]). In humans a pooled analysis of two small trials showed that blocking the IL-1 pathway with an IL-1 receptor antagonist (anakinra) early after myocardial infarction decreased the incidence of heart failure without a significant change in the risk of recurrent myocardial infarction[104]. The CANTOS trial reported in 2017 randomized 10061 subjects with stable coronary disease, a history of MI, and elevated high-sensitivity C-reactive protein (hsCRP) as a biomarker for inflammation, testing the role of canakinumab – a monoclonal antibody against IL-1β - versus placebo. Treatment lowered the rate of recurrent MI, though this came with a higher risk of fatal infection.[105] While not specifically powered for HF outcomes, a sub-group analysis found subjects hospitalized for HF on active treatment had lower levels of hsCRP that correlated with reduced hospitalization rate, and a composite of re-hospitalization and HF-related mortality.[106] This is the first evidence that anti-inflammatory therapy may provide benefit in HF, though in a select group of patients with ischemic heart disease. Sorting out the risk/benefit ratio is needed to move the approach forward. Not surprisingly, the astronomic cost of canakinumab at US market price of $73,000 per year, to be taken life-long after the diagnosis of heart failure is made, represents a major barrier for its widespread use. At the current price canakinumab is not cost-effective to be used for prevention of recurrent cardiovascular events and is estimated to need a price cut of 98% to reach this.[107]

Promising therapies that fell short in clinical trials

Relaxin peptide is secreted by the corpus luteum in early pregnancy and binds to relaxin family peptide receptor (RXFP1) to upregulate VEGF, anti-fibrotic matrix metalloproteinases, NO-mediated vasodilation, and downregulate TGFβ.[108] These effects led to its therapeutic assessment in HFrEF. The RELAX-AHF trial in acute decompensated HF[109] studied 1161 patients, and while breathlessness (primary endpoint) did not improve, 6 month mortality following brief exposure fell surprisingly. This triggered the 6545-patient RELAX-AHF-2 trial using a similar design which missed its primary endpoints (180 day mortality and reduction in 5-day worsening of heart failure). While the biology is intriguing, relaxin's fate remains in doubt.

Endothelin antagonism seemed a promising HF target given the hormone's role in pathological fibrosis, hypertrophy, hypertension, and upregulation in in HFrEF[110]. Despite encouraging preclinical data, multiple clinical trials found no benefit in HFrEF, some reporting adverse effects.[111] The cause likely involves competing effects in different cell types, receptor subtype selectivity, non-linear dose response signaling, and other factors.

Sarcoplasmic reticulum (SR) calcium cycling is abnormal in HF, contributing to depressed systolic and diastolic function. Limited Ca^{2+} uptake by the SR-ATPase[112] (SERCA2a) and diastolic leakage out of the SR by the ryanodine receptor (RyR2), both contribute to a fall in systolic Ca^{2+} and higher diastolic levels. SERCA2a expression and function declines in human and experimental heart failure[112], and its genetic upregulation benefits experimental HF models[113] and human myocardial tissue.[114] Translation to humans was less successful. In the CUPID IIb trial,[115] the largest HF gene-therapy study to date, intracoronary delivery of recombinant adeno-associated virus expressing SERCA2a in 250 HFrEF patients did not alter primary or secondary outcomes. Inadequate dosing with poor myocardial uptake of the viral cargo is proposed as a potential cause, and interest in the target remains particularly with small molecule approaches.

Inhibition of promiscuous RyR2 Ca^{2+} release has been effected using binding protein regulators to stabilize receptor open probability. FKBP12.6 has been targeted with small molecules known as Rycals® to reduce its dissociation from the RyR and thus diastolic Ca^{2+} leakage.[116] However, early-phase clinical testing in HFrEF was discontinued.

Biased ligand activation of GPCRs has the potential to affect receptor signaling beyond the primary G-protein, such as transactivating β-arrestin mediated cascades. TRV027 was developed to achieve this for the AT1R. In pre-clinical HF models, TRV027 lowered systemic resistance while improving contractility and renal blood flow.[117, 118] Based on this, a ~600 patient Phase-2b acute HF dose-finding study of TRV027 (BLAST-AHF) was performed[119], but found no benefit over placebo on the primary composite endpoint of 30-day survival, heart failure hospitalization, worsening heart failure, dyspnea score, or length of hospital stay.

Glucagon-like peptide GLP-1 is an endogenous incretin that enhances glucose-dependent insulin secretion and suppresses glucagon release.[120] Preclinical data suggested GLP-1 agonism benefits ischemia-reperfusion,[121, 122] and a pilot clinical study tested recombinant GLP-1 in severe HFrEF, finding improved LV function, clinical status and quality of life.[123] A subsequent large trial of liraglutide, a long acting GLP-1 derivative, in T2D patients without HF but at high CV risk found less all-cause and CV mortality,[124, 125] but non-fatal MI, stroke, and HF-hospitalizations incidence were unchanged. Subsequent trials of HFrEF patients, however, found some adverse effects (pro-arrhythmia and worsened heart failure)[126], or were neutral[127].

Emerging therapies from experimental models

Epigenetic modifiers: Histone deacetylase inhibitors, bromodomain and extraterminal (BET) bromodomain inhibitors, microRNAs, and long noncoding RNAs

Stress response signaling in HF is prominently affected by transcriptional changes regulated by epigenetic modifiers that facilitate or impede access to particular portions of the genome.[128] Genomic DNA is wound tightly around histones, but this changes upon acetylation, methylation, phosphorylation, and other modifications to regulate transcription. Relevant proteins are grouped as “writers” that turn on transcription, “erasers” that turn it off, and “readers” that enhance assembly of transcriptional machinery. Pharmacologic agents that

affect each of these processes can profoundly modify the cell's stress/growth response, and have applications in various diseases including cancer and cardiovascular disease.[129] However, given their ubiquitous involvement in signal transduction, and key roles in development and normal physiology, therapeutic epigenetic modification would seem likely to need careful application with target specificity.

Histone deacetylase inhibition (suppressing "writers") obtained by genetic models or pharmacological tool compounds have generated considerable interest given their anti-hypertrophic, anti-fibrotic, and other HF ameliorating properties, particularly in mice.[130] Surprisingly, even broad inhibitors appear tolerated in these animals, though large animal data remain scant. Further, there are some conflicting results based on the mode of inhibition and/or inhibitor used. At present, HDAC inhibitors are FDA-approved for treating lymphoma.[131]

Bromodomain and extra terminal (BET) proteins are epigenetic "readers" that bind to acetylated histones and augment the assembly of transcriptional machinery. The BET protein bromodomain-containing protein 4 (BRD4) displays increased expression in animal models of heart failure, and BRD4 blockade with the small molecule JQ1[132] reduces stress-induced left ventricular hypertrophy and dysfunction. Mechanisms of benefit from BRD4 inhibition in heart failure include inhibition of Nuclear Factor kappa-light-chain-enhancer of B cells (NFκB) and Transforming Growth Factor-β−signaling networks.[133] JQ1 improved disease in animal models with pre-existing heart failure and in human induced-pluripotent stem cell-derived cardiomyocytes, and did not suppress physiologic hypertrophy from exercise.[133] There are two planned trials testing the BET inhibitor RVX000222 (Resverlogix Corp) in high risk diabetic patients with coronary artery disease (NCT02586155) and apabetalone (Resverlogix) in pulmonary arterial hypertension (NCT03655704). No HF trials have yet been initiated.

Noncoding RNAs, both long (long non-coding RNAs or lncRNAs) and short (microRNAs or miRNAs) are master regulators of signal transduction. LncRNAs modify gene transcription and protein translation by recruiting histone modification complexes, X-chromosome inactivation, and regulating miRNAs among the mechanisms. LncRNAs have a role in cardiac pathologic remodeling to stress,[134–136] and activation of fibroblasts following cardiac injury.[137] The pre-clinical work has yet to translate to a therapeutic effort in humans. MicroRNAs regulate protein translation of messenger RNA. Though many miRNAs have been implicated in animal models of heart failure, and manipulated to therapeutically improve heart disease, their translational role has so far been more as potential disease biomarkers. Limitations to translation include the complexity of their introduction, particularly for miRNA mimetics, lack of organ/tissue specificity, multiplicity of mRNA targets for each miRNA (~1000 each), and longevity of the response. MiRNA mimetics have been tested in cancer trials, with mixed results.[138] MiRagen Therapeutics, Inc has two clinical trials at early stages for MRG201 (a miR-29 analogue), an anti-fibrotic miRNA that is being tested for keloid treatment (NCT03601052, Phase 2) and MRG110 (a miR-92a inhibitor) for wound healing (NCT03603431, Phase 1). No HF or cardiovascular studies are currently in progress.

Calcium-calmodulin kinase II

The serine-threonine kinase Ca^{2+}/Calmodulin activated kinase II (CaMKII) is a multimeric complex consisting of two hexameric assembled rings, with isoform subunits encoded by four different genes. The γ and δ isoforms are present in the heart, with the δ isoform being predominant. Each subunit contains an N-terminal catalytic domain, a C-terminal association domain, and a regulatory segment that functions as the autoinhibitory domain by sterically blocking substrate access to the catalytic site. The autoinhibitory domain contains sites for phosphorylation, O-GlcNAC modifications, oxidation, and for calmodulin recognition. Calcium activated calmodulin binding disrupts autoinhhibition of the catalytic domain and autophosphorylates the kinase to maintain activation independent of Ca^{2+}.[139] CamKII is also activated by methionine oxidation, independently of Ca^{2+}-calmodulin signaling.[140] Physiological activation of CamKII and in disease states leads to phosphorylation of L-type Ca^{2+} channels, increasing Ca^{2+} currents,[141–143] which facilitate sarcoplasmic reticulum (SR) Ca^{2+} release. CamKII also phosphorylates phospholamban, promoting SR Ca^{2+} uptake,[144] and phosphorylation of the ryanodine receptor, which increases both diastolic SR Ca^{2+} leak as well as systolic release (reviewed in[139]). As increased intracytoplasmic Ca^{2+} concentration is a common pathway in cardiac hypertrophy, heart failure, cardiac ischemia, and arrhythmias, it is not surprising that augmented CamKII activity has been associated with all of the these. Inhibition of CamKII activity has demonstrated to improve outcomes in several animal models of heart failure.[145] CamKII activity increases in human HFrEF, and its myocyte-specific overexpression induces heart failure and sudden cardiac death in mice. Increased neurohormonal activation, a hallmark of the heart failure syndrome, causes increased CamKII activation by β-adrenergic stimulation[145] and CamKII oxidation by angiotensin and aldosterone promotes cardiac injury.[140, 146]

A variety of CamKII inhibitors including competitive (KN-93 and KN-62), peptide-based (AC3-I, CN19o, CN19β) and ATP binding inhibitors, have been tested in preclinical models. Some have off-target effects and others remain inadequately screened. Rimacalib (SMP-114, Dainippon Sumitomo Pharma) completed phase II trials for treatment of rheumatoid arthritis, and other therapy programs are currently underway.[147] One concern over CamKII inhibitors is the risk of cognitive side effects if they penetrate the central nervous system, so must be avoided.

G-protein receptor kinase inhibitors

Upon GPCR agonism, the heterotrimeric G-protein complex dissociates with GTP binding to Gα-subunits to engage primary signaling. There are several mechanisms for reversing this, including removal of GTP to restore the complex and turn off receptor agonism, and phosphorylation of the receptor sub-units by G-protein receptor kinases (GRK) that recruit β-arrestins to reduce signaling and internalize the receptors. The first mechanism is controlled by regulators of G protein signaling (RGS), GTPase activating proteins that catalyze removal of GTP from Gα to turn receptor signaling off.[148] Reducing RGS2 removes early compensatory responses against pressure-overload in mice[68]. Alternatively, increasing RGS3 or RGS2 improves β2AR signaling in failing myocytes, and is observed *in*

vivo when HF with dyssynchronous contraction is treated with biventricular pacing – called cardiac resynchronization therapy[149].

The heart expresses multiple GRKs, with GRK2 and GRK5 being the most prominently linked to HF pathophysiology.[150] GRK2 has received the most attention to date. Its expression increases in experimental and human heart failure and is linked to desensitization of β-adrenergic receptors. Mice with myocyte-targeted GRK2 overexpression develop worse HF upon exposure to chronic isoproterenol[151] and conditional deletion of GRK2 is protective.[152]

Given the critical interaction of GRK2 with Gβγ dimers for membrane translocation and receptor modification, the first tactic taken was to express a carboxy-terminal GRK2 fragment (βARKct) that competitively binds to Gβγ,[153] and expression of this fragment after the onset of infarction is protective.[152] Translation using *in vivo* gene therapy has been reported in rabbit[154] and pig MI models.[155] Another approach is to inhibit kinase activity, and interestingly, a clinically used antidepressant serotonin reuptake inhibitor, paroxetine, was found to do this, as did a compound that also blocks Rho kinase-1.[156] However, the doses required are too high for human translation and more potent selective small molecules based on these structures are under development.[156] As with β-blockers, inhibiting GRK2 offsets β-adrenergic pathway desensitization, thereby increasing a signaling pathway thought to be detrimental to HF. However, where modulation is applied in this pathway matters, and the signaling coupled with GRK2 suppression does not mimic that from βAR agonism. For example, suppressing GRK2 also reduces direct signaling from Gβγ subunits, that activate ERK[157] and PLCε and are linked to hypertrophy.[158]

In addition to phosphorylating GPCRs, GRK5 contains a calmodulin-activated nuclear translocation sequence resulting in non-canonical control over gene transcription. In addition to phosphorylating class II HDAC-5 to disinhibit key pro-hypertrophic transcription factors (e.g. MEF2, NFAT, and SRF), GRK5 also directly stimulates NFκB and NFAT transcriptional activity (reviewed in[150]). This is eliminated if either the calmodulin binding domain is impeded or the nuclear localization sequence is removed. Genetic expression of solely the N-terminus calmodulin binding region as a sort of decoy to blunt GRK5 nuclear translocation reduces pathological hypertrophy both in vivo and in vitro.

Antioxidants: Thioredoxin, mitoTempo, and NO pathway enhancement

Despite the recognized role of oxidative stress in the pathophysiology of HF, antioxidant therapies have been disappointing. However, prior strategies were generally broad ROS scavengers, whereas more targeted intracellular approaches show greater promise. Two approaches, treatment with mitoTEMPO and increasing thioredoxin-2 signaling, both target mitochondrial-specific ROS. MitoTEMPO reduces both cytosolic and mitochondrial ROS, and treatment with mitoTEMPO improved LV function and reduced arrhythmia in a guinea pig HF model.[159] Thioredoxin proteins are essential for scavenging ROS and controlling apoptosis in the setting of ROS accumulation. Cardiac-specific deletion of the mitochondrial-specific thioredoxin-2 leads to LV dysfunction in mice,[160] and loss of function thioredoxin-2 mutations in humans are associated with dilated cardiomyopathy.[161] Augmentation of thioredoxin-2 signaling via inhibition of apoptosis stress kinase-1,

improves ventricular function in these mice, suggesting this as a relevant target to rescue heart failure in the setting of reduced thioredoxin-2 activity.[162] Gene therapy efforts to enhance thioredoxin and small molecular activators are being pursued.

Antioxidants to augment nitric oxide signaling have also been studied, but the results proved disappointing. Oxidation of NO synthase results in a decline in NO generation and rather generation of O_2^-. Supplementation with the critical cofactor tetrahydrobiopterin (BH4) to recouple NOS was effective in animal models,[163] but translation to humans was unsuccessful. One likely reason was that oral BH4 is first oxidized to BH2, a form that impairs NOS function, and must be first reduced to BH4 to be effective. This was less well achieved in humans than in mice, so levels required for antioxidant signaling were not achieved. Other studies, however, have found BH4 has benefits beyond antioxidant effects, suppressing inflammatory responses[164] and impacting T-cell function,[165] and so may prove therapeutic by other mechanisms.

Modulating intracellular calcium handling by S100A1

S100A1 is a multifaceted regulator of intracellular calcium handling, linking mitochondrial ATP generation to excitation-contraction coupling via interactions with SERCA2a, RyR2, and myocardial F1-ATPase.[166] S100A1 expression declines in human heart failure and experimental overexpression improves ventricular function and remodeling after infarction, and adeno-associated virus gene therapy improved cardiac performance in a porcine ischemic cardiomyopathy model.[167] Further development as gene therapy in humans is ongoing.

Summary

The armamentarium for HFrEF treatment has undergone various paradigm transitions, from one that was mostly hemodynamic in orientation, to those aimed at reversing maladaptive signaling pathways and/or enhancing beneficial ones. Advancing these molecular signaling discoveries to clinical therapy must confront the background therapy that we already have achieved, blocking βAR, AT1R, AldoR, restoring chamber synchrony (if contraction is dyssynchronous), and each of these confers many beneficial molecular signaling effects by themselves. Yet the recent positive results for combining neprilysin inhibition with AT1R, and the results of SGLT2 inhibition, indicate that this is not an impossible task, and that significant advances lay ahead. Perhaps the next paradigm will include an integration of existing and novel hemodynamic modulators with more patient-specific targeting of maladaptive molecular pathways, as opposed to the historical one size fits all approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table of Abbreviations:

HFrEF, HFpEF	heart failure with a (reduced, preserved) ejection fraction
β1AR, β2AR	beta-1 or beta-2 adrenergic receptor
PKA	protein kinase A
ERK1/2	extracellular response kinase ½
Gai	inhibitory G protein alpha subunit
Gas	stimulatory G protein alpha subunit
cAMP	cyclic adensine monophosphate
cGMP	cyclic guanosine monophosphate
GRK2, GRK5	G-receptor kinase 2; 5
SERCA2a	sarcoplasmic reticular calcium ATPase 2a
RAAS	renin angiotensin aldosterone system
AT1R	angiotensin type 2 receptor
AldoR	aldosterone receptor
ACE	angiotensin converting enzyme
ARB	angiotensin receptor blocker
NEP	neprilysin
ANP	A-type natriuretic peptide
BNP	B-type natriuretic peptide
NO	nitric oxide
SGLT2	sodium-glucose co-transporter 2
SLC5	sodium-glucose co-transporter family
T2D	type 2 diabetes mellitus
CVD	cardiovascular disease
OM	omecamtiv mercarbiil
PDE	phosphodiesterase

PKG	protein kinase G
TRPC6	transient receptor potential canonical channel subtype 6
RGS2/4	regulator of G-protein signaling type 2 or type 4
sGC	soluble guanylate cyclase
APJ	Apelin
PLC	phospholipase C
TGFb	tissue growth factor beta
IL-1b	interleukin 1-beta
hsCRP	high sensitivity C reactive protein
RyR2	ryanodine receptor type 2
GPCR	G protein coupled receptor
GLP-1	glucogon like peptide – 1
BRD4	bromodomain-containing protein 4
BET	bromodomain and extra terminal
miRNA	microRNA
lncRNA	long non-coding RNA
CaMKII	calcium calmodulin activated kinase II
MEF2	myocyte enhancer factor 2
NFAT	nuclear factor of activated T cells
SRF	serum response factor
ROS	reactive oxygen species
BH4	tetrahydrobiopterin

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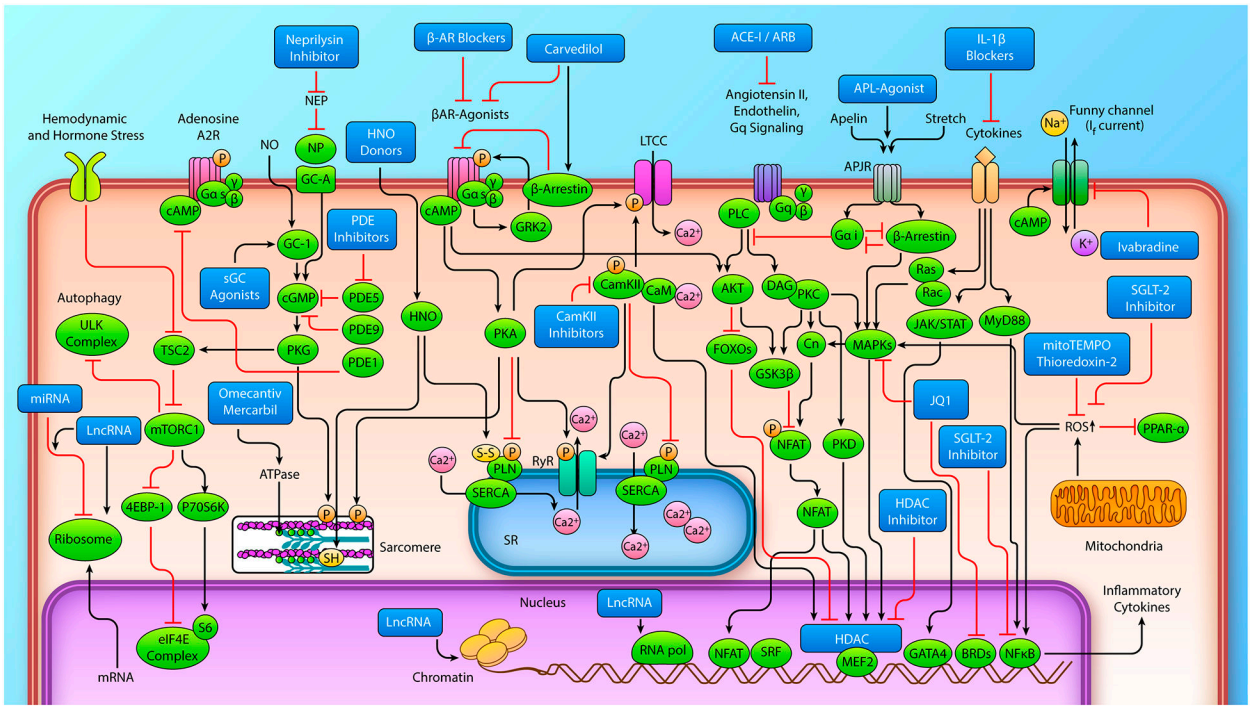

Figure 1. Myocyte signaling approaches for the treatment of Heart Failure with Reduced Ejection Fraction.

Individual therapeutic approaches are identified in the figure by the dark blue boxes. Abbreviations are: miRNA, micro ribonucleic acid; mRNA, messenger ribonucleic acid; lncRNA, long noncoding ribonucleic acid; PI3K, phosphoinositide 3-kinase; TSC2, tuberous sclerosis complex-2; mTORC1, mechanistic target of rapamycin complex 1; 4EBP-1, eukaryotic translation initiation factor 4E binding protein 1; p70S6K, ribosomal protein S6 kinase; eIF4E, eukaryotic translation initiation factor 4E; S6, ribosomal protein S6; ULK, UNC-51 like kinase; NO, nitric oxide; NP, natriuretic peptide; ANP, atrial natriuretic peptide; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; PKG, protein kinase G; PKC, protein kinase C; PKD, protein kinase D; SH, thiol modification; S-S, disulfide; DAG, *diacylglycerol*; HNO, nitroxyl; β-AR, β-adrenergic receptor; cAMP, cyclic adenosine monophosphate; GRK2, G protein-coupled receptor kinase 2; PKA, protein kinase A; PLN, phospholamban; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; FOXO, forkhead box O; SR, sarcoplasmic reticulum; RyR, ryanodine receptor; LTCC, L-type calcium channel; CAMKII, calcium-calmodulin-dependent kinase II; CaM, calmodulin; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; APL-agonist, apelin agonist; IL-1β, interleukin-1β; GSK3-β, glycogen synthase kinase 3-β; NFAT, nuclear factor of activated T cells; MAPKs, mitogen-activated protein kinase; SRF, serum response factor; MEF2, myocyte enhancer factor 2; HDAC, histone deacetylase; GATA4, GATA binding protein 4; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; RNA pol, *RNA polymerase*; SGL-T2 inhibitors, sodium-glucose transporter 2 inhibitors; PPAR-α, peroxisome proliferator activated receptor-α; MyD88, myeloid differentiation primary response gene 88; JAK, janus kinase; STAT, signal transducer and activator of transcription; Gsα, stimulatory G protein α-

subunit, Gqα, G protein-q α-subunit; PLC, Phospholipase C; P, phosphorylation modification; ROS, reactive oxygen species. (Illustration Credit: Ben Smith).

Table 1.

New pharmacologic signaling approaches for HFrEF under active clinical investigation

Drug class	Proposed mechanism of action	Name of drug (Company)	Current clinical research phase
Direct sarcomere activators	Directly activates myosin	Omecamtiv mercarbil (Cytokinetics)	Phase 3
Direct sarcomere activators	Directly activates myosin	MYK-491 (MyoKardia, Inc.)	Phase 1b/2a
Phosphodiesterase inhibitors	Inhibits PDE9, augments cGMP	CRD-733 (Cardurion Pharmaceuticals)	Phase 1
Phosphodiesterase inhibitors	Inhibits PDE1, augments cGMP and cAMP	ITI-214 (Intracellular Therapies, Inc.)	Phase 1b/2a
Nitroxyl donors	Antioxidant, enhances calcium sensitivity and calcium handling, augments NO signaling	BMS-986231 (Bristol-Myers Squibb)	Phase 2b
Soluble guanylate cyclase stimulators	Augments cGMP-PKG signaling	Vericiguat (Bayer/Merck)	Phase 3
Apelin	Gαi activation and Inhibition of β-arrestin, ERK activation	CLR325 (Novartis); AMG986 (Amgen)	Phase 3; Phase 1
Anti-inflammatory agents	Blocks inflammatory interleukin 1β	Canakinumab (Novartis)	Phase 3

HFrEF, Heart failure with reduced ejection fraction; PDE, phosphodiesterase; cGMP, cyclic guanosine monophosphate; cAMP, cyclic adenosine monophosphate; NO, nitric oxide; PKG, Protein Kinase G; ERK, extracellular signal-regulated kinase

Table 2.

Promising therapies for HFrEF that fell short in clinical trials

Drug class	Name of drug (Company)	Patient population studied	Reason for failure
Synthetic relaxin	Serelaxin (Novartis)	Acute decompensated heart failure	No benefit
Endothelin antagonists	Bosentan (Actelion), macitentan (Actelion), ambrisentan (Gilead)	Chronic HFrEF	No benefit, signal for harm
Sarcoplasmic reticulum ATP-ase gene therapy	SERCA2a gene therapy (Celladon Corporation)	Chronic HFrEF	No benefit, possible underdosing, issues with delivery
Ryanodine Receptor stabilizers	Rycals (ARMGO)	Chronic HFrEF	No benefit. Early termination
Biased G-protein coupled receptor signaling	TRV027 (Trevena)	Acute decompensated heart failure	No benefit
Glucagon-like peptide 1 agonism	Liraglutide (Novo Nordisk)	Chronic HFrEF	No benefit, signal for harm

HFrEF, Heart failure with reduced ejection fraction; ATP, adenosine triphosphate

Table 3.

Emerging therapies for HFrEF that have shown promise in experimental models

Mechanistic approach	Drug class	Model studied
Epigenetic modifiers	Histone deacetylase inhibitors	Genetic deletion, ischemia-reperfusion, pressure overload, mice
Epigenetic modifiers	Bromodomain and extraterminal bromodomain (BET) inhibitors	Presure overload and ischemic cardiomyopathy, mice; induced pluripotent stem cell cardiomyocytes, human
Epigenetic modifiers	Long noncoding RNAs	Pressure overload and ischemic cardiomyopathy, mice; embryonic stem cell-derived cardiomyocytes, human
Epigenetic modifiers	microRNAs	
Calcium-calmodulin kinase II inhibitors	Calcium-calmodulin kinase II inhibitors	Ischemic cardiomyopathy, chronic catecholamine infusion, mice
G-protein receptor kinase inhibitors	G-protein receptor kinase 2 and 5 inhibitors	Chronic catecholamine infusion, mice; ischemic cardiomyopathy, mice, rabbits, and pigs
Mitochondrial ROS scavengers	MitoTEMPO	Combined pressure overload and chronic catecholamine infusion, guinea pig
Mitochondrial ROS scavengers	Apoptosis stress kinase-1 inhibitors to augment thioredoxin-2 signaling	Spontaneous cardiomyopathy in cardiac-specific thioredoxin-2 genetic deletion, mice
Antioxidants	Tetrahydrobiopterin to enhance eNOS-generation of NO, suppress inflammation	Pressure overload, mice
Intracellular calcium modulation	S100A1	Ischemic cardiomyopathy, pigs

HFrEF, Heart failure with reduced ejection fraction; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; NO, nitric oxide