

Clinical update

Cardiac inotropes: current agents and future directions

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Intrinsic inotropic stimulation of the heart is central to the regulation of cardiovascular function, and exogenous inotropic therapies have been used clinically for decades. Unfortunately, current inotropic drugs have consistently failed to show beneficial effects beyond short-term haemodynamic improvement in patients with heart failure. To address these limitations, new agents targeting novel mechanisms are being developed: (i) istaroxime has been developed as a non-glycoside inhibitor of the sodium-potassium-ATPase with additional stimulatory effects on the sarcoplasmic reticulum calcium pump (SERCA) and has shown lusitropic and inotropic properties in experimental and early clinical studies; (ii) from a mechanistic point of view, the cardiac myosin activators, directly activating the acto-myosin cross-bridges, are most appealing with improved cardiac performance in both animal and early clinical studies; (iii) gene therapy approaches have been successfully employed to increase myocardial SERCA2a; (iv) nitroxyl donors have been developed and have shown evidence of positive lusitropic and inotropic, as well as potent vasodilatory effects in early animal studies; (v) the ryanodine receptor stabilizers reduce pathological leak of calcium from the sarcoplasmic reticulum with initial promising pre-clinical results; and finally, (vi) metabolic energy modulation may represent a promising means to improve contractile performance of the heart. There is an urgent clinical need for agents that improve cardiac performance with a favourable safety profile. These current novel approaches to improving cardiac function provide the hope that such agents may soon be available.

Keywords Inotropes • Drugs • Therapies

Introduction

In 1986, two review articles on new positive inotropic agents for the treatment of congestive heart failure were published,^{1,2} presenting a number of promising developments. However, over 24 years later, the only inotropic agent recommended, and weakly at that, in the European Society of Cardiology (ESC) Guidelines for the treatment of chronic heart failure is digitalis, a drug introduced in the eighteenth century.³ In the setting of acute heart failure, inotropic agents are only recommended in patients with systolic blood pressure <90 mmHg and evidence of inadequate organ perfusion despite other therapeutic interventions.

Why have so many promising inotropic drugs failed to demonstrate beneficial clinical outcomes in patients with heart failure? The therapeutic hypothesis is compelling; central to the pathogenesis of systolic heart failure is decreased left ventricular (LV)

contractile function. The initial insults (myocardial infarction, other cardiomyopathies, hypertension, etc.) that cause decreased LV function set into motion an inexorable series of maladaptive haemodynamic, remodelling, and neurohormonal responses that result in heart failure, clinical deterioration, and ultimately death. It would seem intuitively obvious that improving LV function should halt the progression of disease and improve clinical outcomes. A recent analysis supports this concept, where clinical device or drug trials that demonstrate long-term improvement in LV function are associated with improved survival.⁴ Furthermore, the success of cardiac resynchronization therapy in reducing heart failure events and improving survival clearly supports the hypothesis.⁵ However, there is no similar supportive evidence for inotropic agents. While there may be many reasons for the failure of currently available inotropes to improve clinical outcomes, the adverse effect of these agents on myocardial energetics and intracellular calcium may play

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an important role. While most inotropic agents increase energy consumption and intracellular calcium, inotropic stimulation through cardiac resynchronization therapy does not. Thus, the success story of cardiac resynchronization by biventricular pacing therapy may indicate that the future search for inotropic intervention in heart failure is not hopeless, and that inotropic mechanisms that avoid these liabilities may be clinically beneficial.

What defines an inotropic intervention?

Inotropic interventions comprise all means that increase muscular contractile force, and in particular, the contractile force of the myocardium. The mechanisms underlying the regulation of myocardial force production can best be explained at the level of the smallest force-producing unit, the acto-myosin cross-bridge using a simplified two-stage cross-bridge model (Figure 1). During a cross-bridge cycle, the myosin head attaches to actin, rotates to produce force, which is maintained during the so-called on-time (Figure 2). Thereafter, the cross-bridge detaches again to enter its non-force-producing state for the duration of the off-time. The on-time and the unitary force production of the cross-bridge define the force–time integral of the individual cross-bridge cycle. Accordingly, contractile force depends on the number of cross-bridges attached per unit of time. The cross-bridges are activated by calcium binding to troponin C with the subsequent conformational changes of tropomyosin and troponin I to facilitate acto-myosin interaction. The muscle relaxes when calcium is pumped back into the sarcoplasmic reticulum (SR) by the sarcoplasmic reticulum calcium pump (SERCA) and eliminated outside the cell by the sodium–calcium exchanger (NCX).⁶ Inotropy, i.e. the number of cross-bridges activated, depends upon: (i) the amount of calcium available to bind to troponin C, (ii) the calcium affinity of troponin C, and (iii) alterations at the level of the cross-bridge cycle including promotion of the force-producing cross-bridge state, cross-bridge unitary force production, and prolongation of the on-time, and thus the duration of the force-producing state.

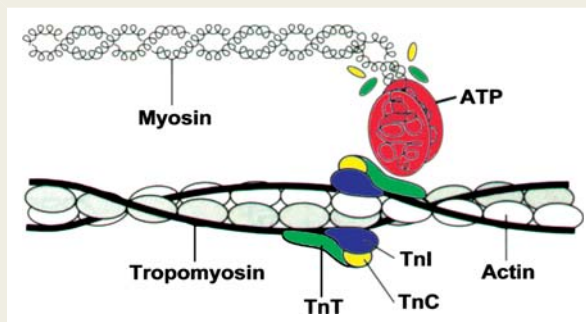


Figure 1 Acto-myosin interaction. The myosin head carrying the ATPase site combines with actin to produce force. Calcium binding to troponin C (TnC) results in a conformational change of tropomyosin, troponin I (TnI), and troponin T (TnT), allowing the myosin head to attach to actin, facilitating the acto-myosin cross-bridge to cycle (see also Figures 2 and 6).

Furthermore, the ability of attached cross-bridges to increase calcium affinity of troponin C and activate neighbouring cross-bridges (i.e. co-operativity) may also increase contractile force.

The mechanisms underlying the increase in inotropy may also influence the velocity of contraction and relaxation as well as energy consumption of the myocardium. It is assumed that one molecule of ATP is hydrolysed during one individual cross-bridge cycle. Accordingly, the most efficient way to increase contractile force would be to prolong cross-bridge attachment time, but this mechanism might reduce the velocity of force development and relaxation. However, it is also recognized that there is a basal rate of non-force-generating ATP hydrolysis by myosin,⁷ such that mechanisms that increase the likelihood of force-generating hydrolysis with cross-bridge formation would potentially increase performance with no adverse effect on energetics. Thus, efficiency (economy) of contraction and contractile performance may diverge depending on the inotropic mechanism.

How does the heart regulate its inotropic state?

Endogenous inotropic mechanisms include (i) length-dependent activation of cross-bridges, (ii) contraction frequency-dependent activation of contractile force, and (iii) catecholamine-mediated inotropy.⁶ The most important mechanism to regulate the basal contractile force of the heart is length-dependent activation of cross-bridges by increasing the length of the individual sarcomere (Frank-Starling mechanism). This length-dependent activation occurs without an increase in calcium release, partially by spatial changes and increased co-operativity.⁸ Frequency-dependent up-regulation of contractile force is calcium dependent. With increasing heart rate, more calcium enters the cardiomyocyte, is accumulated into the SR, and is available for release during the next contraction, resulting in more recruited cross-bridges and increased contractile force.⁹ Catecholamines increase contractile

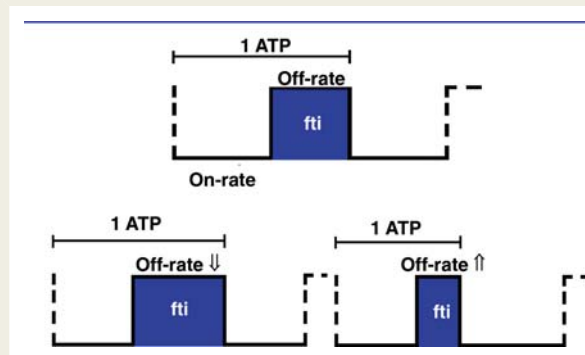


Figure 2 Cartoon of a simple two-state on-off cross-bridge model. Assuming that one molecule of ATP is hydrolysed during each cycle, the duration of the on-state determines cross-bridge economy: prolonged attachment increases and shortened decreases cross-bridge economy. Force development of the muscle depends on the number of cross-bridges attached per unit of time. fti, cross-bridge force-time integral.

force by the β -adrenoceptor-adenylyl cyclase system or by stimulation of α -receptors. Through protein kinase A, the β -adrenoceptor system phosphorylates L-type calcium channels to increase calcium influx and ryanodine receptors (RyRs) to increase SR calcium release resulting in activation of cross-bridges. In addition, phosphorylation of phospholamban accelerates SR accumulation of calcium and relaxation which is supported by phosphorylation of troponin I due to reduced calcium sensitivity of troponin C (positive lusitropy). At the cross-bridge level, cyclic AMP (cAMP)-mediated increase in contractility has been reported to reduce the attachment time of the individual cross-bridge. Consequently, cAMP-mediated inotropy increases the rate of force development and rate of relaxation at the expense of a reduced economy of contraction.⁹

Alterations of the inotropic state in heart failure

In heart failure, excitation–contraction coupling is significantly altered, largely by abnormal calcium accumulation of the SR (Figure 3). Calcium enters the cell following activation of the L-type calcium current during the upstroke of the action potential. This calcium triggers the release of a larger amount of calcium by activating the RyR, which is the calcium release channel of the SR. Released calcium binds to troponin C to activate acto-myosin cross-bridges, inducing myocyte contraction. For relaxation, calcium is transported back to the SR by SERCA and eliminated outside the cell through the NCX. In heart failure, SR calcium uptake is abnormal due to SR calcium leak through RyR, decreased re-uptake of calcium secondary to decreased SERCA protein levels, and increased calcium elimination outside the cell due to increased levels of the NCX. Disturbed SR calcium accumulation is also the main mechanism underlying inversion of the force-frequency relation (see above, ‘How does the heart regulate its inotropic state?’). In the failing myocardium,

frequency-dependent up-regulation of SR calcium load is absent, which is associated with a decline of contractile force at higher heart rates.⁹

Current inotropes

Current inotropic drugs include cardiac glycosides, β -adrenoceptor agonists, phosphodiesterase (PDE) inhibitors, and calcium sensitizers (Figure 4, Table 1).^{1,2} Cardiac glycosides inhibit the sodium-potassium-ATPase (Na^+/K^+ -ATPase) resulting in sodium accumulation which in turn promotes cellular calcium accumulation by influencing driving forces of the NCX. Providing intact SR function, calcium accumulates in the SR, ready for release during the next twitch. As explained above, β -adrenoceptor stimulation increases intracellular cAMP that activates protein kinase A to phosphorylate key calcium-cycling proteins. Phosphodiesterase inhibitors prevent cAMP degradation, thus increasing cAMP activation of protein kinase A. Because β -adrenoceptor density is reduced in heart failure, PDE inhibitors have been assumed to be more effective in heart failure patients. The beneficial effects of both catecholamines and PDE inhibitors are directly a result of their ability to increase intracellular calcium, which is also the direct mechanism of the adverse effects of these agents, including myocardial ischaemia and arrhythmias. Calcium sensitizers increase contractile force without increasing intracellular calcium release. The molecular mechanism of current calcium sensitizers is at the level of troponin C through increased calcium affinity or more downstream through alterations of cross-bridge kinetics (1, 2).

Current clinical use of inotropes

According to current ESC guidelines, cardiac glycosides (digoxin) are indicated in patients with heart failure and atrial fibrillation to

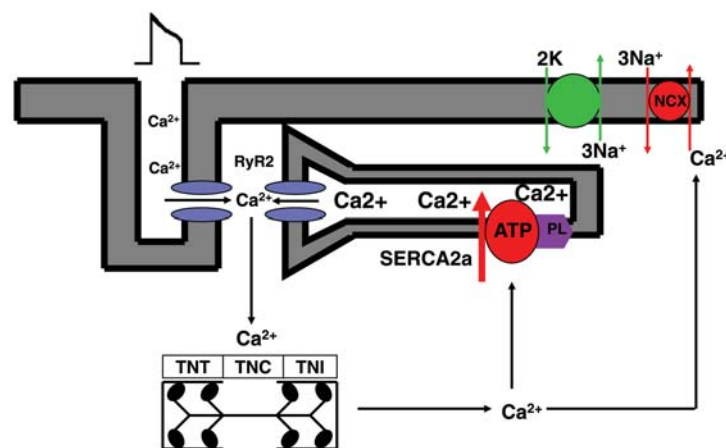


Figure 3 Excitation–contraction coupling. Calcium enters the cell through the L-type calcium channel during the action potential and triggers the release of a larger amount of calcium from the sarcoplasmic reticulum. This calcium binds to troponin C resulting in activation of contractile proteins. The muscle relaxes when calcium is removed into the sarcoplasmic reticulum by sarcoplasmic reticulum calcium pump or outside the cell by the sodium-calcium exchanger. RyR2, ryanodine receptor 2; TNT, troponin T; TNC, troponin C; TNI, troponin I; PL, phospholamban.

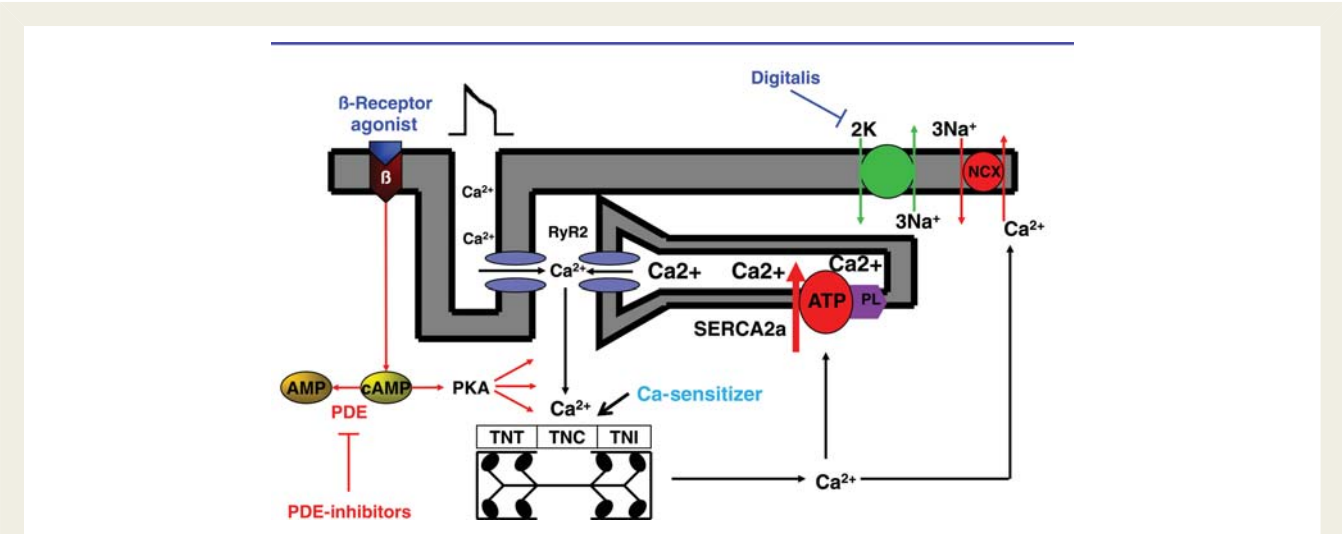


Figure 4 Inotropic mechanisms and current inotropic interventions. Activation of the β -adrenoceptor stimulates adenyl cyclase to produce cAMP, which activates protein kinase A (PKA) to phosphorylate intracellular calcium-cycling proteins. Phosphodiesterases (PDEs) degrade cAMP. Phosphodiesterases are inhibited by Phosphodiesterase inhibitors. Digitalis inhibits transport of three sodium ions for two potassium ions through Na/K-ATPase. Calcium sensitizers increase the affinity of troponin C for calcium.

Table 1 Inotropic mechanisms and drugs	
Inotropic mechanism	Drugs
Sodium-potassium-ATPase inhibition	Digoxin
β -Adrenoceptor stimulation	Dobutamine, dopamine
Phosphodiesterase inhibition	Enoximone, milrinone
Calcium sensitization	Levosimendan
Sodium-potassium-ATPase inhibition plus SERCA activation	Istaroxime
Acto-myosin cross-bridge activation	Omecamtiv mecarbil
SERCA activation	Gene transfer
SERCA activation plus vasodilation	Nitroxyl donor; CXL-1020
Ryanodine receptor stabilization	Ryanodine receptor stabilizer; S44121
Energetic modulation	Etomoxir, pyruvate

control the ventricular rate.³ In patients with sinus rhythm, digoxin may be given to symptomatic patients with chronic systolic heart failure to improve ventricular function and patient well-being, and to reduce hospitalization, but without improving survival. Non-glycoside inotropic agents should only be used in patients with acute heart failure with low blood pressure or cardiac output in the presence of signs of hypoperfusion or congestion. Available agents include cAMP-elevating drugs such as dobutamine, dopamine, milrinone, and enoximone.¹⁰ However, cAMP-mediated inotropic stimulation may impair survival, in particular, in patients with coronary artery disease. This adverse effect has been demonstrated by several clinical trials, including the OPTIME-CHF trial that randomized patients with acute heart failure to milrinone or placebo infusion. Milrinone was not superior to placebo regarding the primary endpoint of number of days hospitalized for

cardiovascular causes within 60 days after randomization, and milrinone was associated with more adverse events, with significant increases in atrial fibrillation/flutter, ventricular tachycardia/fibrillation, and sustained hypotension.¹¹ In addition, milrinone patients suffering from coronary artery disease had worse outcomes, including increased mortality.¹² Moreover, in the ESCAPE trial, use of inotropes was associated with a significantly increased risk of all-cause mortality.¹³

Use of the calcium sensitizer levosimendan may be more favourable. Levosimendan has a unique mechanism of action. It binds to troponin C depending on the actual calcium concentration. This may result in drug binding only during high systolic calcium levels but not during diastole when calcium is low. By this on–off mechanism, levosimendan may increase calcium sensitivity only during systole without impairing diastolic relaxation.¹⁴ In addition, levosimendan activates ATP-dependent potassium channels in smooth muscle cells resulting in vasodilation.¹⁵ At higher concentrations, levosimendan also acts as a PDE inhibitor.¹⁴ A number of clinical trials have been performed demonstrating inotropic and vasodilating properties of levosimendan in patients with heart failure including cardiogenic shock: an increase in stroke volume and cardiac output and a decrease in pulmonary capillary wedge pressure (PCWP).¹⁰ Levosimendan possesses unusual pharmacokinetics with a biological half-life of its active metabolite extending over several days, explaining the recommendation to administer the drug for only 24 h using continuous infusion. However, some caution in the use of levosimendan, particularly with bolus loading doses, may be warranted given the results of the only placebo-controlled acute heart failure trial, REVIVE II, which demonstrated an early increase in mortality, as well as increased atrial fibrillation/flutter, ventricular ectopy, and sustained hypotension.¹⁶ A recent meta-analysis of studies in patients with acute severe heart failure showed that levosimendan has favourable haemodynamic effects superior to placebo or dobutamine and

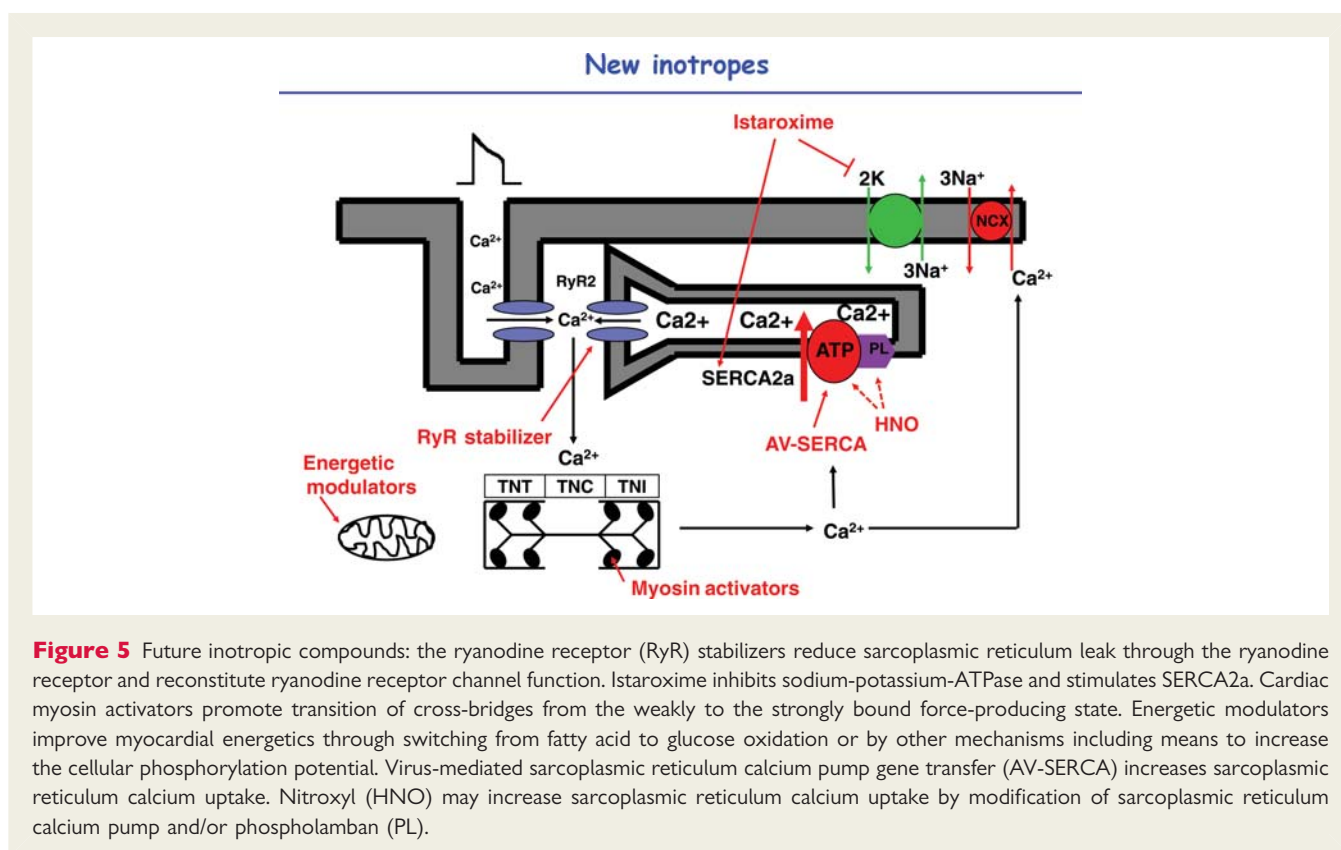


Figure 5 Future inotropic compounds: the ryanodine receptor (RyR) stabilizers reduce sarcoplasmic reticulum leak through the ryanodine receptor and reconstitute ryanodine receptor channel function. Istaroxime inhibits sodium-potassium-ATPase and stimulates SERCA2a. Cardiac myosin activators promote transition of cross-bridges from the weakly to the strongly bound force-producing state. Energetic modulators improve myocardial energetics through switching from fatty acid to glucose oxidation or by other mechanisms including means to increase the cellular phosphorylation potential. Virus-mediated sarcoplasmic reticulum calcium pump gene transfer (AV-SERCA) increases sarcoplasmic reticulum calcium uptake. Nitroxyl (HNO) may increase sarcoplasmic reticulum calcium uptake by modification of sarcoplasmic reticulum calcium pump and/or phospholamban (PL).

suggested that levosimendan was associated with reduced mortality compared with dobutamine.¹⁷

Future directions

Promising new inotropic agents have been developed during the last decade (Table 1). They are based on pathophysiological defects identified in heart failure and include (i) istaroxime, which is an inhibitor of Na^+/K^+ -ATPase and an activator of SERCA, (ii) cardiac myosin activators, (iii) increasing myocardial SERCA through gene therapy, (iv) nitroxyl donors, (v) stabilizers of the RyR, and (vi) energetic modulation (Figure 5).

Istaroxime—a new luso-inotropic agent?

Istaroxime [(E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione] has been identified in the search for new inotropic agents acting at the Na^+/K^+ -ATPase. Istaroxime does not have a glycoside-like structure and in addition to its inhibitory effects on Na^+/K^+ -ATPase, it has been suggested to stimulate SERCA.¹⁸ Inhibition of Na^+/K^+ -ATPase increases intracellular sodium, which reduces the driving force for the NCX, decreasing calcium elimination outside the cell. Moreover, increased sodium may stimulate the NCX to function in the reverse mode of transporting calcium intracellularly. Calcium influx into the cytosol may, however, be harmful in the failing heart with reduced SERCA activity and elevated diastolic calcium levels. Under

those circumstances, Na^+/K^+ -ATPase inhibition by elevating cytosolic calcium may not only impair diastolic function, but it may also induce delayed after-depolarization and cardiac arrhythmias. Therefore, additional effects on top of Na^+/K^+ -ATPase inhibition, which promote calcium uptake of the SR, may be crucial to the potential success of this type of inotropic agent. In a study of guinea pig myocytes, Micheletti *et al.*¹⁹ demonstrated that istaroxime increased twitch amplitude and accelerated relaxation without the after-contractions seen with digoxin. In various dog studies, istaroxime increased the maximum rates of rise and fall in LV pressure and decreased end-diastolic pressure and volume without a change in the heart rate and the blood pressure. Most importantly, these inotropic and lusitropic effects were different from those of digoxin and have not been associated with an increase in myocardial oxygen consumption.^{20,21} Thus, from animal experiments istaroxime shows a favourable profile with increased inotropy and accelerated relaxation without associated increased energy consumption.

The HORIZON trial evaluated the haemodynamic, echocardiographic, and neurohormonal effects of intravenous istaroxime in 120 patients hospitalized with heart failure and reduced ejection fraction.²² In this randomized, double-blind, placebo-controlled, dose-escalating study, three doses of istaroxime or a placebo were given as intravenous infusions over 6 h to patients with a history of heart failure and a PCWP >20 mmHg. A reduction in PCWP was the primary endpoint, which was attained in all three dose groups during the entire observation period of 6 h. There was an increase in systolic blood pressure and a transient increase in cardiac index with the highest dose and a decrease in heart rate

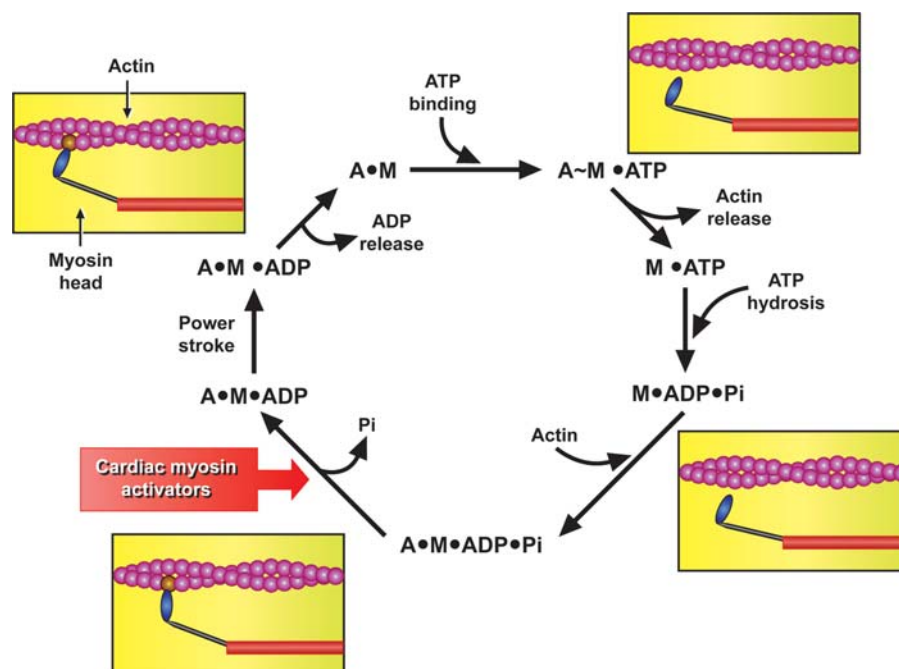


Figure 6 Mode of action of cardiac myosin activators. The agents promote actin-dependent phosphate release (P_i -release) moving the cross-bridge into its strongly bound force-producing state (see text). A, actin; M, myosin.

and diastolic and systolic volume, without a change in ejection fraction. As indicators for improved diastolic function, E-wave deceleration time and E_a velocity increased, and the E/E_a ratio decreased. Istaroxime shortened QT_c and was well tolerated with a short half-life of 1 h. Thus, the haemodynamic profile of istaroxime reflects inotropic and lusitropic effects without any indication of vasodilatory properties. The limitation of this study is related to the fact that patients included presented with milder forms of acute heart failure, not requiring inotropic interventions according to current guidelines.

Cardiac myosin activators—increasing myocardial performance

Cardiac myosin activators represent a new class of compounds, which directly influence the cross-bridge cycle. These molecules accelerate the rate of actin-dependent phosphate release of the weakly bound acto-myosin cross-bridge (the rate-limiting step of the cross-bridge cycle). This promotes transition to the force-producing on-state of the cross-bridge. (Figure 6)²³ As a consequence, more cross-bridges enter the force-producing state, more cross-bridges are activated per unit of time, and contractile force increases (Figure 2). Several compounds have been investigated so far.²⁴ These agents stimulate myosin-ATPase and increase fractional shortening of myocytes without increasing intracellular calcium transients. The increase in myocyte shortening is associated with an increase in time-to-peak contraction with unaltered velocity of contraction.

The molecule omecamtiv mecarbil (formerly CK-1827452) has recently been studied in two different dog models of heart failure.²⁵ Both included tachycardia-pacing-induced failure on top of myocardial infarction in one model and pressure overload by constriction of the ascending aorta in the other. In both models, omecamtiv mecarbil increased stroke volume and cardiac output, and decreased LV end-diastolic pressure and heart rate. In addition, omecamtiv mecarbil increased LV systolic ejection time (SET) by 26%. Importantly, these improvements in cardiac function were not associated with increased myocardial oxygen consumption.

Omecamtiv mecarbil has advanced into clinical studies. The first-in-human study assessed the effect of omecamtiv mecarbil in ascending dose cohorts of healthy volunteers ($n = 34$) and demonstrated dose- and concentration-dependent increases in the SET, stroke volume, fractional shortening, and ejection fraction.²⁶ A subsequent study in patients with heart failure presented similar findings.²⁷ Due to concerns that prolongation of SET might adversely impact diastolic-filling, particularly during exercise, another study, enrolling 94 patients with documented ischaemic cardiomyopathy, exercise-induced angina, reduced ejection fraction, and symptomatic heart failure, evaluated the effect of intravenous omecamtiv mecarbil on symptom-limited exercise tolerance.²⁸ In these patients, there was no deleterious effect of omecamtiv mecarbil on exercise tolerance. No doubt, the cardiac myosin activators are a very interesting new development in inotropic heart failure therapy. While the characteristic increase of SET may be a matter of concern, as long as relaxation is not prolonged and heart rate not too high, the increased ejection period should be well tolerated, as suggested by the exercise study.

Gene therapy approaches to increase sarcoplasmic reticulum calcium pump activity—stimulating the calcium pumps

Various gene therapy strategies have been proposed to correct abnormal excitation–contraction coupling in heart failure and increase inotropy. Most approaches are related to reduced SR calcium uptake, however, abnormal SR leak has also been considered. It has been shown in isolated myocytes that overexpression of the RyR-regulatory protein FKBP12.6 increases SR calcium content and fractional shortening.²⁹

A number of studies have been performed to evaluate the possibility of SERCA gene transfer. Up-regulating SERCA2a, the cardiac isoform of the sarco-endoplasmic reticulum calcium ATPase, in different animal models of heart failure results in improvement in systolic and diastolic function^{30,31} and may reduce arrhythmias.³² A recent paper on a sheep model of myocardial infarction and mitral regurgitation showed that SERCA up-regulation improves function and reduces the remodelling processes.³³ The Calcium Up-regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) study, enrolled with a total of 39 patients with severe heart failure randomized to adeno-associated virus-mediated transfer of SERCA2a or placebo, has been recently presented.³⁴ As a phase 2 study, there was no single primary endpoint, but when a broad range of efficacy and safety endpoints were evaluated, there were very encouraging signals in improvement in symptoms and ventricular remodelling.³⁵ Problems related to gene transfer approaches to increase inotropy include (i) immunological reactions, (ii) duration of gene expression, (iii) control of gene expression, and (iv) unknown toxic effects, which are related to the vector used to transfer a gene. Currently, new-generation adeno-associated viruses have brought considerable progress in the field.

Nitroxyl—nitric oxide's soon-to-be famous sibling?

Nitric oxide (NO) is well known as an important signalling molecule central to the regulation of vascular tone and other cardiovascular processes, but NO's one-electron-reduced and protonated sibling, nitroxyl (HNO), is currently less well known. While HNO and NO are both gaseous signalling molecules and can be potent vasodilators, HNO appears to have additional unique signalling pathways and mechanisms independent of NO.^{36,37} *In vitro* experiments have demonstrated HNO-induced vasorelaxation in isolated large conduit and small resistance arteries, as well as intact coronary and pulmonary vascular beds, and HNO is a potent arterio- and venodilator in intact animal studies. While some of these vasorelaxant properties may be mediated by soluble guanylate cyclase (sGC), other mechanisms are also important in HNO-induced vasodilation, including increased circulating neuropeptide calcitonin gene-related peptide levels and activation of vascular smooth muscle potassium channels (perhaps both K_v and K_{ATP} -channels). While the importance of these non-sGC mechanisms in patients is unclear, there is one characteristic that clearly differentiates HNO from

traditional nitrovasodilators: the potential absence of tolerance or tachyphylaxis.³⁸ If confirmed in human studies, the absence of vascular tolerance could provide an important clinical advantage for HNO in the setting of heart failure. Additional effects of interest include inhibiting platelet aggregation and limiting vascular smooth muscle proliferation.³⁹

While these vascular properties are intriguing, the positive inotropic effect of HNO is potentially of greater clinical interest. Early *in vitro* experiments suggested positive inotropic and lusitropic properties of HNO, while subsequent studies in healthy and heart failure dog models with the HNO donor Angeli's salt ($\text{Na}_2\text{N}_2\text{O}_3$) demonstrated significant improvements in load-independent LV contractility, associated with reductions in pre-load volume and diastolic pressure.^{40,41} The mechanisms of these beneficial inotropic and lusitropic effects continue to be elucidated, but they appear to be independent of cAMP/protein kinase A (PKA) and cGMP/PKG signalling,⁴² with no modification of L-type calcium channel activity,⁴³ and related to modification of specific cysteine residues on either phospholamban⁴⁴ and/or SERCA2a,⁴⁵ resulting in augmented SR calcium transients. These and other studies³⁹ were encouraging, but the clinical utility of HNO was limited by the poor pharmacological properties of the available HNO donors, such as $\text{Na}_2\text{N}_2\text{O}_3$. Recently, a clinical development programme has used the new HNO donor CXL-1020 in animal studies, which have confirmed its positive inotropic and lusitropic effects.⁴⁶ Clinical studies are currently being conducted (clinicaltrials.gov NCT01092325, clinicaltrials.gov NCT01096043). Nitroxyl donors, such as CXL-1020, may be a new generation of inodilators that avoid the safety issues of current inodilators, such as catecholamines, PDE inhibitors, and levosimendan, and offer significant promise, however, the extent of the vasodilating properties may determine their clinical utility.

Ryanodine receptor stabilizers—stopping the leak

As discussed above, calcium leak through RyRs significantly contributes to abnormal calcium cycling in human heart failure. Accordingly, restoration of RyR function seems to be an interesting target for its treatment. A leak of calcium from the SR may not only decrease SR calcium load and availability for systolic contraction, but it may also promote diastolic dysfunction due to diastolic activation of contractile proteins. In addition, SR calcium leak may be a trigger for arrhythmias and contribute to altered gene expression in heart failure. Finally, a leak has unfavourable energetic consequences because ATP consumption for SR calcium accumulation increases with recycling calcium. Several compounds that reduce calcium leak through the RyR have been developed. JTV519, a 1,4-benzothiazepine, was one of the first compounds that restored abnormal RyR function and preserved contractile performance in heart failure models.^{47,48} In addition, JTV519 improved diastolic and systolic function in isolated myocardium from failing human hearts.⁴⁹ In addition to RyR stabilization, JTV519 has inhibitor properties on L-type calcium channels, potassium channels, and possibly other transporters. Subsequently, molecules that may specifically act on cardiac RyRs have been

developed, including S44121. The study drug S44121 is currently being evaluated in a phase 2 multicentre clinical study (ISRCTN Registration number 14227980).

Energetic modulators—fuelling the engine

Disturbed energetic metabolism is considered to play a major role in human heart failure.⁵⁰ This may result from inadequate vessel formation during cardiac hypertrophy, altered substrate uptake of the myocyte or disturbed mitochondrial oxidative phosphorylation, and ATP availability for contractile processes. Several drugs that switch energy metabolism from fatty acids to glucose oxidation have been investigated. Etomoxir, an inhibitor of mitochondrial carnitine palmitoyltransferase 1, had shown promising results in animal experiments. It was also shown that substrate switching is associated with changes in gene expression such as myosin heavy chain gene or SERCA, but in a recent study in rats with aortic banding, an improvement of cardiac function has not been observed.⁵¹ A clinical trial had to be stopped prematurely because of liver toxicity of the substance in some patients.⁵²

Administration of the glycolytic substrate pyruvate may result in profound inotropic effects under experimental conditions as well as in patients with heart failure. Pyruvate has numerous molecular effects that may contribute to its inotropic action. These include: (i) an increase in phosphorylation potential, (ii) a reduction of inorganic phosphate, (iii) a decrease in hydrogen ion concentration, and (iv) a modulation of the cytosolic redox state. The most important mechanism for its inotropic action may be an increase in the phosphorylation potential and an increase in free energy of ATP hydrolysis. In isolated muscle strip preparations from patients with end-stage heart failure, pyruvate resulted in a concentration-dependent increase in developed force and a decrease in diastolic force.⁵³ There was a dose-dependent prolongation of time-to-peak tension and relaxation time. This was associated with increased intracellular calcium transients and increased SR calcium content. The data support the hypothesis that pyruvate increases energy availability to the SERCA resulting in improved SR calcium handling.⁵³ When pyruvate was injected into the coronary circulation of patients with dilated cardiomyopathy, it exhibited a profile of an ideal inotropic agent with an increase in cardiac index and stroke volume index, a decrease in PCWP and heart rate. Mean aortic pressure and system vascular resistance did not change.^{54,55} These pyruvate data suggest that energetic modulation has a significant potential for the treatment of heart failure. A major difficulty for using pyruvate to treat acute heart failure in patients results from the fact that high arterial concentrations are needed which can only be achieved by intra-arterial application of pyruvate. Nevertheless, the favourable pronounced inotropic effects of pyruvate suggest that efforts should be invested to search for energetic targets in the treatment of heart failure.

Conclusion

Despite the compelling therapeutic hypothesis that increasing ventricular performance should improve clinical outcomes, the

development of positive inotropes in the past has generated a litany of failures. These failures have been largely due to increases in cardiovascular complications directly related to the mechanism of action of these agents. Newer agents targeting different mechanisms of action hold new promise for the future, as long as complications of myocardial ischaemia, arrhythmias, and hypotension can be avoided.

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References

- Colucci WS, Wright RF, Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. 2. *N Engl J Med* 1986;**314**:349–358.
- Colucci WS, Wright RF, Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. 1. *N Engl J Med* 1986;**314**:290–299.
- Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, Stromberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Piori SG, Swedberg K, Vahanian A, Camm J, De Caterina R, Dean V, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur Heart J* 2008;**29**:2388–2442.
- Kramer DG, Trikalinos TA, Kent DM, Antonopoulos GV, Konstam MA, Udelsom JE. Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction: a meta-analytic approach. *J Am Coll Cardiol* 2010;**56**:392–406.
- Cleland JG, Daubert JC, Erdmann E, Freemantle N, Gras D, Kappenberger L, Tavazzi L. The effect of cardiac resynchronization on morbidity and mortality in heart failure. *N Engl J Med* 2005;**352**:1539–1549.
- Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol* 2008;**70**:23–49.
- Stewart MA, Franks-Skiba K, Chen S, Cooke R. Myosin ATP turnover rate is a mechanism involved in thermogenesis in resting skeletal muscle fibers. *Proc Natl Acad Sci USA* 2010;**107**:430–435.
- Holubarsch C, Ruf T, Goldstein DJ, Ashton RC, Nickl W, Pieske B, Pioch K, Ludemann J, Wiesner S, Hasenfuss G, Posival H, Just H, Burkhardt D. Existence of the Frank-Starling mechanism in the failing human heart. Investigations on the organ, tissue, and sarcomere levels. *Circulation* 1996;**94**:683–689.
- Hasenfuss G, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* 2002;**34**:951–969.
- Teerlink JR, Metra M, Zaca V, Sabbah HN, Cotter G, Gheorghiade M, Cas LD. Agents with inotropic properties for the management of acute heart failure syndromes. Traditional agents and beyond. *Heart Fail Rev* 2009;**14**:243–253.
- Cuffe MS, Califf RM, Adams KF Jr, Benza R, Bourge R, Colucci WS, Massie BM, O'Connor CM, Pina I, Quigg R, Silver MA, Gheorghiade M. Short-term intravenous milrinone for acute exacerbation of chronic heart failure: a randomized controlled trial. *JAMA* 2002;**287**:1541–1547.
- Felker GM, Benza RL, Chandler AB, Leimberger JD, Cuffe MS, Califf RM, Gheorghiade M, O'Connor CM. Heart failure etiology and response to milrinone in decompensated heart failure: results from the OPTIME-CHF study. *J Am Coll Cardiol* 2003;**41**:997–1003.
- Elkayam U, Tasissa G, Binanay C, Stevenson LW, Gheorghiade M, Warnica JW, Young JB, Rayburn BK, Rogers JG, DeMarco T, Leier CV. Use and impact of inotropes and vasodilator therapy in hospitalized patients with severe heart failure. *Am Heart J* 2007;**153**:98–104.

14. Hasenfuss G, Pieske B, Castell M, Kretschmann B, Maier LS, Just H. Influence of the novel inotropic agent levosimendan on isometric tension and calcium cycling in failing human myocardium. *Circulation* 1998;**98**:2141–2147.
15. Yokoshiki H, Sperelakis N. Vasodilating mechanisms of levosimendan. *Cardiovasc Drugs Ther* 2003;**17**:111–113.
16. Cleland JG, Freemantle N, Coletta AP, Clark AL. Clinical trials update from the American Heart Association: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE. *Eur J Heart Fail* 2006;**8**:105–110.
17. Delaney A, Bradford C, McCaffrey J, Bagshaw SM, Lee R. Levosimendan for the treatment of acute severe heart failure: a meta-analysis of randomised controlled trials. *Int J Cardiol* 2010;**138**:281–289.
18. Rocchetti M, Besana A, Mostacciolo G, Micheletti R, Ferrari P, Sarkozi S, Szegedi C, Jona I, Zaza A. Modulation of sarcoplasmic reticulum function by Na⁺/K⁺ pump inhibitors with different toxicity: digoxin and PST2744 [(E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride]. *J Pharmacol Exp Ther* 2005;**313**:207–215.
19. Micheletti R, Mattera GG, Rocchetti M, Schiavone A, Loi MF, Zaza A, Gagnol RJ, De Munari S, Melloni P, Carminati P, Bianchi G, Ferrari P. Pharmacological profile of the novel inotropic agent (E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride (PST2744). *J Pharmacol Exp Ther* 2002;**303**:592–600.
20. Mattera GG, Lo Giudice P, Loi FM, Vanoli E, Gagnol JP, Borsini F, Carminati P. Istroxime: a new luso-inotropic agent for heart failure. *Am J Cardiol* 2007;**99**:33A–40A.
21. Sabbah HN, Imai M, Cowart D, Amato A, Carminati P, Gheorghide M. Hemodynamic properties of a new-generation positive luso-inotropic agent for the acute treatment of advanced heart failure. *Am J Cardiol* 2007;**99**:41A–46A.
22. Gheorghide M, Blair JE, Filippatos GS, Macarie C, Ruzyllo W, Korewicki J, Bubenek-Turconi SI, Ceracchi M, Bianchetti M, Carminati P, Kremastinos D, Valentini G, Sabbah HN. Hemodynamic, echocardiographic, and neurohormonal effects of istroxime, a novel intravenous inotropic and lusitropic agent: a randomized controlled trial in patients hospitalized with heart failure. *J Am Coll Cardiol* 2008;**51**:2276–2285.
23. Vale RD, Milligan RA. The way things move: looking under the hood of molecular motor proteins. *Science* 2000;**288**:88–95.
24. Teerlink JR. A novel approach to improve cardiac performance: cardiac myosin activators. *Heart Fail Rev* 2009;**14**:289–298.
25. Shen YT, Malik FI, Zhao X, Depre C, Dhar SK, Abarzua P, Morgans DJ, Vatner SF. Improvement of cardiac function by a cardiac Myosin activator in conscious dogs with systolic heart failure. *Circ Heart Fail* 2010;**3**:522–527.
26. Teerlink JR, Malik FI, Clarke CP, Saikali KG, Escandon RD, Lee JH, Wolff AA. The selective cardiac myosin activator, CK-1827452, increases left ventricular systolic function by increasing ejection time: results of a first-in-human study of a unique and novel mechanism. *J Card Fail* 2006;**12**:763.
27. Cleland JGF, Malik FI. The selective cardiac myosin activator, CK-1827452, increases systolic function in heart failure. *J Card Fail* 2008;**14**:67.
28. Greenberg BH, Chou W, Escandon R, Lee JH, Saikali KG, Malik F, Wolff AA, Shaburishvili T. Phase II safety study evaluating the novel cardiac myosin activator, CK-1827452, in patients with ischemic cardiomyopathy and angina. *J Card Fail* 2009;**15**:S67–S67.
29. Prestle J, Janssen PM, Janssen AP, Zeitz O, Lehnart SE, Bruce L, Smith GL, Hasenfuss G. Overexpression of FK506-binding protein FKBP12.6 in cardiomyocytes reduces ryanodine receptor-mediated Ca(2+) leak from the sarcoplasmic reticulum and increases contractility. *Circ Res* 2001;**88**:188–194.
30. Sakata S, Lebeche D, Sakata N, Sakata Y, Chemaly ER, Liang LF, Takewa Y, Jeong D, Park WJ, Kawase Y, Hajjar RJ. Targeted gene transfer increases contractility and decreases oxygen cost of contractility in normal rat hearts. *Am J Physiol Heart Circ Physiol* 2007;**292**:H2356–H2363.
31. Schmidt U, del Monte F, Miyamoto MI, Matsui T, Gwathmey JK, Rosenzweig A, Hajjar RJ. Restoration of diastolic function in senescent rat hearts through adenoviral gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase. *Circulation* 2000;**101**:790–796.
32. del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doye AA, Gwathmey JK, Hajjar RJ. Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc Natl Acad Sci USA* 2004;**101**:5622–5627.
33. Beeri R, Chaput M, Guerrero JL, Kawase Y, Yosefy C, Abedat S, Karakikes I, Morel C, Tisovsky A, Sullivan S, Handschumacher MD, Gilon D, Vlahakes GJ, Hajjar RJ, Levine RA. Gene delivery of sarcoplasmic reticulum calcium ATPase inhibits ventricular remodeling in ischemic mitral regurgitation. *Circ Heart Fail* 2010;**3**:627–634.
34. Jaski BE, Jessup ML, Mancini DM, Cappola TP, Pauly DF, Greenberg B, Borow K, Ditttrich H, Zsebo KM, Hajjar RJ. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail* 2009;**15**:171–181.
35. Stiles S. CUPID: first-in-human gene therapy for advanced heart failure promising in small study. <http://www.theheart.org/article/1083549.do>.
36. Irvine JC, Ritchie RH, Favaloro JL, Andrews KL, Widdop RE, Kemp-Harper BK. Nitroxyl (HNO): the Cinderella of the nitric oxide story. *Trends Pharmacol Sci* 2008;**29**:601–608.
37. Paolucci N, Jackson MI, Lopez BE, Miranda K, Tocchetti CG, Wink DA, Hobbs AJ, Fukuto JM. The pharmacology of nitroxyl (HNO) and its therapeutic potential: not just the Janus face of NO. *Pharmacol Ther* 2007;**113**:442–458.
38. Irvine J, Kemp-Harper BK, Widdop RE. Chronic administration of the HNO donor, Angeli's salt does not lead to tolerance, cross-tolerance or endothelial dysfunction: comparison with GTN and DEA/NO. *Antioxid Redox Signal* 2010; doi:10.1089/ars.2010.3269. Published online ahead of print 19 September 2010.
39. Bullen ML, Miller AA, Andrews KL, Irvine J, Ritchie RH, Sobey C, Kemp-Harper B. Nitroxyl (HNO) as a vasoprotective signaling molecule. *Antioxid Redox Signal* 2010; doi:10.1089/ars.2010.3327. Published online ahead of print 2 November 2010.
40. Paolucci N, Katori T, Champion HC, St John ME, Miranda KM, Fukuto JM, Wink DA, Kass DA. Positive inotropic and lusitropic effects of HNO/NO⁻ in failing hearts: independence from beta-adrenergic signaling. *Proc Natl Acad Sci USA* 2003;**100**:5537–5542.
41. Paolucci N, Saavedra WF, Miranda KM, Martignani C, Isoda T, Hare JM, Espey MG, Fukuto JM, Feelisch M, Wink DA, Kass DA. Nitroxyl anion exerts redox-sensitive positive cardiac inotropy in vivo by calcitonin gene-related peptide signaling. *Proc Natl Acad Sci USA* 2001;**98**:10463–10468.
42. Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, Di Benedetto G, O'Rourke B, Gao WD, Wink DA, Toscano JP, Zaccolo M, Bers DM, Valdivia HH, Cheng H, Kass DA, Paolucci N. Nitroxyl improves cellular heart function by directly enhancing cardiac sarcoplasmic reticulum Ca²⁺ cycling. *Circ Res* 2007;**100**:96–104.
43. Kohr MJ, Kaludercic N, Tocchetti CG, Dong Gao W, Kass DA, Janssen PM, Paolucci N, Ziolo MT. Nitroxyl enhances myocyte Ca²⁺ transients by exclusively targeting SR Ca²⁺-cycling. *Front Biosci (Elite Ed)* 2010;**2**:614–626.
44. Froehlich JP, Mahaney JE, Keceli G, Pavlos CM, Goldstein R, Redwood AJ, Sumbilla C, Lee DI, Tocchetti CG, Kass DA, Paolucci N, Toscano JP. Phospholamban thiols play a central role in activation of the cardiac muscle sarcoplasmic reticulum calcium pump by nitroxyl. *Biochemistry* 2008;**47**:13150–13152.
45. Lancel S, Zhang J, Evangelista A, Trucillo MP, Tong X, Siwik DA, Cohen RA, Colucci WS. Nitroxyl activates SERCA in cardiac myocytes via glutathiolation of cysteine 674. *Circ Res* 2009;**104**:720–723.
46. Wang MJ, Mazhari R, Ihsar I, Wang A, Sabbah MS, Sabbah HN. Intravenous infusion of CXL-1020, a novel nitroxyl (HNO) donor, improves left ventricular systolic and diastolic function in dogs with advanced heart failure. *J Card Fail* 2009;**15**:S73–S74.
47. Yano M, Kobayashi S, Kohno M, Doi M, Tokuhisa T, Okuda S, Suetsugu M, Hisaoka T, Obayashi M, Ohkusa T, Kohno M, Matsuzaki M. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. *Circulation* 2003;**107**:477–484.
48. Lehnart SE, Wehrens XH, Reiken S, Warrier S, Belevych AE, Harvey RD, Richter W, Jin SL, Conti M, Marks AR. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell* 2005;**123**:535–536.
49. Toischer K, Lehnart SE, Tenderich G, Milting H, Körfer R, Schmitt JD, Schöndube FA, Kaneko N, Laughrey CM, Smith GL, Hasenfuss G, Seidler T. K201 improves aspects of the contractile performance of human failing myocardium by reduction in Ca²⁺ leak from the sarcoplasmic reticulum. *Basic Res Cardiol* 2010;**105**:279–287.
50. Ingwall JS. Energy metabolism in heart failure and remodelling. *Cardiovasc Res* 2009;**81**:412–419.
51. Schwarzer M, Faerber G, Rueckauer T, Blum D, Pytel G, Mohr FW, Doenst T. The metabolic modulators, etomoxir and NVP-LAB121, fail to reverse pressure overload induced heart failure in vivo. *Basic Res Cardiol* 2009;**104**:547–557.
52. Holubarsch CJ, Rohrbach M, Karrasch M, Boehm E, Polonski L, Ponikowski P, Rhein S. A double-blind randomized multicentre clinical trial to evaluate the efficacy and safety of two doses of etomoxir in comparison with placebo in patients with moderate congestive heart failure: the ERGO (etomoxir for the recovery of glucose oxidation) study. *Clin Sci (Lond)* 2007;**113**:205–212.
53. Hasenfuss G, Maier LS, Hermann HP, Luers C, Hunlich M, Zeitz O, Janssen PM, Pieske B. Influence of pyruvate on contractile performance and Ca(2+) cycling in isolated failing human myocardium. *Circulation* 2002;**105**:194–199.
54. Hermann HP, Pieske B, Schwarzmüller E, Keul J, Just H, Hasenfuss G. Haemodynamic effects of intracoronary pyruvate in patients with congestive heart failure: an open study. *Lancet* 1999;**353**:1321–1323.
55. Hermann HP, Arp J, Pieske B, Kogler H, Baron S, Janssen PM, Hasenfuss G. Improved systolic and diastolic myocardial function with intracoronary pyruvate in patients with congestive heart failure. *Eur J Heart Fail* 2004;**6**:213–218.