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REVIEW

*Clinical update*

# Cardiac endothelium–myocyte interaction: clinical opportunities for new heart failure therapies regardless of ejection fraction

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Heart failure (HF) is an important global health problem with great socioeconomic burden. Outcomes remain sub-optimal. Endothelium–cardiomyocyte interactions play essential roles in cardiovascular homeostasis, and deranged endothelium-related signalling pathways have been implicated in the pathophysiology of HF. In particular, disturbances in nitric oxide (NO)-mediated pathway and neuregulin-mediated pathway have been shown to contribute to the development of HF. These signalling pathways hold the potential as pathophysiological targets for new HF therapies, and may aid in patient selection for future HF trials.

**Keywords** Heart failure • Endothelium-cardiomyocyte interactions • Nitric-oxide mediated pathway • Neuregulin-mediated pathway • Therapy

## Heart failure is a global health problem

Heart failure (HF) is a major global public health problem, with 26 million sufferers worldwide. Heart failure is the top cause of hospitalizations in those ≥65 years old, with rates of hospitalization on the rise globally. There is a high burden of re-admission[1] and mortality.[2] Multiple studies have demonstrated substantial improvement in HF survival since the late 1990s,[3,4] but outcomes remain dismal particularly for the half of the HF population with HF with preserved ejection fraction (HFPEF),[5] for which there is still no proven therapy. Given the increasing global burden of HF and the projection that HFPEF will become the dominant form of HF in ageing societies, there is an urgent need to explore newer therapies applicable to both HF with reduced ejection fraction (HFREF) and HFPEF.

## The cardiac endothelium in cardiovascular homeostasis

In the pathophysiology of HF, a pivotal role has been attributed to generalized endothelial dysfunction, in particular in the cardiac, pulmonary, renal, and muscular endothelial vasculature (*Figure 1*). Importantly, dysregulation of the communication between cardiac endothelial cells and cardiomyocytes has been implicated in the development of cardiac structural and functional abnormalities, and the restoration of endothelium-related signalling pathways is an emerging new approach to mitigate the progression of HF. Importantly, the cardiac endothelium which directly communicates with subjacent cardiomyocytes in the cardiac capillaries and at the endocardium, should be distinguished from the vascular endothelium in the coronary arterial system. Similarly, as in the rest of the systemic circulation, the latter coronary vascular endothelial cells merely

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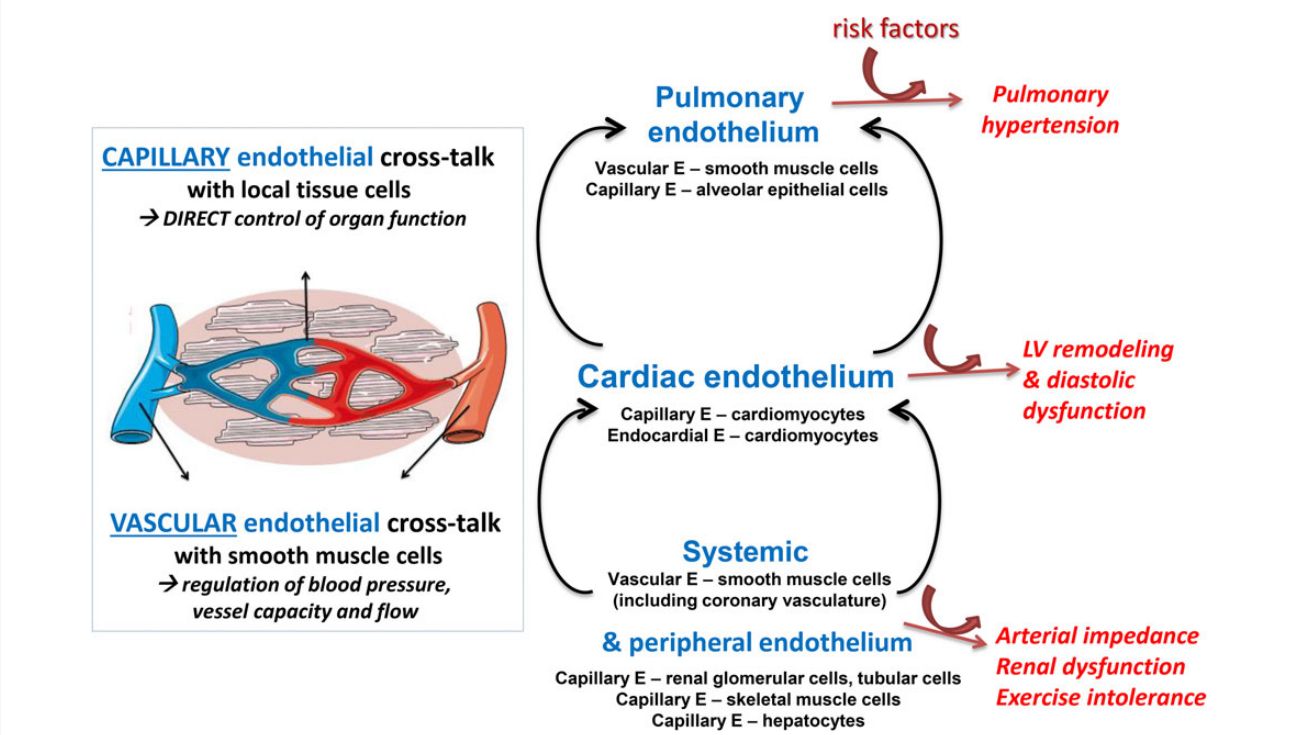


**Figure 1** Schematic presentation of the endothelial system in the organism, and its cell-to-cell interactions in different compartments of the circulation. The endothelium is not commonly thought of as a system and rather studied according to the interests of a specific clinical organ-oriented specialty. In cardiology, most attention has been directed to the (coronary) vascular endothelium in vasomotor control, and to the pathogenesis and management of coronary atherosclerosis and myocardial ischaemia. Yet the arterial endothelium comprises only a minute fraction of the endothelial surface. On the other hand, capillary endothelial cells which directly overlie organ-specific tissue cells, account for the largest number of endothelial cells in the circulation. The role of capillary endothelial cells has long been underestimated, except for the interactions with astrocytes and neurons in the brain. In particular, the interactions between capillary endothelial cells with adjacent organ cells, such as hepatocytes and Kupffer cells in the liver, tubular and glomerular epithelial cells in the kidney, alveolar epithelial cells in the lungs, or myofibre cells in skeletal muscle are likely to be important but have scarcely been studied. In the heart, endothelial cells and adjacent cardiomyocytes communicate in an obligatory fashion, inherent to (i) the anatomical proximity of endothelial cells and cardiomyocytes, both in the endocardium and at the level of the microcapillaries, (ii) the adjustable release of various cardioactive diffusible factors by endothelial cells, which control the activity of surface receptors and signalling molecules in cardiomyocytes. Risk factor-induced activation and dysfunction of endothelial cells is a common pathophysiological step during many diseases and leads to ventricular dysfunction, pulmonary and systemic hypertension, kidney failure, and exercise intolerance.

conduct and mediate perfusion of the heart through interaction with subjacent smooth muscle.

The heart is a highly organized pluricellular organ consisting of different, equipotent cell types including endothelial cells, fibroblasts, and cardiomyocytes. Communication between these cell types in a network structure is crucial for cardiac development, autoregulation, and adaptation.[6,7] The concept that cell–cell communication in the heart is important for cardiac performance was formulated almost 30 years ago by Brutsaert *et al.*,[8] from experimental observations that selectively damaging the endocardial or microvascular endothelium of isolated papillary muscles influenced the mechanical performance of the underlying cardiac muscle.[9,10] To date, a growing number of endothelial-derived cardio-active factors have been identified (*Figure 2*), including nitric oxide (NO), endothelin-1, neuregulin-1 (NRG-1), angiotensin II, angiopoietins, prostaglandins, connective tissue growth factor, fibroblast growth factor, vascular endothelial growth factor, Dickkopf-3, apelin, and endothelial miRNAs (*Table 1*). Importantly, half-life and/or duration of signalling activity of these substances appear to vary, reflecting a specific modulatory time-window for each of these factors. This window may be long and sustained (e.g. for most growth factors) or can be very brief, confining modulation to a brief interval in the cardiac cycle. In the latter case, the moment of release must be tightly coordinated within the cardiac cycle, as is the case for NO.[11]

Recent experimental observations and pharmacological developments have identified pharmacological tools and clinical opportunities to treat HF based on the concept of endothelium–cardiomyocyte interactions. To date, two endothelial pathways, the NO-mediated pathway and the neuregulin-mediated pathway, have progressed to the stage of clinical trials in human HF patients. We aim to review these endothelial pathways and discuss their potential clinical applications in this paper.

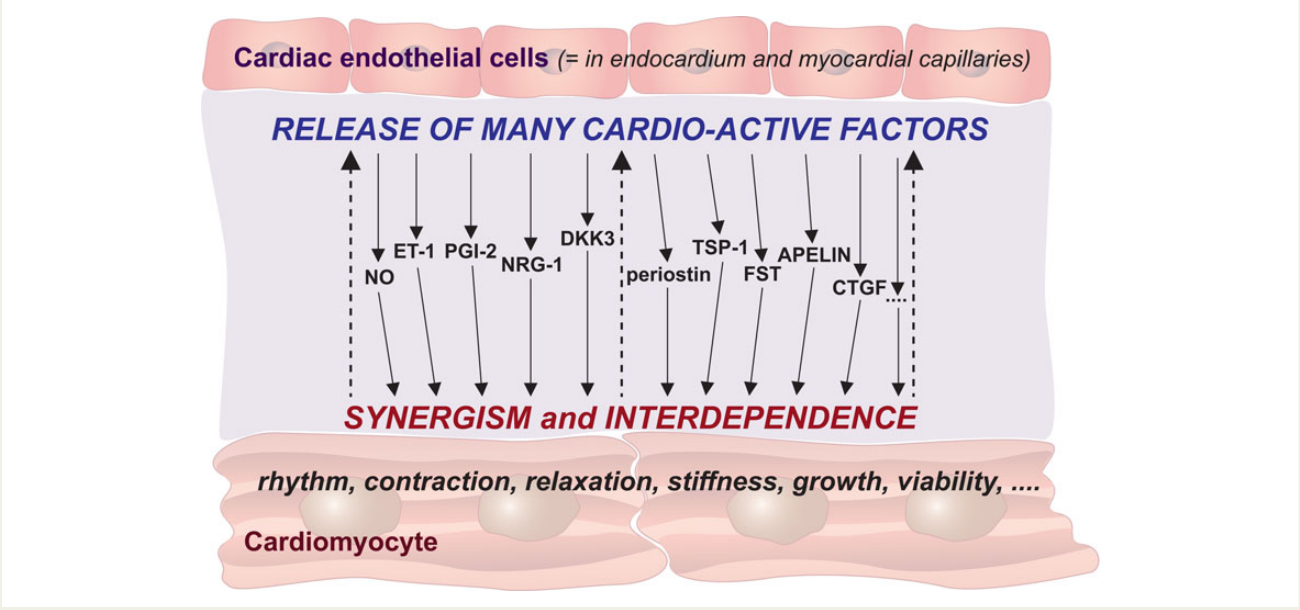


**Figure 2** Cardiac endothelium–cardiomyocyte communication. Cardiac endothelial cells (in endocardium and myocardial micro-capillaries) secrete many factors in an adjustable fashion, including proteins and diffusible substances like nitric oxide (NO). The cardiac activity of some of these factors like nitric oxide, prostacyclin (PGI2), endothelin-1 (ET-1), and neuregulin-1 (NRG-1) has been recognized. For many other factors, like Dickkopf-3 (DKK3), periostin, TSP-1 (thrombospondin-1), follistatin (FST), apelin, connective tissue growth factor (CTGF) effects on the heart have only emerged recently. The synthesis, secretion, and activities of these factors are closely linked, interrelated, and interactive. Most factors modulate the actions of factors on the same target cell, actions which may be mutually additive, synergistic, or inhibitory. They may even result in novel effects, not seen with either agent alone, also influenced by the milieu in which the interactions occur. With this background, targeting cardiac endothelial function with a (pharmacological) intervention which abruptly imposes dominancy of one of these intertwined pathways may be risky. A gentle, gradual, balanced, and sustained rehabilitation of endothelial function appears a preferable goal.

**Table 1 Overview of selected endothelium-derived factors with modulatory effects on cardiomyocyte function**

| Factor | Receptor/Pathway | Contractility/Relaxation | Hypertrophy/Heart failure | References |
|---|---|---|---|---|
| Nitric oxide | sGC | + and − Inotropic effects<br>Increased relaxation | Attenuates cardiac remodelling | 15,27 |
| Endothelin-1 | $ET_A$ $ET_B$ | Mainly + inotropic<br>Increased relaxation | Increases hypertrophy and fibrosis | 127 |
| Prostacyclin | PG-$I_2$ receptor | + and − Inotropic effects | Anti-hypertrophic | 7,128,129 |
| Neuregulin-1 | Erbb4 | Reduces contractility | Attenuates HF progression | 130,131 |
| Periostin | $\alpha$-V/$\beta$-3 and $\alpha$-V/$\beta$-5 integrins | ? | Increases fibrosis and myocyte proliferation | 132,133 |
| Thrombospondin-1, -2, -4 | Cell adhesion receptors | Role in stretch mediated contractility augmentation (TSP-4) | Anti-hypertrophic<br>Anti-fibrotic | 134–137 |
| Follistatin and follistatin-like factor (Fstl1) | Antagonists of TGF-$\beta$ superfamily cytokines | ? | Anti-apoptotic (Fstl1)<br>Anti-hypertrophic (Fstl1 and 3) | 138–141 |
| Connective tissue growth factor (CTGF) | Integrin receptors | ? | Pro-fibrotic<br>Pro-hypertrophic | 142,143 |
| Dickkopf-3 (DKK-3) | Wnt signalling | ? | Anti-hypertrophic | 144 |
| Apelin | Apelin receptor | + Inotropic | Anti-hypertrophic | 145–147 |
| miR-146a | 16K Prolactin pathway | ? | Induces peripartum cardiomyopathy | 148 |

# The nitric oxide-soluble guanyl cyclase-cyclic guanosine monophosphate pathway

## Nitric oxide plays an important role in cardiovascular homeostasis

Initially thought to exert its effects primarily on the vasculature, including on the coronary vasculature, it is increasingly recognized that NO plays an obligatory role in the regulation of myocardial function via its autocrine and paracrine effects**.** NO is synthesized from the amino acid L-arginine, through a process catalysed by NO synthase (NOS). Expressed in both constitutive [endothelial (eNOS), neuronal (nNOS)] and inducible (iNOS) forms, it can be found in the cardiac endothelium (eNOS, iNOS), myocardium (eNOS, nNOS, iNOS), nerve fibres (nNOS), and inflammatory cells (iNOS).

The fundamental signalling pathway of NO is via stimulation of soluble guanyl cyclase (sGC), leading to the production of the second messenger cyclic guanosine monophosphate (cGMP). Depending on the site of cGMP activation and the site of NO release, the consequent biological actions are different.

## Nitric oxide and natriuretic peptides contribute to spatially distinct cyclic guanosine monophosphate pool

Apart from the NO-sGC-cGMP pathway, synthesis of cGMP is also regulated by the natriuretic peptide (NP)-particulate GC (pGC)-cGMP pathway. Initially thought to be contributing to a common cGMP pool, studies have shown that cGMP produced by pGC and sGC is compartmentalized to distinct sub-cellular regions, leading to different responses.[12,13] NP activation leads to increased cGMP production around the sub-sarcolemmal region, whereas NO signalling results in an increase in the cytosolic pool of cGMP. The spatial boundaries of the distinct cGMP pools are poorly defined, restricted by the activity of cGMP-hydrolysing phosphodiesterases (PDE); PDE-2 limits the sub-sarcolemmal cGMP pool and PDE-5 controls the cytosolic cGMP pool.[12,14] In addition to compartmentalization, these cGMP pools appear to be functionally distinct. While cGMP produced by pGC and sGC enhances myocardial lusitropy, only cGMP produced by sGC blunts myocardial response to β-adrenergic stimulation.[13]

## Nitric oxide and cardiac function

NO modulates cardiac function through its inotropic, lusitropic, and chronotropic effects. It is generally accepted that NO exerts bimodal effects on inotropy.[15,16] At low and physiological NO concentrations, the NO-sGC-cGMP pathway enhances inotropy via activation of protein kinases A (PKA) and G (PKG) which increases $Ca^{2+}$ concentration.[17] Higher amounts of NO result in negative inotropy;[18] mediated by PKG, the increased cGMP production leads to blockade of sarcolemmal $Ca^{2+}$ channels and reduction in the sensitivity of troponin C to $Ca^{2+}$. The definitions of 'low' and 'high' concentrations are controversial, given that the amounts of NO produced *in vivo* may not correspond to amounts of bioactive NO delivered. It is therefore not surprising that not all studies demonstrated these bimodal effects on inotropy. At higher concentrations, NO also enhances lusitropy by accelerating myocardial relaxation and improving myocardial distensibility.[15,17] Phosphorylation of troponin I reduces cardiac myofilaments' sensitivity to calcium and promotes diastolic cross-bridge detachment.[19] Phosphorylation of titin by PKA and/or PKG increases its compliance, hence improving lusitropy.[20] The challenge is identifying the 'sweet spot' where lusitropy is enhanced without excessive compromise on inotropy.

In addition to these direct actions on cardiac function, the general vasodilatatory properties of the NO pathway may also indirectly contribute to cardiac function by altering cardiac pre- and afterload conditions. In particular, an altered afterload of the left ventricle (LV) will, through changes arterial impedance, modulate LV systolic duration and the timing of arterial reflected waves, and thereby optimize the crosstalk between the LV and peripheral circulation without necessarily affecting arterial systemic blood pressure.[21,22]

The chronotropic effects of NO differ according to the site of action. Animal studies demonstrated positive chronotropic effects via cGMP-dependent stimulation of the hyperpolarization-activated pacemaker current ($I_f$).[23] Yet, experiments using isolated neonatal and adult ventricular myocytes demonstrated negative chronotropic effects, modulated at the post-synaptic level.[24]

Through effects on mitochondrial respiration, NO promotes free fatty acids as the preferred myocardial energy substrate and protects against excessive oxygen consumption. In canine models of experimental HF, a drop in myocardial NO production was associated with a shift in myocardial substrate utilization to glucose.[25]

## Role of nitric oxide in heart failure

There is increasing recognition of the central role that NO imbalance plays in contributing to the abnormal cardiac and vascular phenotypes seen in chronic HF, regardless of EF.

HFREF, with post-myocardial infarction as the prototype, is characterized by eccentric remodelling and LV dilatation. In post-infarct models of HFREF, there was a greater degree of adverse remodelling in NOS knock-out mice, compared with wild-type mice; this was mediated via the NO signalling pathway.[26]

In HFPEF, concentric LV remodelling with increased LV wall thickness is thought to be driven by a pro-inflammatory state, which results in increased oxidative stress through the activation and migration of leucocytes and increased superoxide production. NO is then diverted to peroxynitrite by its interaction with superoxide.[27] Low bioavailability of NO and high peroxynitrite levels result in low intracellular cGMP and reduced PKG activity.[28,29] A deficient NO-sGC-cGMP pathway increases diastolic cytosolic $Ca^{2+}$ and delays myocardial relaxation. Myocardial stiffness also increases with a deficient NO-sGC-cGMP pathway since there is hypophosphorylation of titin via PKG or PKA, with resultant decreased compliance. Accordingly, rodent studies showed that LV diastolic dysfunction and leftward shift of the LV pressure–volume relationship occurred when NO synthesis was inhibited.[30] Conversely, infusions of NO donors improved LV diastolic compliance via phosphorylation of titin.[31] If this trial could be repeated in multicentre studies including a large number of patients, it could be a major breakthrough in the treatment of patient with HFPEF. Beyond the direct actions on the myocardium, NO-sGC-cGMP deficiency may also create a mismatch of the crosstalk between the LV and the systemic arterial impedance. Moreover, it also underlies

the vasomotor dysfunction in systemic, pulmonary, renal, muscular, and coronary circulations. Pulmonary vascular endothelial dysfunction with deficient NO-sGC-cGMP signalling leads to pulmonary vasomotor dysfunction, vascular remodelling, and pulmonary hypertension.[32] This secondary pulmonary hypertension directly affects right-ventricular (RV) function.[33] NO deficiency also drives renal vasomotor dysfunction, contributing to the development of cardiorenal syndrome (concomitant cardiac and renal dysfunction).[34] In the coronary circulation, NO-sGC-CMP deficiency causes inappropriate coronary vasoconstriction, triggers smooth muscle proliferation and vascular remodelling, and reduced myocardial perfusion reserve.[35]

### Propagation of heart failure by worsening nitric oxide deficiency

The neurohormonal activation and systemic inflammation in HF result in endothelial dysfunction, down-regulation of eNOS expression, uncoupling of eNOS, and increased production of reactive oxygen species.[36] Chronic inactivity in HF, with altered local endothelial shear stress, further down-regulates eNOS. In addition, the altered redox state causes NO resistance by reducing the levels of NO-sensitive sGC. sGC exists in two different forms, the NO-sensitive reduced sGC and NO-insensitive oxidized sGC. In HF, the balance is shifted in favour of oxidized sGC with reduced NO-sGC-cGMP signalling activity. These eventually culminate in dysfunctional endothelium with attenuated NO activity, which in turn propagates the progression of HF.

### Endothelial dysfunction is prevalent in heart failure and portends a worse prognosis

Endothelial dysfunction is highly prevalent in HF, regardless of EF. It was first demonstrated in HFREF patients two decades ago. Since then, there has been a wealth of data showing endothelial dysfunction among HFREF patients in various circulatory beds, and involving large conduit[37] and small resistance vessels,[38] in addition to the microvasculature.[39] Endothelial dysfunction independently predicts adverse cardiovascular outcomes[40] and correlates with the extent of exercise intolerance.[41] Similarly, endothelial dysfunction was present in 42% of HFPEF patients (compared with 28% of hypertension and none of healthy controls), and correlated with the extent of exercise intolerance[42] and pulmonary hypertension.[43] Akiyama *et al.*[44] extended this finding, by further demonstrating that peripheral endothelial dysfunction independently predicted cardiovascular events. The high prevalence and independent prognostic impact suggest that endothelial dysfunction plays an important pathophysiological role in HFPEF.

## The neuregulin-erythroblastic leukaemia viral oncogene homolog pathway

### Neuregulins and their receptors

NRG-1 is a growth factor belonging to the epidermal growth factor (EGF) family, expressed in the nervous system, the cardiovascular system, mammary glands, the intestine, and kidneys.[45] In the heart (*Figure 3*), NRG-1 is expressed and released by the endocardial and microvasculature endothelium. NRG-1 binds to two of the four erythroblastic leukaemia viral oncogene homolog (ErbB) receptors, namely ErbB3 and ErbB4, of which ErbB4 seems to be the most important in the heart. Ligand binding activates these receptors and initiates dimerization, usually with ErbB2, although ErbB4 homodimer signalling has been described. Dimerization triggers the activation of signalling molecules, such as the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3-OH kinase (PI3K)/Akt pathways. Paracrine communication through NRG-1 between cardiac endothelial cells and cardiomyocytes has been confirmed in co-culture studies,[7] adding NRG-1 to a multiplicity of endothelial factors where interdependence contributes to the regulation of cardiovascular function.

### *In vitro* effect of neuregulin-1 on cardiomyocytes and myocardial performance

Cultured cardiomyocytes exposed to NRG-1 show less cytochrome *c* release, caspase-3 activation, myofibrillar disorganization, and apoptotic cell death when exposed to oxidative stress and anthracyclines.[46–48] Treatment of cultured adult rat ventricular myocytes with NRG- 1 induces DNA synthesis and cardiomyocyte proliferation through ErbB4 signalling.[49] In isolated cardiac muscle, NRG-1 reduced contractility and inotropic responsiveness to adrenergic stimulation. Most likely, this effect is linked to activation of eNOS; ErbB receptors and eNOS co-localize in the caveolae.[50] Interestingly, sympatholytic effects of NRG-1 have been recapitulated in the brain cardiovascular control system, where NRG-1 decreases heart rate and renal sympathetic nerve activity.[51,52]

The beneficial effects of NRG-1 on cell viability, structure, and growth at the cardiomyocyte level, and the sympatholytic effects at the level of muscle and central nervous system offer an attractive therapeutic profile to NRG-1.

### Endogenous neuregulin-1/erythroblastic leukaemia viral oncogene homolog signalling in cardiac disease

NRG-1/ErbB expression and signalling change during disease states. In compensatory states of ventricular hypertrophy, ErbB2 and ErbB4 expression increase, whereas depressed levels are found in terminal stages of HF.[53] Depression of NRG-1/ErbB signalling may contribute to progressive myocardial dysfunction. In addition, co-morbidities like diabetes may predispose to such depression.[54] Interestingly, in patients who had left-ventricular unloading with ventricular assist devices, depressed levels of ErbB receptors reversed.[55,56]

Ischaemia/reperfusion injury is a potent activator of myocardial NRG-1/ErbB signalling.[57] Depressed endothelial NRG-1synthesis impairs recovery of cardiac contractile function after an ischaemic insult.[58]

### Cardiac therapeutic effect of neuregulin-1 in animals

The therapeutic effect of an EGF-domain fragment of rhNRG-1β or a kringle and Ig domain-containing version (known as GGF2) was demonstrated in a series of animal models of HFREF. In rats with

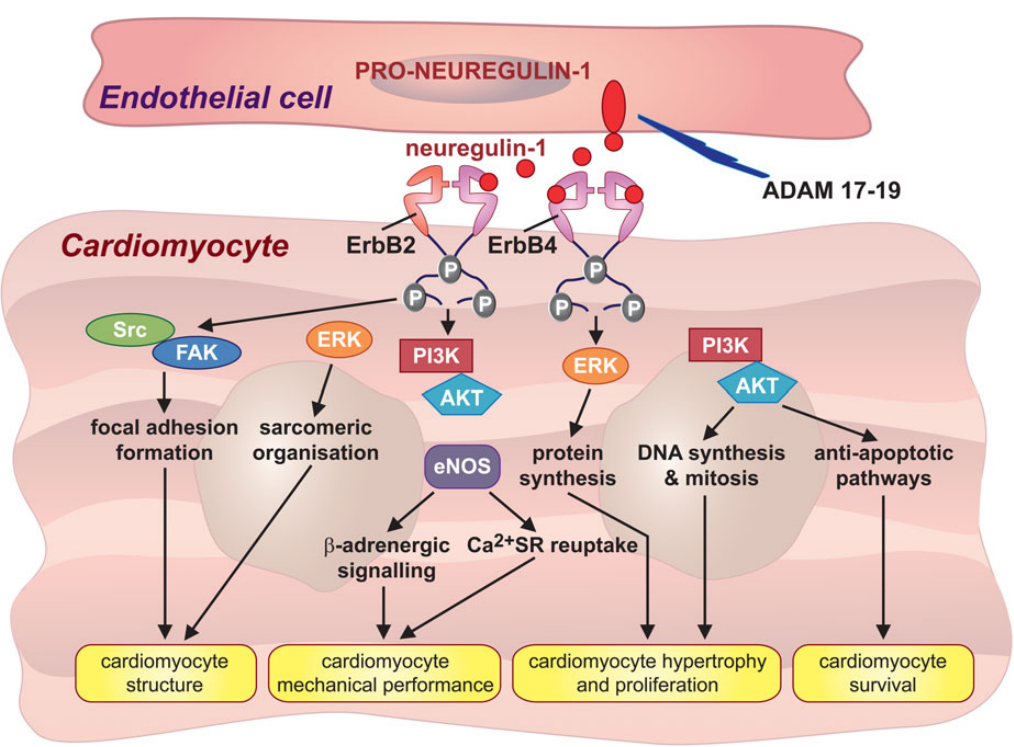


**Figure 3** NRG-1/ErbB signalling in the cardiomyocyte. NRG-1 regulates cardiomyocyte structure, mechanical performance, hypertrophic growth, proliferation, and viability. NRG-1, expressed as a transmembrane pro-protein is spliced by specific proteases, belonging to the desintegrin and metalloproteinase (ADAM) family and include ADAM 17 and ADAM 19. After binding to ErbB4, homodimerization of ErbB4 or heterodimerization with ErbB2, different signalling pathways are activated. These include the ErbB2/FAK/Src pathway, the activation of PI3K/Akt, the activation of ERK1/2, and the phosphorylation of eNOS, leading to an increase in NO production. This has been shown to attenuate adrenergic inotropic stimulation and phosphorylate phospholamban leading to increased SERCA2a activity and calcium uptake by the sarcoplasmic reticulum. Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; ErbB2/4,erythroblastic leukaemia viral oncogene homolog 2/4; ERK1/2, extracellular signal-; PI3K, phosphoinositide-3-kinase; Src, proto-oncogene tyrosine-protein kinase.

systolic dysfunction induced by myocardial infarction, rhNRG-1β administered daily for 5 days improved cardiac function.[59] Similar effects were reported in models of anthracycline and virally induced cardiac injury in mice. Next, in a model of diabetic cardiomyopathy, rhNRG-1 improved LV function and reduced LV collagen volume fraction.[60]

In large animals with rapid-pacing-induced HF, rhNRG-1β similarly improved LV function. GGF2 treatment of rats or swine with myocardial infarction-induced myocardial dysfunction is associated with improved systolic function and reduced progressive remodelling.[61,62]

## Current clinically available interventions targeting endothelium–cardiomyocyte communication

The central hypothesis of this review is that generalized endothelial dysfunction is an important characteristic of chronic HF, and that alleviating endothelial dysfunction may improve HF symptoms and/or prognosis. Medical interventions may restore endothelial function globally or partially, or may only substitute one of more endothelial communication pathways (*Figure 4*). For example, physical exercise may have a rather global, pleiotropic effect on endothelial function, whereas administration of a NO synthase agonist will be rather pathway specific. Since endothelial cells are present throughout the organism, endothelium-targeted interventions may improve the function of each organ in the organism. Retrospectively, it may be difficult to pinpoint how exactly this improvement emerged. Besides normalizing tissue perfusion or organ's haemodynamic loading conditions, the endothelium-targeted intervention may have enhanced a tissue-specific homeostatic process in the micro-capillaries, where endothelial cells similarly as in the heart directly interact with the organ's specific cells. Hence, a general principle in 'endothelium therapeutics' is to act gently and patiently, allowing endothelium-controlled homeostasis to recover, and not to shoot for abrupt changes of haemodynamic parameters like blood pressure or vascular resistance with excessive pharmacological dosages. Therapies directed at the NO-cGMP pathway may have a more direct and greater impact on haemodynamics than therapies directed at the NRG-1 pathway.

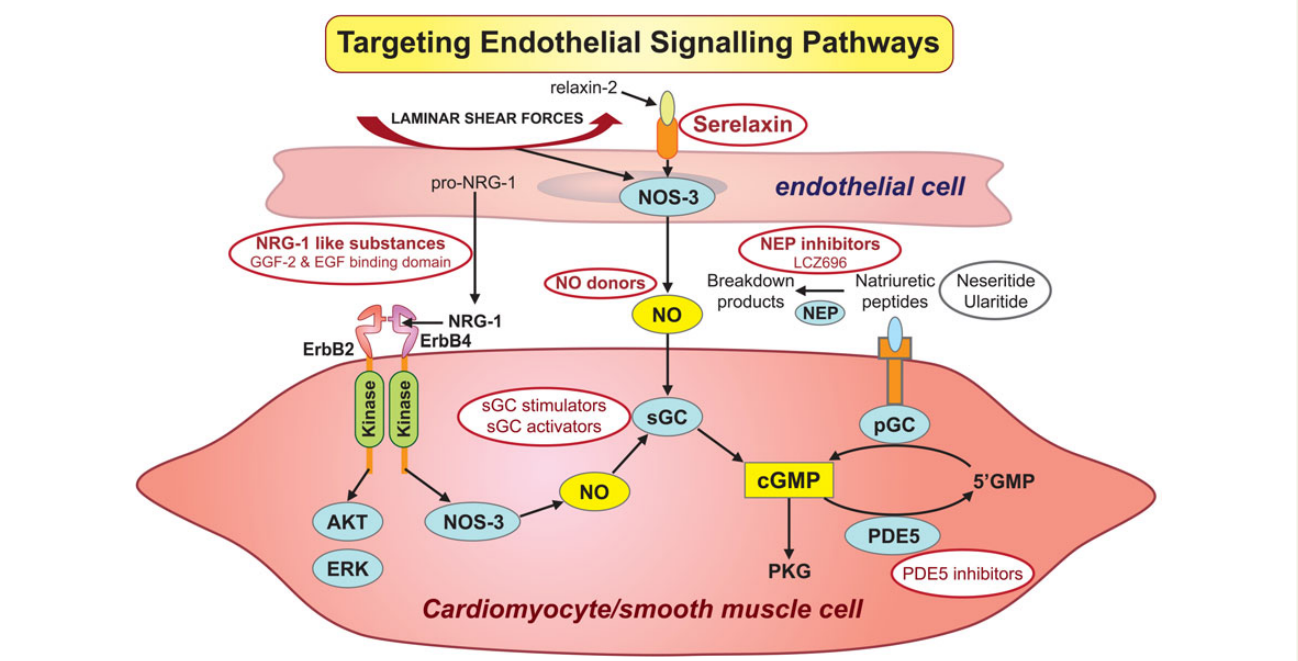


**Figure 4** Cardiac endothelium-cardiomyocyte signalling pathways tested in clinical trials in 2014. Overview of currently tested drugs (and their action) targeting endothelium–cardiomyocyte crosstalk. As shown, these drugs act at different levels. For example, serelaxin increases the concentration of endothelial activators, and hence the endogenous release of endothelial factors, especially nitric oxide (NO). Neuregulin-1 (NRG-1)-like substances and NO donors mimic and thus replace endothelium-released factors. Soluble guanyl cyclase (sGC) and particulate guanyl cyclase (pGC) activators and phosphodiesterase-5 (PDE-5) inhibitors intensify the signalling cascade triggered by endothelial factors in the cardiomyocyte. Use of these drugs may be indicated in different phenotypes of patients, or at different stages of this disease. The combination of some drugs may be physiologically interesting, while other combinations may be dangerous given the risk to over-activate one of the pathways. GGF-2, Glial Growth Factor 2; EGF, epidermal growth factor; NEP neutral endogenous peptidase; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; ERK, extracellular-signal-regulated kinase.

## Organic nitrates and nitric oxide donors

Therapeutic concentrations of organic nitrates preferentially vasodilate the veins and conduit arteries, lowering the peripheral arterial resistance only at higher doses. In the myocardium, NO has positive lusitropic effects with earlier onset of ventricular relaxation and improvement in ventricular compliance.[15,63] Intravenous infusion of nitroprusside has been shown to improve ventricular distensibility and a reduction in LV peak systolic pressure.[64] The effects of NO on myocardial energetics are synergistic with its effects on cardiac contractility, by reducing myocardial energy wastage brought about by the myocardial contraction against reflected arterial pressure waves in late LV ejection.[65]

The use of organic nitrates is not without limitations. Long-term use of organic nitrates is characterized by the development of tolerance. In addition, NO resistance occurs frequently in HF as a result of the oxidative stress. In the presence of oxidative stress, the shift of sGC redox equilibrium towards the oxidized form renders sGC unresponsive to NO. The abundance of superoxide anions in HF may lead to interaction with NO with formation of peroxynitrite. Peroxynitrite has numerous cytotoxic effects; through its interaction with lipids and proteins, it disrupts mitochondrial function and cellular signalling, eventually leading to cellular dysfunction and death.

The addition of hydralazine to chronic nitrate therapy prevents nitrate tolerance and prevents the increase in vascular superoxide production.[66] In a small study of 28 HFREF patients, hydralazine has been shown to restore NO responsiveness.[67] Hence, the synergistic combination of hydralazine and organic nitrates is thought to exert beneficial effects on the endothelium.

Benefits of organic nitrates were first demonstrated in the Vasodilator Heart Failure Trial (V-HeFT I), where improvements in left-ventricular function and survival were seen following the addition of isosorbide dinitrate and hydralazine to a HF therapeutic regimen of diuretics and digoxin.[68] The concept of augmenting NO bioavailability was subsequently tested in the African-American Heart Failure Trial (A-HeFT), where a fixed combination of isosorbide dinitrate and hydralazine was tested in a group of advanced HF patients.[69] This study was terminated prematurely due to benefits in the treatment arm, with improvements in mortality, HF admissions, and quality of life among those receiving isosorbide dinitrate and hydralazine in addition to standard HF therapy.

## Phosphodiesterase-5 inhibition

Phosphodiesterases are a group of enzymes which catalyse cyclic nucleotide hydrolysis, thereby controlling the intracellular concentration of second messenger molecules including cAMP and cGMP.

Phosphodiesterase-5 (PDE-5) inhibitors increase the intracellular concentration of cGMP, which leads to enhancement of endothelial function,[70] improvement of renal response to NPs,[71] and attenuation of adverse LV remodelling.[72]

PDE-5 inhibitors have been explored in the management of pulmonary hypertension secondary to left-sided HF. In general, great caution should be exercised when using pulmonary-specific vasodilators in HF: selective pulmonary arterial vasodilatation without concomitant left-ventricular afterload reduction risks precipitating acute pulmonary oedema due to sudden increases in RV output emptying into a congested left atrium. This deleterious effect has been described with inhaled NO.[73,74] Prostaglandins[75] and endothelin receptor antagonists[76] have been associated with progression of chronic HF. However, PDE-5 inhibitors have not been associated with this risk of worsening left HF. In fact, Guazzi *et al.*[77] demonstrated an improvement in exercise capacity and quality of life in small groups of patients with HFREF, as well as improvement in left- and RV function in HFPEF patients with pulmonary hypertension.[78] Unfortunately, these favourable findings were not seen in a multi-centre trial involving the use of sildenafil in 216 HFPEF patients (regardless of pulmonary hypertension).[79] There was no significant improvement in exercise capacity, quality of life, or haemodynamics. In addition, there was no significant increase in cGMP levels between the two groups. Failure to increase cGMP levels with PDE-5 inhibition suggests that the predominant problem may be decreased cGMP production, rather than increased cGMP breakdown by PDE-5.

## Soluble guanyl cyclase stimulators and activators

Given that regulation of cardiovascular homeostasis by NO is mediated through sGC-cGMP-dependent downstream mechanisms, while the undesirable effects of NO appear independent of this signalling pathway, sGC has recently emerged as an attractive therapeutic target for HF. Two classes of compounds are known to influence the activity of sGC independent of NO, sGC stimulators and sGC activators.

sGC exists in two isoforms, with distinct sensitivity to NO, depending on the state of its prosthetic ferrous haem group. A reduced ferrous haem group is necessary for NO-dependent sGC stimulation. Conversely, oxidation of the haem group results in its dissociation, rendering the enzyme dysfunctional and resistant to NO stimulation.[80] sGC stimulators target the NO-sensitive, reduced sGC; they sensitize sGC to NO in the presence of low levels of NO and directly increase the activity of sGC in the absence of NO. In contrast, sGC activators specifically and effectively target NO-unresponsive sGC, irrespective of the bioavailability of endogenous NO.

sGC activators have potent vasodilatory effects, and have been shown to exert haemodynamic and other non-vascular effects in animal studies.[81,82] Hypertensive rats treated with sGC activators demonstrated reductions in blood pressure and remodelling. In addition, sGC activators have renoprotective effects independent of their blood pressure lowering effects. In canine models of experimental HF, reductions in preload and afterload with concomitant improvements in renal perfusion and cardiac output were demonstrated following the administration of cinaciguat, a sGC activator.[83] These effects were dose-dependent. Similar beneficial effects were shown in rats with cardiorenal syndrome—there were attenuation of blood pressure, reduced fibrosis, and inflammation with improvement in cardiac and renal function, and reduced mortality.[84] Clinical trials involving HF patients have not replicated similar results. The efficacy and safety of intravenous cinaciguat was evaluated in a phase II study involving patients admitted for acute decompensated HF. This study was discontinued prematurely due to excess hypotension in the patients.[85] (see below recommendation 3 under 'Considerations for future clinical trials in HF').

sGC stimulators are potent vasodilators, exerting similar favourable effects on blood pressure, LV remodelling, and cardio- and renoprotective effects.[86,87] Experiments involving hypertensive rats demonstrated lower myocardial remodelling, attenuated cardiac, and renal fibrosis with improvement in renal function, following administration of riociguat.[88] In canine models of HF, BAY 41-2272 resulted in improvements in systemic vasoconstriction without significant effects on blood pressure, pulmonary hypertension, cardiac output, and renal function.[89] The oral sGC stimulator BAY 60-4552 was evaluated in patients with HFREF and secondary pulmonary hypertension, and demonstrated reductions in left- and RV preload and afterload.[90] The Acute Hemodynamic Effects of Riociguat in Patients with Pulmonary Hypertension Associated with Diastolic Heart Failure (DILATE-1) study was a phase IIa trial evaluating the oral sGC stimulator riociguat in HFPEF patients with pulmonary hypertension. Following a single dose of riociguat 2 mg, there were significant increases in stroke volume and cardiac index, with concomitant decreases in afterload and RV area.[91] Other phase II and III trials involving the use of sGC stimulators in HF are currently ongoing. The Soluble Guanylate Cyclase stimulator Heart Failure Studies (SOCRATES) program consists of two parallel-group, randomized, double-blind, multi-centre phase II studies evaluating the pharmacodynamics, pharmacokinetics, safety, and tolerability of the new oral sGC stimulator BAY1021189 in patients with worsening HF requiring hospitalization (ClinicalTrials.gov Identifier: NCT101951638). Eligible patients will be recruited into either SOCRATES-REDUCED (patients with LVEF $<45\%$) or SOCRATES-PRESERVED (patients with LVEF $\geq 45\%$), where four dose regimens of this drug will be explored over 12 weeks.

## Neprilysin inhibitors

LCZ 696 is a complex molecule which comprises the neprilysin inhibitor prodrug AHU377 together with an angiotensin II receptor blocker (ARB) valsartan in the same compound.[92] Inhibition of neprilysin enhances the availability of biologically active NPs. The latter peptides act in parallel to the NO-sGC-cGMP pathway via activation of the NP-pCG-cGMP signalling system. NPs enhance natriuresis, diuresis, and vasodilatation. They also augment myocardial relaxation through a cardiac endothelium-dependent signalling pathway[93] and reduce myocardial hypertrophy through increased generation of cGMP.[94,95] Inhibition of angiotensin receptor results in suppression of the renin–angiotensin–aldosterone system, which has been proved to be beneficial in HF.

LCZ 696 has been successfully evaluated in HFREF patients. The PARADIGM-HF (Prospective comparison of ARNI with ACEI (angiotensin-converting enzyme inhibition) to Determine Impact on Global Mortality and morbidity in Heart Failure) study is a phase

III, randomized, double-blind, parallel group, two-arm, event-driven trial evaluating the safety and efficacy of LCZ 696 and enalapril in chronic symptomatic HFREF patients.[96] This trial has recently been completed and published; LCZ 696 resulted in a 16% reduction in all-cause mortality, 20% reduction in cardiovascular mortality and 21% reduction in HF hospitalizations among chronic HFREF patients.[97] Although augmentation of the NP pathway by LCZ 696 may be expected to boost endothelial function, other beneficial neurohormones may also be involved and endothelial function was not specifically measured in this landmark trial. Future studies in this cohort may provide insight to the extent to which beneficial effects of LCZ696 may be attributable to improvement in endothelial function.

LCZ696 was also evaluated in the PARAMOUNT [Prospective comparison of ARNI (angiotensin receptor neprilysin inhibitor) with ARB on Management Of heart failUre with preserved ejection fracTion] trial; it was a phase II, randomized, parallel-group, multicentre trial comparing LCZ 696 against valsartan over 36 weeks in 301 HFPEF patients.[98] LCZ 696 reduced N-terminal prohormone of brain natriuretic peptide (NT proBNP) to a greater extent than valsartan at 12 weeks, but the difference was not significant at 36 weeks. Favourable changes in left-atrial volume and dimensions were observed at 36 weeks, without concomitant changes in diastolic function. A large phase III trial has recently commenced to evaluate the benefit of LCZ 696 on morbidity and mortality in HFPEF [Efficacy and Safety of LCZ 696 Compared with Valsartan, on Morbidity and Mortality in Heart Failure Patients With Preserved Ejection Fraction (PARAGON-HF), ClinicalTrials.gov Identifier: NCT01920711].

## Serelaxin

Serelaxin is the recombinant form of human relaxin-2, a naturally occurring peptide hormone that mediates systemic haemodynamic and renal adaptive changes during pregnancy.[99] Serelaxin binds to relaxin family peptide (RXFP) receptors that are located in the heart, blood vessels,[100] and kidneys. Serelaxin reduces pulmonary capillary wedge pressure and systemic vascular resistance, and increases cardiac index in stable and acute HF patients.[101,102] These haemodynamic effects of serelaxin are predominantly a consequence of receptor-mediated actions on endothelial cells, including the release of NO, a reduction of endothelin-1 levels, and an increase in vascular endothelial growth factors.[103–107] Apart from these endothelial effects, serelaxin directly or indirectly exerts anti-inflammatory effects, promotes cell survival, and mitigates reparative fibrosis.[108]

In the phase III RELAX-AHF[101,102] trial in patients with acute HF, a 48 h infusion of serelaxin significantly reduced symptoms and signs of congestion and lowered NT-proBNP levels compared with placebo. It also shortened length of hospital stay. Interestingly, at Day 180, serelaxin reduced all-cause mortality and cardiovascular death, although these data were not primary endpoints of the trial and need to be confirmed in an adequately powered prospective trial.

## Exercise

Exercise enhances endothelial function, in particular NO bioavailability, and it improves NO-mediated vasodilation in chronic HF[109] primarily through increased endothelial shear stress. In addition, activation of various interconnected endothelial pathways, resulting from altered plasma levels of circulating insulin, glucose, lipids, interleukins, all known to affect endothelial (dys)function, may also contribute to the beneficial effects of exercise. *In vivo* studies have demonstrated an upregulation of vascular eNOS expression in humans and animals exposed to physical training.[110] In addition, endothelial shear stress promotes NO release through the increase in $Ca^{2+}$ influx[111] and activation of endothelial $K^{+}$ channels.[112] This improvement in NO-mediated vasodilatation is associated with improvements in peak oxygen consumption, indicating that an improvement in endothelial function enhances cardiorespiratory fitness.[41]

However, these favourable findings were not replicated in the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION) study, a multi-centre, randomized-controlled trial evaluating the efficacy of home-based exercise training following a period of supervised exercise training in chronic HFREF patients.[113] However, the non-adherence rate of 40% in the exercise arm and crossovers to exercise in the control arm could have contributed, in part, to the neutral findings. Endothelial function was neither a selection criterion nor an assessment parameter, and it is conceivable that a study sample enriched with endothelial dysfunction may have demonstrated benefits of exercise training. Supporting this postulation, a sub-analysis of women in HF-ACTION, in whom age-related endothelial dysfunction has been shown to be more prevalent than in men,[114] showed that exercise training in women with HF was associated with a larger reduction in rate of the combined endpoint of all-cause mortality and hospital stay than in men.[115]

The benefits of exercise training are similarly demonstrated in HFPEF, where exercise training has been shown to be associated with improvements in peak oxygen consumption, physical functioning score, and echocardiographic indices of diastolic function.[116,117] However, a study performed by Kitzman *et al.*[118] showed that neither carotid arterial stiffness nor brachial artery flow-mediated vasodilatation accounted for improvements in peak oxygen consumption seen with exercise training. The duration of endurance training may not have been sufficient to produce detectable changes in conduit arterial function, and potential benefits on the microvasculature mediated by NO-dependent vasodilatation, cannot be excluded. Indeed, Borlaug *et al.*[42] showed that microvascular reserve, assessed by change in EndoPAT fingertip plethysmography in response to exercise, was reduced in patients with HFPEF compared with age-matched healthy controls, and both systemic vascular conductance and microvascular reserve were related to peak VO2 in HFPEF. Thus, although conduit arterial endothelial function seems to be preserved in HFPEF, impaired microvascular function may limit exercise performance in older patients with HFPEF by decreasing diffusive oxygen transport to the active muscle.

## Human recombinant neuregulin-1

The effects of recombinant human NRG-1 (rhNRG-1) in patients with stable HFREF on optimal medical therapy have been described in two recent phase II clinical trials.[119,120] A first trial, in which a daily intravenous infusion of rhNRG- 1 was given to patients during 10 consecutive days, showed an increase in left-ventricular performance and reverse LV remodelling 30 days later. On Day 90, LV function improved even further and LV dimensions further decreased.[119] A second trial, in which patients received a consecutive 12 h intravenous infusion of rhNRG-1 during 10 days, showed a 30% increase in cardiac output and a 20–30% decrease in pulmonary artery wedge

pressure and systemic vascular resistance of 30%. Twelve weeks after infusion, LVEF was increased by 12%.[120] Importantly, in these studies, short-term rhNRG-1 administration was safe and well tolerated. Larger trials are now ongoing. A phase II clinical trial has been set out to determine, over a period of 12 months, the efficacy and safety of NRG-1 as a treatment in 120 patients with chronic HFREF (ClinicalTrials.gov Identifier: NCT01251406), and a phase III clinical trial has been set-up with the purpose of evaluating the ability of NRG-1 to improve survival (ClinicalTrials.gov Identifier: NCT01541202), cardiac remodelling (ClinicalTrials.gov Identifier: NCT01439893), and efficacy and safety of subcutaneous NRG-1 administration in HFREF (ClinicalTrials.gov Identifier: NCT01214096). A Phase I clinical trial with Glial growth factor 2, an NRG-1 isoform, is also trying to determine its effects on patients with LV dysfunction and symptomatic HF (ClinicalTrials.gov Identifier: NCT01258387).

A potential risk of systemically treating patients with NRG-1 is the activation of neoplastic signalling and cancer growth through the induction of ErbB3/ErbB2 oncogenic complex in ErbB2 amplified cells. To overcome this risk, Lee *et al.*[121] designed an engineered bivalent neuregulin-1 (NN) that demonstrates reduced neoplastic potential in comparison with NRG-1, and still retains its cardioprotective properties. Although its clinical effects still have to be demonstrated, NN could represent a more translationally relevant therapy to rescue ErbB signalling in chronic diseases such as HF.

## Considerations for future clinical trials in heart failure

The HF clinical trial landscape, and particularly that of HFPEF, is littered with 'gravestones' of recent neutral trials. Some of these have included trials of agents known to have positive effects on endothelial function, such as ACE inhibitors and ARBs in HFPEF (PEP-CHF, CHARM Preserved, I-PRESERVE). However, endothelial dysfunction was neither a selection criterion nor specifically assessed in these prior trials. Hence, not the medical action of the drugs, but the design of the clinical trials may have contributed to the failure of many of these HF trials. The design of a trial (e.g. patient selection and study endpoints) should be better adapted to both the action and the clinical expectations of the drug of interest. In the further clinical development of tools to target endothelial signalling in HF, we recommend the following general principles (*Figure 5*):

First, patient selection may be based on phenotypic characteristics associated with endothelial dysfunction (e.g. metabolic dysfunction, obesity, diabetes, males vs. females), rather than solely on vague traditional HF parameters like LVEF or stand-alone biomarkers; in other words future trials may be designed to be more pathophysiology-specific. This approach will obviously significantly scale down the number of patient candidates for drug development, but this may be better than vague studies in a broad, heterogeneous (and commercially more interesting) HF population. Although we believe that phenotypic characteristics of a patient more conveniently reflect patient-specific disease processes than any stand-alone biomarker or HF parameter, direct assessment of endothelial function in endothelium-targeted HF trials would be most logical. Such assessments will further homogenize and characterize the HF study population in terms of endothelial features, and allow longitudinal follow-up of endothelial function during the course of the clinical trial. However, accurate functional assessment of the endothelial system is complex, and functional testing of the endothelium should not be confined to a mere assessment of endothelial regulation of vascular motricity.

To date, most currently available indices of endothelial function have scientific downsides and it is not clear which of these indices is to be preferred. Some of the indices are often difficult to obtain, have not been rigorously standardized or show large intra- and inter-observer variability. Notably, they merely reflect a narrow window of endothelial function such as NO-dependent effects or inflammatory changes, or lack specificity for endothelial dysfunction. Endothelial assessments applied to HF include (i) invasive or non-invasive measures of vasomotor function, (ii) serum markers of endothelial (dys)function such as endothelin-1, NRG-1, C-reactive protein, von Willebrand factor, E-selectin, thrombospondin-1, endothelial miRNAs (miR-146a), and endothelial microparticles, (iii) urine markers of endothelial dysfunction such as albuminuria, or (iv) circulating cells such as endothelial progenitor cells. A recent study involving community-based adults without cardiovascular diseases demonstrated associations between endothelial microparticles and cardiometabolic risk factors, highlighting the importance of such risk factors on endothelial function.[122] Other markers of endothelial function have shown correlations with adverse events or prognosis of HF.[123,124] Yet, their use in clinical cardiology or in scientific trials is still very limited. Obviously, further progress and brilliant discoveries in the assessment of endothelial function in HF are awaited.

Second, intermediate endpoints such as quality-of-life indices, exercise capacity, and some biomarkers of matrix remodelling and anatomic cardiac indices may be considered, as these may be useful surrogates for HF mitigating drug activities, induced by these 'endothelial' interventions. Two recent studies illustrated this, showing anti-fibrotic effects of NRG-1[125] and LCZ696 in the remodelling myocardium.[126] These intermediate endpoints may be coupled with biomarkers of HF prognosis, such as serum BNP, LV diameters, left-atrial size, or heart-rate variability. Too many active HF drugs have been terminated based on their disappointing effect on total mortality, despite a beneficial effect on the progression of chronic HF. This is unfortunate since in HF, e.g. for the very symptomatic and elderly patients, drugs can be useful even if they do not reduce total mortality.

Finally, preference should be given to pleiotropic drugs acting at various sites, including the endothelium, in order to re-establish overall physiological homeostasis. For drugs acting through endothelial-mediated signalling pathways, this would necessitate prolonged exposure to the lowest possible dosages even if at first no measurable changes are observed. For example, as half-life and duration of signalling activity varies significantly among the different interdependent endothelium–cardiomyocyte signalling pathways, one may expect a profound mismatch in their interaction in HF. This mismatch may be further hampered if only one of these pathways would be targeted for short periods and at high dosages of a drug. In contrast, chronic exposure to lower dosages would, i.p. in HF patients with concomitant pulmonary hypertension, allow for an auto-amplifying shear stress-induced pulmonary endothelial-mediated haemodynamic reversal back into a low impedance pulmonary vascular system. Similarly, in the systemic circulation, as most of

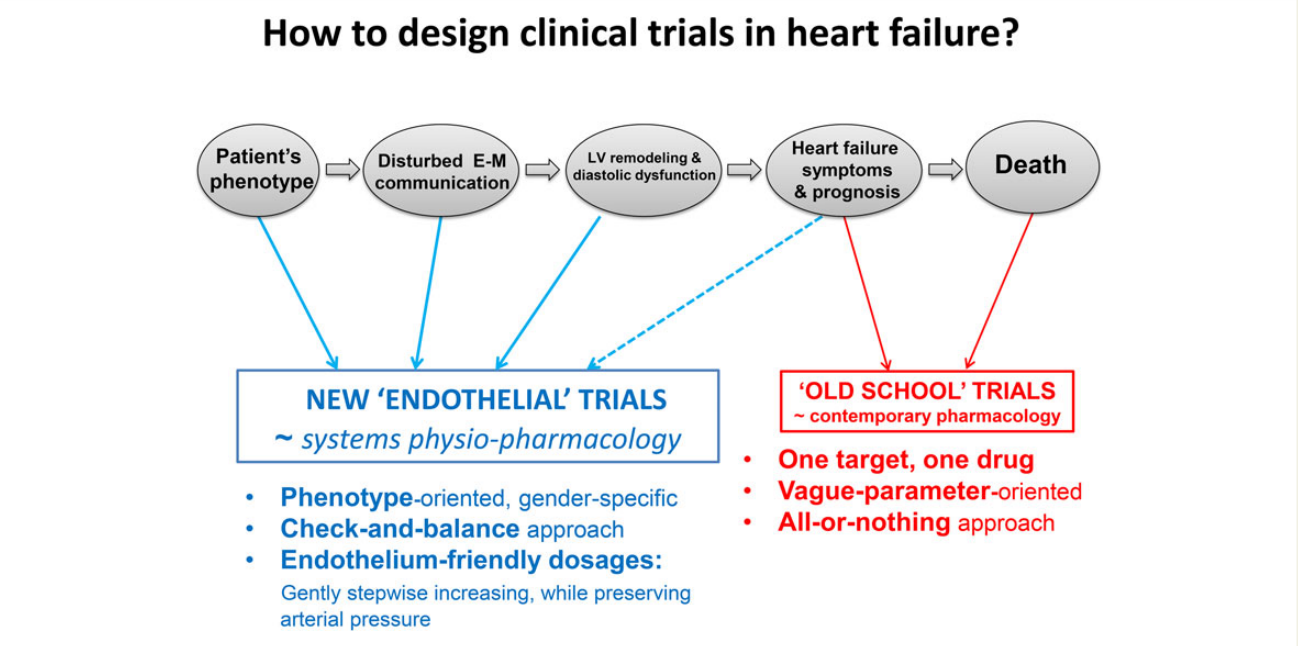


**Figure 5** Designing clinical trials to test endothelium–cardiomyocyte pathways in heart failure. When a drug is tested in a clinical trial there should be evidence that the drug-related disease pathway is disturbed in the study population. Therefore, when 'endothelial' drugs are put into trial, one should first carefully define a study population in which cardiac endothelial dysfunction is central in the pathogenesis. One way to do this is to start from phenotypic characteristics and co-morbidities known to reflect or to be associated with endothelial dysfunction (independent from LVEF). Selecting endpoints closer to the presumed cardiac effect of the drug (e.g. markers of reverse cardiac and matrix remodelling, and of diastolic dysfunction) would allow a cheaper 'check-and-balance' strategy. Also, it may suffice to define the patient population positively responding to the trial drug. If required, the results could then later be employed to test effects on mortality and hospitalization in a pathophysiological coherent and drug-responsive population.

these drugs are vasodilators, focus in HF should be on the modulation of arterial impedance while avoiding arterial pressure drop. A prolonged exposure at low dosages of endothelium-mediated drugs would thus be expected to optimize the ventricular–arterial cross-talk through simultaneous endothelium-mediated effects on timing and rate of ventricular relaxation and on arterial impedance while avoiding arterial pressure drop. In summary, preference may be given to low and prolonged dosages of pleiotropic drugs, while avoiding short treatments at high dosages of single target drugs which may be detrimental to endothelial–cardiomyocyte balance. In addition, this low-dosage pleiotropic drug strategy would greatly complement the more physiological, but still largely undervalued exercise training.

Accordingly, a phenotype-based check-and-balance approach may allow a stepwise progression of new clinical tools in cheaper clinical studies to define which patients are optimal candidates for treatment.

# Conclusions

Endothelium-controlled signalling pathways play a crucial homeostatic role in cardiac tissue. Disturbance of these pathways accounts for the adverse myocardial remodelling and dysfunction seen in HF, and portends a worse prognosis in these patients. Endothelial pathways are emerging as an important pathophysiological target in the treatment of HF regardless of EF. Attention should be paid to patient selection and study endpoints in future clinical trials, in order to avoid new disappointments in the clinical development of this target.

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