Cardiovascular diseases in general and heart failure (HF) in particular are major contributors to death and morbidity in the Western world, where they are also recognized as important drivers of healthcare expenditure. The health and economic burden of these disorders is projected to increase with the aging of populations around the world.\textsuperscript{1–3} On the basis of accumulating evidence that chronic overactivity of the renin–angiotensin–aldosterone system (RAAS) plays a fundamental role in HF pathophysiology, drugs inhibiting key components of the RAAS have become a cornerstone of contemporary cardiovascular drug therapy.\textsuperscript{4–6} For example, angiotensin-converting enzyme inhibitors (ACEi) reduce biosynthesis of angiotensin-II (Ang-II), 1 of the strongest vasoconstrictors, prohypertrophic and profibrotic hormones in man. Moreover, ACEi may prevent proteolysis of bradykinin, thus enhancing bradykinin-mediated vasodilatory effects that may counteract the profound vasoconstriction seen in patients with HF.\textsuperscript{7} Excessive levels of Ang-II have been implicated in many cardiovascular diseases, and in addition to ACEi, the detrimental actions of Ang-II can be abrogated by direct angiotensin-receptor blockers (ARB). However, despite encouraging results from many clinical trials, ACEi- and ARBs-based pharmacotherapy is still far from optimal. ACEi may lose their efficacy over time because of redundant Ang-II–generating pathways and the so-called aldosterone escape,\textsuperscript{8} whereas conventional ARBs do not possess the bradykinin-enhancing properties of ACEi and are considered less effective in HF compared with ACEi.\textsuperscript{4,5}

The natriuretic peptides (NPs), consisting of atrial NP (ANP), B-type NP (BNP), C-type NP (CNP), and urodilatin, are predominantly generated by the heart, vasculature, kidney, and central nervous system in response to wall stress and number of other stimuli. Importantly the NPs, particularly ANP and BNP, represent the body’s own blood pressure (BP)–lowering system. Besides promoting vasodilation, NPs counteract pathological growth, fibrosis, and dysfunction of heart, kidneys, brain, and the vasculature. Current NP-augmenting strategies include the design of a number of synthetic NPs and inhibition of neprilysin, the key enzyme responsible for NP breakdown. Dual-acting angiotensin-receptor neprilysin inhibitors (ARNi) are under scientific scrutiny for the treatment of hypertension and HF.

This review summarizes the current knowledge on RAAS blockade and NP-augmenting drugs as single or combined strategies in HF. We will discuss challenges that have been met with some of these compounds and novel therapeutic agents currently being evaluated, which could strengthen our pharmacological armamentarium for HF.

**Renin–Angiotensin Aldosterone System**

The RAAS is fundamental in the overall regulation of cardiovascular homeostasis through the actions of important hormones, which regulate vascular tone, and specifically BP through vasoconstriction and renal sodium and water retention. These hormones, specifically Ang-II and aldosterone, also possess direct actions that are important in HF by mediating cardiomyocyte hypertrophy and cardiac fibrosis with activation of collagen synthesis and fibroblast proliferation (Figure 1).\textsuperscript{9–11} RAAS is also causally involved in the pathophysiology of cardiorenal syndrome in HF, which carries a particularly poor prognosis. Thus, blockade of RAAS has become a central therapeutic strategy for HF using RAAS modulating drugs, such as ACEi, ARBs, and mineralocorticoid receptor antagonists (MRA).\textsuperscript{4}

To date these agents have had a positive impact on HF with improvements in symptoms, outcomes, and survival. Indeed their use is increasingly widespread and their use is moving from symptomatic HF into earlier stages of mild and asymptomatic myocardial dysfunction to delay the progression of HF. Recently, a pivotal trial was completed with the MRA epleronone in patients with systolic HF and mild symptoms.\textsuperscript{12} Importantly, MRAs compared with placebo reduced the risk of the both death and hospitalization, thus delaying disease progression and providing further momentum to this continuously expanding therapeutic modality. In addition, the MRA spironolactone is under investigation in the ongoing...
TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial) trial for efficacy in HF with preserved ejection fraction (HFPEF), a disease entity for which no specific treatment recommendations exist.4,13

Direct renin inhibition upstream of ACE, well-known for decades as a RAAS-blocking concept, prevents the generation of Ang-I and thus, Ang-II. The first-in-class drug aliskiren is currently being evaluated in 2 clinical HF trials, the Aliskiren Trial on Acute Heart Failure Outcomes (ASTRONAUT) and the Aliskiren Trial of Minimizing Outcomes for Patients with Heart Failure (ATMOSPHERE).14–16 ASTRONAUT set out to evaluate the primary and the secondary composite end points of cardiovascular death or HF rehospitalization at 6 and 12 months, respectively, in 1782 patients recently hospitalized with HF and reduced systolic left ventricular (LV) function. The very recently published study showed that aliskiren in addition to standard therapy failed to reduce primary or secondary end points but led to significantly larger decrease from baseline in NT-proBNP levels.16 Subgroup analysis demonstrated increased all-cause mortality at 12 months for patients with a history of diabetes mellitus randomized to aliskiren, whereas patients with non–diabetes mellitus showed net benefit compared with placebo. The reason for such a bidirectional effect of aliskiren on all-cause mortality depending on the presence or on the absence of diabetes mellitus deserves further evaluation. In a similar but considerably larger HF cohort (planned enrollment, n=7000), ATMOSPHERE will compare effects on mortality and morbidity by aliskiren, enalapril, or dual aliskiren+enalapril treatment. Recent evidence suggest similar rates of angioedema with aliskiren and ACEi.17

Perhaps the latest advance in RAAS blockade in HF has been the development of innovative AT1 receptor antagonists, which possess dual actions that go beyond simple antagonism of Ang-II binding. Like conventional ARBs, these molecules target the superfamily of G-protein–coupled receptors. Activation of G-protein–coupled receptors by an agonist leads to intracellular dissociation of a heterotrimeric G-protein into Gα and Gβγ subunits, resulting in activation of second messenger–mediated cellular responses. The 13 arrestins are a second group of proteins, which activate specific signaling pathways in a G-protein–independent manner. Studies have shown that some ligands can selectively activate either G-protein or 13 arrestin pathways.18 We recently reported the actions of a novel 13-arrestin–biased ligand for the Ang-II type 1 (AT1R), TRV120027.19 TRV120027 antagonizes G-protein signaling like an ARB, but, unlike conventional AT1 receptor antagonists, it activates 13 arrestin and downstream signals. In rodents, TRV12007 has vasodilating effects similar to a conventional ARB, but, unlike an ARB, enhanced cardiac contractility while decreasing myocardial oxygen consumption.
consumption.20 In a large animal model of HF, TRV12007, in combination with furosemide, has potent renal and systemic vasodilating properties and preserved glomerular filtration rate (GFR), despite a reduction in BP.21 Currently, TRV12007 has entered early trials in acute decompensated HF (ADHF).

Together, although efficient RAAS blockade can be achieved at multiple levels, ACEi and MRA remain the cornerstone of contemporary HF pharmacotherapy.4 Despite initial enthusiasm because of their greater tolerability compared with ACEi, ARBs offer little cardioprotective benefit after MI, and do, therefore, no longer seem on the A-list of recommended medical therapy for HF.4,22 Novel ARBs, including contractility-enhancing compounds, seem promising. RAAS blockade afforded by direct renin-inhibitors may eliminate some of the shortcomings of current strategies but no clinical outcome data in HF are available yet. Moreover, regarding their safety profile important adverse effects do not seem to occur less frequently than with ACEi.17

Natriuretic Peptide System

The NP system (NPS; Figure 2) has emerged as an increasingly important autocrine, paracrine, and endocrine system linked to particulate guanylyl cyclase (GC) receptors, the second messenger cGMP and its effector molecule protein kinase G.23 Originally discovered by de Bold et al.24 who reported that the heart synthesized and released a factor that both not only augmented natriuresis by the kidney, but also possessed BP-lowering properties. This cardiac factor was identified as ANP and recent studies have reported that genetic variations of the ANP gene, which increases circulating levels of ANP and protects against human hypertension.25 On the basis of the renal and vascular actions of ANP an intravenous drug, known as carperitide, has been approved for HF in Japan. Studies have also well established that ANP mediates its action via the GC-A receptor, which is widely expressed throughout a number of tissues and especially in the adrenal cortex in which ANP is a potent inhibitor of aldosterone independent of its robust renin inhibitory actions.26,27 In the kidney, alternative processing of the ANP precursor, proANP by an unknown protease generates an ANP-like peptide called urodilatin, which regulates renal sodium and water handling.28 Indeed studies evaluating the effects of synthetic ularitide in patients with ADHF (SIRIUS I29 and SIRIUS II30) have shown favorable effects on hemodynamic, neurohumoral, and symptomatic profiles without any compromise in renal function, despite a modest and dose-dependent decrease in BP. Moreover, a larger Phase III trial is currently ongoing, evaluating the efficacy and safety of ularitide in patients with ADHF (TRUE-AHF [Trial to Evaluate the Efficacy and Safety of Ularitide (Urodi-latin) Intravenous Infusion in Patients Suffering From Acute Decompensated Heart Failure]).31 To complement ANP the cardiac hormone BNP, which is approved as nesiritide in the United States and Canada for the treatment of ADHF, Like ANP, BNP is a ligand for the GC-A receptor and possesses similar pleiotropic actions, which include natriuresis, aldosterone suppression, and vasodilatation. Although the seminal VMAC (Vasodilatation in the Management of Acute CHF) clinical trial32 lead to the US Food and Drug Administration approval of nesiritide for ADHF, 2 subsequent meta-analyses suggested that nesiritide administration may be associated with increased short-term risk of death33 and worsening renal function34 in patients with ADHF. However, several other studies, including Acutely Decompensated Heart Failure Registry (ADHERE),35-39 failed to demonstrate these adverse associations with nesiritide therapy. Thus, the ASCEND-HF clinical trial was designed to address these safety and efficacy concerns that were raised since its approval. In the ASCEND trial, nesiritide improved symptoms in the European, but not in the United States patient cohort with ADHF and was not superior to conventional therapy in improving mortality in patients with ADHF.40 Notably, these neutral findings could have been related to excessive hypotension with doses that are potentially vasodilating, and thus offsetting the beneficial renal actions of nesiritide. To underscore the importance of the GC-A receptor beyond the actions discussed above and relevant to HF are the antihypertrophic and antiapoptotic actions, which may contribute to long-term favorable antiremodeling actions if a GC-A agonist can be given chronically.23,41 Indeed, in a recently completed human trial in mild systolic HF, 8 weeks of BNP administered twice daily by subcutaneous injection improved symptoms and reduced LV mass as determined by MRI.42

BNP is the third member of the NP family and is produced in endothelial cells43,44 and renal epithelial cells.45,46 BNP mediates its biological action through the activation of the GC-B receptor and potentially the non–cGMP-mediated pathway.47 CNP is the third member of the NP family and is produced in endothelial cells43,44 and renal epithelial cells.45,46 CNP mediates its biological action through the activation of the GC-B receptor and potentially the non–cGMP-mediated pathway.47,48 Although having an important action to promote bone growth, evidence has supported that CNP has important CV actions as well. These include hyperpolarization of vascular smooth muscle,49 antithrombotic actions,50 promotion of re-endothelialization,51 and potent antifibrotic properties.52 The use of CNP as a HF therapeutic has been limited by both its rapid enzymatic degradation and lack of renal-enhancing actions. A designer CNP-based NP has been engineered, which is now in clinical trials for HF and this will be discussed below.

A key component of the NPS is the ectoenzyme neutral endopeptidase (neprilysin), which is also known as neprilysin. This membrane-bound enzyme is widely expressed, but is most abundant in the kidney. Neprilysin serves as the principal mechanism for enzymatic removal of the native NPs with susceptibility to degradation greatest for CNP>ANP>BNP.54 Furthermore, many other substrates for neprilysin exist, some of them with opposing physiological actions. These include endothelin-1, kinin peptides, opioid peptides, Substance P, amyloid beta protein, and gastrin.55-57 Importantly, neprilysin hydrolyzes Ang-I to Ang-(1–7),58 and because Ang-(1–7) opposes the action of Ang-II, the hydrolysis of Ang-I to Ang-(1–7) by neprilysin potentially has beneficial CV effects. Inhibition of NEP (NEPi) has been advanced as a therapeutic modality. If neprilysin only targeted NPs, NEPi would augment the vasodilating and natriuretic actions afforded by increased levels of these peptides. However, neprilysin’s ability to catabolize numerous substrates also means that sole NEPi yields broader effects than anticipated, and explains why NEPi is best combined with the inhibition of other vasoactive peptides. Candoxatril was the first potent, orally available neprilysin inhibitor. Candoxatril mediated not only a dose-dependent increase in plasma ANP, natriuresis, and
cGMP in humans, but also increased circulating Ang-II. Importantly, Candoxatril’s effects on BP in patients with hypertension were not clinically meaningful. Candoxatril was also investigated in HF. In a canine model of severe HF, which is characterized by both NP elevation and RAAS activation, candoxatril was natriuretic and suppressed aldosterone.

In human HF, candoxatril increased ANP and BNP levels, promoted natriuresis, and decreased clearance of exogenously administered ANP. However, systemic and pulmonary vascular resistances were not altered.

Early strategies to enhance the salutary actions of the NPS have clearly met challenges. Clinical efficacy of recombinant drugs, such as nesiritide, carperitide, or ularitide has been limited by hypotension and their short bioavailability. For the class of single-acting NEPi, as discussed below, their effectiveness to promote the endogenous NPs and to improve overall cardiorenal function was only finally realized when combined with RAAS modulators.

Designer Natriuretic Peptides
Therapeutic use of the native NPs has been highly attractive, given their diverse intrinsic protective properties, which include natriuresis, diuresis, RAAS suppressing, inhibition of fibrosis, vasodilatation, and angiogenesis. In an effort to overcome the shortcomings of recombinant NPs outlined above, the concept of designer NPs has emerged as an innovative advancement in drug discovery for the treatment of various CV diseases. Designer NPs are novel peptides that have been engineered through modifications in their amino acid (AA) structures or through use of genetically altered forms of native NPs. The rationale behind this concept is to produce chimeric NPs whose pharmacological and beneficial biological profiles go beyond those of the native NPs while minimizing undesirable effects.

CD-NP (Cenderitide)
The most advanced designer NP to date was designed by investigators in the Cardiorenal Research Laboratory at Mayo Clinic and first reported in 2008. This novel 37 AA hybrid NP named CD-NP (Figure 3), which is now known as cenderitide, consists of the mature form of native human CNP fused with the 15 AA C terminus of dendroaspis NP, which was first isolated from the venom of the green mamba. This unique first-generation designer NP retains the antifibrotic, antiproliferative, and antihypertrophic effects and venodilatation of CNP, as well as natriuretic and diuretic effects of dendroaspis NP, which are very desirable properties for drugs to combat a number of CV diseases, including HF. Importantly, CD-NP also has antiproliferative actions in cultured human cardiac fibroblasts and stimulates cGMP production in these same cells to a greater extent than equimolar concentrations of BNP. In vitro studies have demonstrated CD-NP is the first NP to activate both the GC-A and the GC-B receptor at physiological doses and is more resistant to proteolytic degradation than ANP, BNP, and
atrial pressure.62 In healthy human subjects, CD-NP infusion renin activity together with a modest reduction in mean perfusion, diuresis and natriuresis, and reductions in plasma significant cardiac-unloading effects, increases in GFR, renal experimental HF induced by rapid ventricular pacing also had effects on mean atrial pressure.62 Furthermore, when compared with conventional recombinant BNP (nesiritide) therapy, an equimolar dose of CD-NP significantly increased GFR and was less hypotensive than BNP.62 Moreover, infusion of CD-NP in experimental HF induced by rapid ventricular pacing also had significant cardiac-unloading effects, increases in GFR, renal perfusion, diuresis and natriuresis, and reductions in plasma renin activity together with a modest reduction in mean atrial pressure.62 In healthy human subjects, CD-NP infusion increased urinary and plasma cGMP levels, suppressed plasma aldosterone, induced a significant diuretic and natriuretic responses and a minimal, yet significant reduction in mean atrial pressure.71 In March 2011, cenderitide received a fast-track designation from the Food and Drug Administration and currently is in Phase II clinical trials targeting postacute patients with HF using chronic subcutaneous infusion technology.72

 CU-Natriuretic Peptide

Building on the encouraging findings of cenderitide in both experimental and human studies and designer NPs technology, a humanized version of cenderitide, called CU-natriuretic peptide (CU-NP) was created. CU-NP is an engineered NP (Figure 3), consisting of the 17 AA ring of native human CNP linked to both the C and N termini of urodilatin, which is a 32 AA cleavage product of intrarenal processed proANP.73 Although CU-NP is in the early stages of drug development, initial experimental studies have demonstrated that intravenous infusion of CU-NP activates cGMP in canine HF and exerts renal-enhancing, cardiac-unloading, and RAAS-suppressing actions without excessive hypotension.74 CU-NP has also direct antihypertrophic effects through the inhibition of the sodium–hydrogen exchanger 1(NHE-1)/calcineurin pathway.75

Figure 3. Amino acid structures of designer natriuretic peptides (NP). CD-NP (cenderitide) consists of amino acids from native human C-type NP (CNP; green) and dendroaspsis NP (light blue). CU-NP consists of amino acids from native human CNP (green) and urodilatin (pink). ANX-042 consists of amino acids from native human B-type NP (BNP; red) and 16 amino acids (yellow) from the C terminus of the alternative spliced transcript of BNP.

Designer NP ANX-042

Another strategy for drug discovery is the biology of alternative RNA splicing which may provide unique opportunities to identify drug targets and therapeutics. We recently reported an alternative spliced transcript for BNP (AS-BNP).76 This alternative spliced BNP transcript is present in failing human hearts and is reduced after mechanical unloading. The transcript would generate a unique 34 AA C terminus although maintaining the remaining structure of native mature BNP. Importantly, unlike BNP, this novel peptide failed to stimulate cGMP in vascular cells or to vasorelax preconstricted arterial rings. From this structure, we designed a shortened 42-AA peptide from AS-BNP, which is currently known as ANX-042 (Figure 3), and demonstrated its ability to stimulate cGMP, like BNP, in canine glomerular isolates and cultured human mesangial cells but lacking similar effects in vascular cells. In a canine-pacing model of HF, systemic infusion of ANX-042 did not alter mean atrial pressure but increased GFR, suppressed plasma renin and Ang-II, while inducing natriuresis and diuresis. Importantly in 2012, ANX-042 was approved as an investigational new drug from the Food and Drug Administration and now has begun a first-in-human clinical trial as a designer renal-enhancing and nonhypotensive NP, which could make ANX-042 a potential novel renal-selective agent for HF.

In summary, the NPs represent the most important endogenous counterpart to RAAS by conferring cardiac, renal, and vascular protection. Therapeutic augmentation of the NPS in HF has been attempted directly using a broad range of recombinant and engineered NPs, or indirectly by preventing NP degradation (through NEP inhibitors). In particular, degradation-resistant NPs, including designer NPs, have shown encouraging early results and are now under evaluation in clinical trials. NEP inhibitors as monotherapy to augment NPs has largely produced neutral effects in clinical studies, and, therefore, its greatest potential presumably lies in the combination with blockers of the RAAS and other neurohormonal systems that are causally inflicted in HF.

RAAS Blockade Combined With NPS Augmentation

Dual ACE/Nephrilysin (Vasopeptidase) Inhibition in HF

As previously described, the RAAS and NPS have a yin/yang relationship with each system, serving as a counter-regulatory constraint on the activity of the other.77 This physiological relationship provides the potential to achieve greater benefits with modulation of both systems than manipulation of individual systems. Specifically, the beneficial effects of inhibition of the RAAS may potentially be augmented by enhancement of
NP activity. Conversely, the disappointing clinical effects of nepriyisin inhibitors as monotherapy may be overcome by combination with RAAS blockade.

Single molecular entities have been developed combining NEP inhibition (NEPi) with both ACEi and ARBs as single molecules (Table).

Early dual NEPi/ACEi agents (vasopeptidase inhibitors), such as sampatrilat, demonstrated promising effect in HF and hypertension but were discontinued because of poor oral bioavailability. The most extensively studied ACEi/NEPi thus far has been omapatrilat. Omapatrilat demonstrated equal potency of inhibition and affinity for both enzymes. In a preclinical HF model, omapatrilat prevented cardiac dysfunction and remodeling and improved survival; also, it produced significant BP reductions in low, normal, and high renin hypertension models, including spontaneously hypertensive rats.

Omapatrilat Cardiovascular Treatment Versus Enalpril (OCTAVE) was the definitive clinical outcome trial to evaluate the beneficial effects of omapatrilat (versus enalpril) in 25,302 untreated or uncontrolled hypertensives. OCTAVE demonstrated improved systolic BP control with omapatrilat and more patients achieving target BP compared with enalapril. The trial, however, reported an increase in prevalence of angioedema in omapatrilat-treated patients, 2.2% versus 0.7%. The mechanism underlying this rare, but potentially life-threatening adverse event is presumably related to enhanced bradykinin levels achieved with blockade of zinc-containing metalloproteinases, such as neprilysin, and potentially aminopeptidase-P and dipeptidyl peptidase-4 as well. Omapatrilat has also been studied in patients with systolic chronic HF. Phase IIB study (IMPRESS [Inhibition of Metalloprotease by Omapatrilat in a Randomized Exercise Symptoms Study of Heart Failure]) comprising 573 patients compared omapatrilat 40 mg/d with lisinopril 20 mg/d for 24 weeks. Omapatrilat reduced the composite end point of death, HF admission, or discontinuation of study treatment for worsening HF compared with lisinopril and produced a greater improvement in New York Heart Association Class III to IV patients. Furthermore, there seemed to be greater preservation of renal function with omapatrilat. There was no significant angioedema signal observed; indeed there were fewer overall adverse events with omapatrilat compared with lisinopril. These favorable findings led to a major outcome study, Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE), which randomized 5770 New York Heart Association Class II to IV systolic patients with HF to enalapril 10 mg twice daily or omapatrilat 40 mg once daily for a mean duration of 14.5 months. The primary end point (death or hospitalization for HF requiring intravenous therapy) was not significantly different compared with enalapril. A twice daily regimen of omapatrilat may have resulted in a smoother pharmacokinetic and pharmacodynamic profile (particularly large postdose falls in systemic BP with once-daily omapatrilat), and this may have translated into fewer major primary end point events.

Vasopeptidase inhibition unfortunately exemplifies yet another therapeutic strategy that, despite strong scientific rationale and positive early trials, has not translated into better pharmacotherapy for patients with HF.

### Triple Inhibitors of ACE, Neprilysin, and ET-Converting Enzyme

Endothelin-1 (ET-1) is a pluripotent vasoconstrictor and multifunctional neurohormone that contributes in the progression of HF and many other cardiovascular diseases. ET-1 plasma levels strongly predict mortality in cardiovascular disease and ET-1 production markedly increases in HF. A multitude of ET-1 receptor antagonists have been tested in acute and chronic HF settings, but the majority failed to improve outcomes. Although the concept of ET-1 receptor antagonists in HF is now widely considered as futile, with the exception of HF because of certain forms of pulmonary arterial hypertension, abrogation of ET-1 biosynthesis by ET-converting enzyme (ECE)-inhibition is a strikingly lesser explored avenue. In experimental HF, ECE-inhibition (ECEi) improved cardiorenal function together with reduction of other key neurohormones, such as Ang-II, renin, and aldosterone. ECE may also cleave NPs to a physiologically relevant degree, accordingly its targeting through ECEi might simultaneously augment and suppress NPs and ET-1 levels, respectively. In human acute HF, ECEi induced favorable hemodynamic changes similar to ET-receptor blockade and on top of ACEi, but no long-term data exist. Dual ECE/neprilysin inhibition reduced adverse LV remodeling and dysfunction in a post-MI HF model. The ECE/neprilysin inhibitor SLV-306 (daglutril) was shown to lower LV filling pressures acutely in human HF, and given over time, to reverse elevated plasma ET-1 levels and pathological cardiac remodeling similar to ACE-inhibition in rats with LV hypertrophy (Table). Daglutril also abrogated big-ET-mediated BP increases and enhanced NP levels in healthy humans. More recently, SLV-338, a similar dual ECE/neprilysin inhibitor prevented experimental hypertension-induced cardiac fibrosis independently of BP lowering. Triple ACE/ECE/neprilysin inhibitors have been designed to suppress biosynthesis of Ang-II and ET-1 and to augment vasodilators, including bradykinin, NPs, and adrenomedullin. In rats with HF post-MI, ACE/ECE/neprilysin inhibition improved LV structure and function more than either ACE or ECE/neprilysin inhibition alone. Although encouraging, these initial results would need to be evaluated in randomized prospective clinical trials.

Unfortunately, further clinical development of triple ACE/ECE/neprilysin inhibitors seems to have been abandoned, perhaps because of previous concerns about safety with vasopeptidase inhibitors. The largely negative results from ET-receptor antagonist HF trials may further have tempered enthusiasm for the field. In addition, unlike with RAAS, it seems that for the case of ET the scientific community has made considerably less distinction between the modalities of receptor antagonism and inhibition of biosynthesis (by ECEi).

### Dual-Acting Angiotensin-Receptor/Neprilysin Inhibitors (ARNi)

On the basis of the above considerations on vasopeptidase inhibitors, newer agents combining NEPi with not an ACEi but ARB have been developed, again as single molecules (ARNi; Table). The rationale for these agents is that ARBs are less likely to interfere with bradykinin...
metabolism and thus less likely to contribute to cough and angioedema. LCZ-696 is a fixed dose combination of valsartan and AHU-377 (NEPi prodrug) in a 1:1 ratio and is the first and most clinically advanced compound in this new class. Preclinically, LCZ-696 was able to lower BP in double transgenic (renin, Ang-II overexpression) rats with associated increases in plasma cGMP, renin concentration and activity, and Ang-II levels indicating that appropriate receptors were targeted as per expected pharmacological actions. Furthermore, enhanced tracheal plasma extravasation was not observed with the ARB/NEPi valsartan/candoxatril, suggesting minimal risk of angioedema with the ARB/NEPi combination, further confirmed by recent patient data on ARB and ACEI.

A large Phase II placebo-controlled study of LCZ-696 has recently been undertaken in patients with mild to moderate
hypertension. The key findings after 8 weeks of follow-up were significantly greater reductions in office and ambulatory BP with LCZ-696 compared with the equivalent dose of valsartan alone. Importantly, LCZ-696 was well tolerated and there were no cases of angioedema reported. Neurohormonal biomarker assessment confirmed the expected augmentation of plasma ANP and cGMP, as well as plasma renin in the LCZ-696 cohorts.

LCZ-696 may also have considerable potential in the setting of systolic chronic HF analogous to the attempt to establish omapatrilat as standard background RAAS blocker (replacing ACEi) in this setting in the OVERTURE study conducted a decade earlier. The PARADIGM-HF (Efficacy and Safety of LCZ696 Compared to Enalapril on Morbidity and Mortality in Patients with Chronic Heart Failure and Reduced Ejection Fraction) study is an ongoing efficacy and safety assessment.

Figure 4. Cardiac antiremodeling effects of angiotensin receptor neprilysin inhibitors (ARNi) in vitro and in vivo. A and B, Effects of angiotensin receptor blockers (ARB) (Valsartan, Val) vs ARNI (Val+NEPi) on Ang-II–stimulated neonatal rat cardiomyocytes hypertrophy (A) and cardiac fibroblasts collagen synthesis (B) as determined by [3H]-leucine and [3H]-proline incorporation, respectively. Data are shown as percentage of unstimulated control (100%). ARB dose dependently attenuated Ang-II–mediated effects in both cell types, with further additional reduction in ARNi-treated cells. Overall, ARNI (Val+NEPi) provided dose-dependent, superior antihypertrophic (A), and antifibrotic (B) effects compared with ARB alone. The effects of neprilysin inhibitor (NEPi) alone in cardiomyocytes were modest, and negligible in fibroblasts. Unstimulated (negative) and Ang-II–stimulated (positive) controls are the first open and solid columns, respectively, from the left. Dashed line demarks level of unstimulated control; ***P<0.001 vs Ang-II–stimulated control, ###P<0.01 ARB+NEPi vs ARB for equal dose of Val, unpaired t test. C–F, Effects of ARNI on cardiac remodeling after myocardial infarction (MI). One week after surgical ligation of the left anterior descending coronary artery in outbred male Sprague-Dawley rats (250 g), animals were randomized to treatment with ARNI (LCZ-696; 68 mg/kg body weight administered perorally) with ARNI (n=11; solid columns) or vehicle (n=6; open columns). After 4 weeks of treatment, ARNI-treated rats exhibited significantly smaller weights of all cardiac chambers, consistent with reduced cardiac hypertrophy (C–F).
of LCZ-696 in patients with stable chronic HF (LV ejection fraction <40%). Before randomization to either LCZ-696 20 mg BID or enalapril 10 mg BID, a single-blind run-in period is undertaken. Patients receive (sequentially) enalapril 10 mg BID, LCZ-696 100 mg BID, and then LCZ-696 200 mg BID for a duration between 5 and 8 weeks. Patients postrandomization are then followed until 2410 primary outcome events (CV death or HF hospitalization) have been achieved.

There are a number of interesting design features built in to PARADIGM-HF that are worthy of comment. Specifically, the single-blind run-in period is designed to first switch patients to a standard comparator agent, enalapril, and to establish that they are able to tolerate a dose equivalent to that achieved in the SOLVD treatment study111 that forms the basis for ongoing use of ACE-inhibitors in systolic HF (mean of 16.6 mg/d). Patients are then evaluated with regard to tolerability of LCZ-696 at progressively increasing doses, before randomization. The other important design feature of PARADIGM-HF is that, unlike in OVERTURE, patients receive twice daily dosing of LCZ-696. This was deliberately designed to minimize the potential (as alluded to earlier) for large drops in BP and other potential hemodynamic disturbance after administration of the full daily dose given on a once daily basis.

We recently evaluated putative antifibrotic and antihypertrophic efficacy of ARNi in cultured neonatal rat cardiac fibroblasts and myocytes using 3H-proline and 3H-leucine incorporation, respectively, as described.112 Cells were stimulated with Ang-II (100 nmol/L) and cotreated with increasing doses of the ARB Valsartan (Val) in the presence and in the absence of LBQ-657, the active metabolite of NEPi prodrug AHU-377. ARNi (ie, Val+NEPi) provided dose-dependent, superior antihypertrophic (1) and antifibrotic (2) effects compared with Val alone. NEPi alone had only modest effect in cardiomyocytes, and, predictably no discernable effect in fibroblasts.113 We further explored the potential use of ARNi to modulate cardiac remodeling after myocardial infarction. One week after experimental induction of myocardial infarction in rats, animals were randomized to 4 weeks of peroral treatment with ARNi (LCZ-696; n=11) or vehicle (n=6). At end point, ARNi-treated rats exhibited significantly reduced cardiac hypertrophy (Figure 4C–4F).

There is considerable further therapeutic potential for ARNi. One obvious area worthy of exploration is that of HFPEF. HFPEF is a heterogenous disorder that is often driven by hypertension and chronic ischemia, 2 conditions where ARB/NEPi may be efficacious. Furthermore, NPs have direct antifibrotic effects in cell culture, and this has also been observed in vivo.114 HFPEF is a disease characterized by pathological myocardial fibrosis, and thus the augmented antifibrotic activity of a combined ARB/neprilysin inhibitor may be of particular benefit in this setting.

The recently published prospective comparison of ARNI with ARB on Management of heart failUrre with preserved ejection fraction (PARAMOUNT) study14 was a phase-2 parallel-group, double-blinded RCT comparing LCZ-696 with valsartan in 301 patients with HFPEF and elevated plasma levels of NT-proBNP. Patients assigned to LCZ-696 showed a greater reduction in NT-proBNP at 12 weeks of follow-up, the primary end point. More patients on LCZ-696 exhibited improved New York Heart Association functional class and of note, reduced LA size compared with valsartan, consistent with reverse LA remodeling. Whether the latter was because of greater reductions of LV and LA wall stress by LCZ-696, or rather reflected distinct drug effects on total arrhythmia burden (>40% of subjects had a history of atrial fibrillation) is debatable, because LV filling pressures estimated by Doppler echocardiography were not different. Of note, attenuation of atrial remodeling was also seen in our experimental study (Figure 4F). Another encouraging signal was that 2 important risk groups, namely patients with diabetes mellitus and those with the highest BP exhibited greater reductions of plasma NT-proBNP by LCZ-696 than valsartan, although the study was underpowered to detect subgroup differences.115 At the same time, no enhanced risk of angioedema or other adverse events were reported in PARAMOUNT. Besides LCZ-696, novel single-molecule ARNi are under preclinical evaluation.116 No data are currently available.

Development of the new drug class of ARNi and initial results hold promise as a breakthrough in the search for better medical therapies for HF. Beyond HF the ongoing evaluation of ARNi should and hopefully will be extended to post-MI LV systolic dysfunction and diabetic nephropathy.

**Future Directions**

Today there continues to be a high clinical need for novel therapeutic agents that optimally control HF and major predisposing cardiovascular diseases, such as hypertension and coronary artery disease. To date, therapeutic strategies targeting the RAAS constitute first-line HF pharmacotherapy and underscore the deleterious effects of this system in HF pathogenesis and progression. Therapeutic augmentation of the NPs by inhibition of NP breakdown or administration of synthetic NPs is another promising area under current investigation. Novel concepts in HF seek to maximize the beneficial properties of the NPs coupled with counteracting RAAS or ET to achieve optimal end-organ protection, including the most recent new class of compounds, ARNi. As such, with the promising results of most recent clinical trials examining ARNi, we think new therapeutic opportunities lie ahead.

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**Disclosures**

Dr Krum has served on the steering committee of the EMPHASIS-HF trial. Mayo Clinic has licensed cenderitide (CD-NP) and CU-NP to Nile Therapeutics and ANX-042 to Anexon Inc. Dr Burnett is the chair of the scientific advisory board of Nile Therapeutics and is a scientific advisor for Anexon Inc. The other authors have no conflicts to report.

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