Natriuretic peptides (NPs) are essential for the maintenance of volume homeostasis, and can be of myocardial, renal, and endothelial origin. Advances in peptide engineering have enabled the design of innovative designer NPs that go beyond native peptides in efficacy, specificity, and resistance to enzymatic degradation. Therefore, designer NPs provide an unparalleled opportunity for the treatment of cardiovascular disease. In this review, we report the conceptual framework of peptide engineering of the NPs that resulted in designer peptides for cardiovascular disease. We specifically provide an update on those currently in clinical trials for heart failure and hypertension. (J Am Coll Cardiol Basic Trans Science 2016;1:557–67) © 2016 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
recently completed international trial of ulitratide (urodilatin [URO]). We focus most on the rapidly developing area of designer NPs that may go beyond the native NPs in the treatment of CV disease. We discuss innovative peptide modification either based on rational design or genomic medicine that may impart enhanced receptor activation and/or reduced enzymatic degradation. We provide insights into the latest generation of designer NPs now being tested in models of CV disease and in clinical trials that may lead to a new generation of peptide therapeutics.

**NATRIURETIC PEPTIDES**

Peptides are biomolecules that consist of amino acids monomers and peptide (acid) bonds. The amino acid composition of these biomolecules is variable, and is considered an important factor that determines unique chemical and physical properties. The number of bound amino acids dictates the length of the peptides: dipeptides are the shortest peptides (2 amino acids and 1 single peptide bond), whereas polypeptides are long, continuous peptide chains. In contrast to biologically complex proteins, peptides have a rather simple biological composition and generally consist of ≤ 50 amino acids.

The family of NPs is a group of polypeptides that plays a pivotal role in maintaining the fluid homeostasis of the body by regulating intravascular volume, vascular homeostasis, and arterial pressure (Figure 1) (10). Recently, a role for the NPs in metabolic homeostasis has also been advanced (11-13). The NP system is highly preserved across species, and currently, the following 6 different NPs have been identified: ANP, BNP, C-type (CNP), D-type (DNP), ventricular NP (VNP), and the renal peptide, URO (14). NPs function as ligands for a set of transmembrane NP receptors (NPRs): CNP, evolutionarily the oldest of the NPs, mainly binds to the extracellular domain of the particulate guanylyl cyclase B receptor (pGcB, NPR-B), whereas all other NPs bind to the transmembrane pGcA (NPR-A) receptor (15). pGcA receptors are expressed in various tissues, including heart, kidney, brain, adrenals, adipocytes, and vasculature (both arteries and veins) (16). pGcB receptors are expressed in kidney, brain and veins, but less so in arteries (17).

A third NPR, called NPR-C, or the clearance receptor (18), actively eliminates endogenous NPs from the circulation using hydrolysis (ranked from the greatest to the lowest degradation rate: VNP = ANP ≅ CNP > BNP = DNP). Studies also suggest a signaling role for NPR-C via modulation of cyclic adenosine monophosphate (19-21). Clearance of NPs is furthermore regulated by the enzyme NEP, which is widely expressed in endothelium and lung with the highest abundance in the kidney. CNP is the least resistant to NEP-mediated hydrolysis (ranked from greatest to lowest degradation rate: CNP > ANP > BNP > DNP) (3,10,15,16). The differences in local NPR expression, degradation and clearance rates, and NP-binding affinity cause all 6 NPs to have unique and NP-specific properties (15).

Importantly, binding of a NP to a NPR activates the membrane-bound pGcA and pGcB receptors, and induces a variety of autocrine, paracrine, and endocrine effects. Activated pGc receptors produce the second messenger cyclic guanosine monophosphate (cGMP) that in turn activates protein kinase G. cGMP can also be produced in a nitric oxide-dependent manner; its production is then regulated via activation of the soluble guanylyl cyclase pathway (22).

ANP and BNP are believed to be the most important in controlling body fluid and blood pressure homeostasis (23,24). ANP has renin-inhibiting properties, is a potent aldosterone inhibitor, and is an antagonist to the mineralocorticoid receptor. In addition, via alternative processing of the ANP precursor (pro-ANP) it also contributes to renal sodium and water handling via generation of URO (25-27). BNP has been identified as an NP highly relevant to HF, which is due to its natriuretic, renin-angiotensin-aldosterone system (RAAS) inhibitory, vasodilating, and lusitropic properties (28-30), as well as its robust performance as a HF diagnostic and prognostic biomarker (31-33). CNP is an autocrine and paracrine factor that currently has limited use as a therapeutic for HF, particularly because of its rapid enzymatic degradation and paucity of renal protective actions (29), although its potent antifibrotic actions provide a therapeutic opportunity (34). Moyes et al. (21) elegantly demonstrated a role for CNP in vascular homeostasis that may involve binding and activation of NPR-C (21). DNP is a unique NP that has only been isolated from the venom gland of the Green Mamba snake (35). Its function has not been entirely clarified. Currently, VNP expression has only been confirmed in the hearts of primitive ray-finned bony fish, in which it is responsible for the maintenance of fluid and salt homeostasis (17).

Overall, NPs possess a wide variety of properties that are of value in diagnosis, prognosis, and treatment of CV disease, especially HF and HTN. Despite current optimal therapies, the prognosis for HF remains poor,
and 50% of patients die within 5 years after first hospitalization (36). The report of a relative deficiency or low bioavailability of NPs in HTN has also provided a therapeutic opportunity for use of designer NPs, especially in special populations, such as those with resistant HTN, in whom there remains a huge unmet therapeutic need (37). Development of new treatment regimens, with novel modes of action is therefore a high priority, and in this light, the concept of targeting (native) NPs for HF and HTN treatment strategies has attracted increased attention (15,38).

**THERAPEUTIC USE OF NATIVE NPS: A FOCUS ON HF**

**NESRITIDE.** Nesiritide is a recombinant version of endogenous BNP. In the early 1990s, Hobbs et al. (39) reported early results of nesiritide in human HF. This synthetic peptide was a potent vasodilator and had rapid dose-dependent hemodynamic effects, including reduction of pulmonary wedge pressure, systemic vascular resistance, and mean arterial pressure (40). In 2001, nesiritide received approval from
Designer Natriuretic Peptides

CARPERITIDE. Carperitide is the recombinant formulation of endogenous ANP that has been approved in Japan for the treatment of acute HF (50).

Beyond the acute action of carperitide on relief of symptoms, the PROTECT trial investigated the effect of carperitide on long-term prognosis after acute HF (53). The PROTECT study enrolled 49 patients who were randomly assigned to a group that received low-dose carperitide (0.01 to 0.05 μg/kg/min) over 72 hours or to a standard medical treatment group. During infusion, the cardiac free-fatty acid-binding protein/serum creatinine ratio was reduced, which is consistent with inhibition of myocardial cell membrane damage. There was no significant difference in serum troponin T and creatinine levels between groups. During the 18-month follow-up, the investigators reported that the incidence of death and rehospitalization were significantly lower with carperitide. These studies therefore demonstrated that low-dose ANP infusion in acute HF improved long-term prognosis. Although the mechanism for long-term beneficial effect of carperitide is not clear, inhibition of the RAAS may be involved.

ULARITIDE. Ularitide is a synthesized version of the endogenous NP URO. Ularitide is a 32-residue ANP that activates cGMP production by binding to the pGC-A receptor (26). It has a fast track designation for treatment of acute HF from the FDA (54).
Endogenous URO is believed to be produced by the kidney through local synthesis and/or processing of renal or circulating pro-ANP. URO plays a pivotal role in regulation of urinary sodium excretion. In rats with HF, URO significantly increased urinary flow, glomerular filtration rate (GFR), sodium excretion, and urinary cGMP excretion in a dose-dependent manner (55). However, in anesthetized dogs with HF that received continuous URO infusion of 2 pmol/kg/min, the increase in urinary sodium excretion was less than in dogs who received BNP infusion (56).

Data from Phase I to IIB clinical phase trials suggest that ularitide may be effective in treatment of acute HF. Ularitide was shown to have vasodilating and renoprotective actions, as well as cardiac unloading properties, including significant improvement of the clinical parameter of dyspnea. Treatment with ularitide further reduced mortality and length of hospital stay, without changing serum creatinine levels. Reported adverse effects were hypotension, cardiac failure, sweating, dizziness, and asthenia (54,57-59).

Ularitide was recently tested in a randomized, double-blind, placebo-controlled Phase III study in patients hospitalized with an episode of acute HF (TRUE-HF [Efficacy and Safety of Ularitide for the Treatment of Acute Decompensated Heart Failure]; NCT01661634). Primary outcomes were safety and short- and long-term efficacy of 48 h of continuous intravenous infusion of 15 ng/kg body weight per min, as assessed by patients’ symptoms and persistence of worsening of HF for 48 h as well as clinical status and CV outcome during 180 days of follow-up. The findings of TRUE-HF have yet to be presented.

**DESIGNER NPs: FOR CV DISEASE**

**STRATEGIC APPROACH TO THE ENGINEERING OF DESIGNER NPS.** As described in Figure 2, there are 5 goals of designer NP engineering. First, safety of a designer peptide is of highest priority, especially with regard to immunogenicity. Second, enhanced receptor activation remains a major goal that may be achieved through defining which amino acids in a structure are either key mediators of receptor binding and/or limit full receptor activation. Third, as enzymatic degradation may be the major mechanisms limiting bioavailability it may probably be enzymatic degradation, the addition of unique amino acids or amino acid substitutions may render a designer peptide immune to degradation and could potentially also markedly enhance efficacy. Fourth, as we have gained insights into peptide design and are able to add or preserve unique elements of a peptide, a major goal is also to design peptides that have properties unique to a specific syndrome (e.g., limiting hypotension in HF or augmenting adipocyte activation in obesity). Fifth, with the progress in sustained delivery systems, developing delivery platforms that permit daily, weekly, or even monthly sustained delivery of a peptide also enhances efficacy and compliance, and has emerged as a key component in peptide engineering.

Advances in peptide engineering has opened the field of designer NPs, resulting in therapeutic drugs that go beyond native endogenous NPs in actions, efficacy, and safety for the treatment of CV disease. Designer NPs transcend structural, biological, functional, and pharmacological properties of endogenous NPs (60) (Central Illustration). The biochemical design of a designer NP can be a de novo creation or based on selective native NP sequences. Specifically, they may be the result of modification and/or addition of amino acid sequences, or genetic modification of the native forms of NPs. The chemical properties of designer NPs can be easily changed, and this significantly contributes to increased overall applicability of synthetic designer peptides. Although our goal has been to use such engineered peptides for CV disease, synthetic peptides are also used for multiple purposes, including antibody production and polypeptide structure and/or functional studies, as well as for development of new enzymes, vaccines, and drugs. Altogether, these novel chimeras play an important role in advancing the panoply of means available for treatment of HF and HTN.

Our approach to novel peptide design is highly unique and does not use traditional computer modeling or use of high-throughput bioinformatics. In contrast, our strategic engineering of designer peptides use a rational drug discovery approach in which we integrate a set of inputs. Specifically, we use a knowledge-based amino acid mutation approach: the ever-increasing synthesis of selective mutations, such as was reported recently by Lee et al. (61) for the designer NP cenderitide (CD-NP), continuously improving our knowledge and information of
properties of key amino acids that may enhance or reduce receptor activation of a native peptide. Furthermore, engineering of designer NPs that are highly resistant to degradation relies upon ours and others’ laboratory-based knowledge, which provides information about sites of enzyme degradation by degrading enzymes, including NEP. In addition, using novel amino acid sequences from venomous snakes such as the Green Mamba, which was used with CD-NP, results in chimeric peptides with highly unique properties and therapeutic benefits that go beyond mammalian peptides (63). In our designer peptide design, we also use a genomic medicine approach: identification of spontaneous or hereditary NP-coding genetic alterations that can then be used to synthesize novel peptides with unique features. For example, this strategy has resulted in engineering of the unique peptides MANP (ZD100) and ASBNP.1 (ANX042) (64,65). Finally, we also rely on careful review of public domain reports from biotechnology and medicinal chemistry that provide key insights that may further guide our strategic designs.

Altogether, this unique strategy has created a broad assortment of designer NPs, each with unique and rather disease-specific actions.

**Table 1.** Highlights of Designer Natriuretic Peptide Particulate Guanylyl Cyclase Activators in Clinical Trials

| Name     | Generic name    | Indication                                      | Current status                                                                 |
|----------|-----------------|-------------------------------------------------|-------------------------------------------------------------------------------|
| Cenderitide | CD-NP           | Post-acute heart failure                        | 2 Phase II studies of 8-day treatment with continuous subcutaneous administration by Insulet OmniPod (Insulet Corp., Billerica, Massachusetts) delivery system in stable heart failure patients has been completed. |
| ANX042   | ASBNP.1         | Cardiorenal syndrome in heart failure           | Phase I study of acute intravenous administration in normal human volunteers has been completed. |
| ZD100    | MANP            | Hypertension                                    | Phase I study of 3-day once daily subcutaneous injection of subjects with resistant-like hypertension has been completed. |
VASONATRIN. Vasonatrin (VNP) represented our first attempt at a designer NP in 1993 (66). VNP is a 27 amino acid peptide chimera of the full-length 22 amino acid structure of human CNP and the 5-amino acid carboxyl-terminus of human ANP. In vitro and in vivo, VNP has both the vasodilating properties of CNP and the natriuretic properties of ANP. It also possesses arterial vasodilating and antiproliferative effects that are unique for this first-in-class chimera that defined the concept of designer NPs going beyond native peptides. The vasorelaxant effects of VNP are dose-dependent, endothelium-independent, and stronger than the effects of ANP and CNP alone (67–69). However, in healthy rats, the natriuretic and diuretic effects of a single bolus of 50 μg/kg VNP were inferior to ANP (66), which reduced enthusiasm for its clinical development.

**CD-NP FOR HF.** CD-NP, developed in 2008, was a major advance in peptide engineering that now has proven efficacy in humans (Table 1). CD-NP is a 37 amino acid hybrid NP that fuses the mature form of native CNP and the 15 amino acid C-terminus of DNP (61). The rationale for the design of CD-NP was in part motivated from results from the ASCEND-HF and ROSE-AHF (Renal Optimization Strategies Evaluation in Acute Heart Failure) trials, which demonstrated excessive hypotension after treatment with the pGC-A activator, nesiritide. During development of CD-NP, the main goal was to design a NP that still possessed the beneficial renal actions of pGC-A and pGC-B activation, but without the unwanted hypotensive properties. We therefore engineered a hybrid that consisted of CNP (pGC-B activator) and the C-terminus of DNP that recognized DNP (pGC-A activator). The C-terminus of DNP itself has less hypotensive actions than DNP but still possesses natriuretic properties. CD-NP is the first designer NP that co-targets both pGC-A and pGC-B receptors, and the biological effects of this designer NP have been a significant advance in NP design.

First, we reported the successful synthesis of the C-terminus of DNP and then of CD-NP (63). Consistent with our goal of a dual pGC-A/pGC-B activator that does not exist in nature, Lee et al. (61), Dickey et al. (70), and Martin et al. (71) elegantly demonstrated the unique ability of CD-NP to co-activate both pGC-A and pGC-B in HEK293 cells, selectively overexpressing each receptor type, which was recently confirmed. In vitro, in human cardiac fibroblasts, CD-NP was a stronger cGMP-activator than equimolar doses of BNP, DNP, or CNP (63,72). This first-in-class designer NP also had antifibrotic, antiproliferative, and antihypertrophic properties that make this drug a promising candidate for HF (29). In vivo, in normal canines, CD-NP was a potent cGMP stimulator that had vasodilating and renal enhancing effects, with less effects on systemic blood pressure (BP) (63). At natriuretic doses, only infusion with nesiritide, and not CD-NP, reduced BP (63). Also, CD-NP, compared with CNP alone, possessed unique and additional renal actions, such as cGMP activation in isolated glomeruli, plasma aldosterone suppression, and potent GFR-enhancing and natriuretic effects (61).

Infusion of CD-NP in healthy volunteers stimulated increases in urinary and plasma cGMP levels, induced a significant diuretic and natriuretic response, and resulted in a minimal, but significant, decrease in systemic BP (Table 1) (73).

CD-NP received fast track designation from the FDA in 2011 for the treatment of post-acute HF. Proof-of-concept studies are underway to reduce post-myocardial infarction HF and enhance outcomes in patients with left ventricular assistant device therapy. A Phase I/II study in with chronic HF stable chronic HF patients with impaired renal function was initiated in 2015 with the goal of enhancing renal function (NCT02603614). This study will evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of CD-NP.

Recently, studies have defined the unique role of key structural components of CD-NP with the ultimate goal of engineering a newer generation CD-NP. Extensive modeling and production of in vitro CD-NP mutants demonstrated and validated the superiority of the structure of CD-NPs to other novel CD-NP-like peptides, establishing the unique structural requirement of the full mature CNP with the 15 amino acid sequence of DNP as a prerequisite for successful co-receptor activation (61).

Originally, CD-NP was developed as a novel therapeutic to enhance renal function with limited effects on BP. However, a more contemporary additional goal in HF treatment is to prevent and or reverse myocardial remodeling, especially cardiac fibrosis. In this light, CD-NP may also appear as an attractive and important therapeutic for HF. This rationale is strengthened by a recent study of Ichiki et al. (72), who demonstrated up-regulation of the pGC-B receptor together with reduced production of CNP in experimental studies in human failing myocardium. In human cardiac fibroblasts, CD-NP was furthermore a stronger inhibitor of collagen production than either BNP or CNP (72). In a model of mild left ventricular diastolic dysfunction with cardiac fibrosis, long-term SQ CD-NP, administered by pump infusion, suppressed the development of both cardiac fibrosis and diastolic dysfunction (71). Altogether, these and ongoing studies suggest that CD-NP is a unique and
potent designer NP that has cardiorenal protective properties and antifibrotic actions.

A key strategy in peptide therapeutics is the development of innovative delivery platforms. In current clinical trials, the OmniPod (Insulet Corporation, Billerica, Massachusetts) insulin delivery system is being used to administer long-term SQ CD-NP in patients with HF. In experimental models of CV disease, novel nanoparticle gel polymer strategies are being tested (74). Furthermore, a novel film delivery system in which CD-NP is released from a patch-like device around the heart is also being tested (75). With the advent of highly innovative delivery systems, long-term delivery of CD-NP or designer NPs will continue to emerge.

ANX-042 (ASBNP.1) FOR HF. Advances in genome-wide investigations have revealed alternative splicing of multiexonic genes. The complexity of the human proteome may be accounted for by alternative splicing of messenger RNA. This provides an opportunity in drug discovery (76). We recently focused on the cardiac peptide BNP, which is encoded by a small multiexonic gene. Its therapeutic use with recombinant BNP (nesiritide) has demonstrated efficacy in HF, but this has been limited by excessive hypotension. In our efforts to identify novel endogenous variants of BNP, we identified an alternative spliced transcript of BNP that resulted from intron retention in failing human hearts. We then used the unique sequence of this alternative spliced BNP (AS-BNP) to design a peptide with unique renal protective actions without associated hypotension (65). Specifically, the designer peptide ANX-042 is a truncated peptide form of an AS-BNP (65). From a structural perspective, this transcript resembles native mature BNP, whereas a unique and distinct longer (34 amino acid) C-terminus is a component due to intron retention. Based on multiple designs, we used the first 16 amino acids of this C-terminal to generate the designer NP ANX-042. ANX-042, based on genomic medicine, presents with highly unique properties that also support its development as a renal-enhancing peptide for HF with limited BP-lowering actions (Central Illustration).

Initial investigations of AS-BNP and ANX-042 demonstrated they lacked the ability to activate cGMP in vascular smooth muscle cells and endothelial cells that are known to possess pGC-A receptors and are responsive to native BNP. Furthermore, in isolated arterial vascular rings, BNP, but not ANX-042, relaxed pre-constricted vessels. Surprisingly, in renal mesangial cells, ANX-042 markedly increased cGMP production greater than BNP, which may be a pGC-B phenomenon rather than pGC-A activation. In addition, in freshly isolated glomeruli, ANX-042 also activated cGMP production. Thus, ANX-042, engineered from the discovery of an alternatively spliced variant of BNP appears to be a selective renal-acting, BNP-like peptide in vitro, but may involve activation of pGC-B and/or yet undefined pGC receptor subtypes in the kidney but not in the systemic vasculature.

In vivo, intravenous administration of ANX-042 in a canine model of pacing-induced HF significantly enhanced diuresis, natriuresis, and GFR without reducing mean arterial pressure (65). Data from a Healthy Volunteer Dose Escalation Study (NCT01638104) furthermore showed that infusion with ANX-042 is safe and results in cGMP-activation (Table 1). ANX-042 obtained FDA approval in 2012 as an investigational new drug and is currently being tested as a potential novel nonhypotensive and renal-enhancing treatment for HF.

MANP FOR HTN. HTN remains the leading cause of HF and strategies to reduce HF are a high health care priority. The need for more effective BP-lowering agents was recently underscored by the SPRINT (Systolic Blood Pressure Intervention Trial), which reported that intensive control of systolic BP to <120 mm Hg in high-risk HTN subjects reduced mortality and adverse CV outcomes, including HF (77). Furthermore, population studies in Olmsted County (Minnesota) as part of the Rochester Epidemiology Project demonstrated that there is a relative deficiency or low bioavailability of NPs in human HTN and a pathophysiological inverse relationship between an increasing aldosterone and decreasing NP in HTN and metabolic disease (78). Such observations from clinical studies and the known BP-lowering, aldosterone-suppressing, and natriuretic actions of ANP via pGC-A have laid the foundation for the development of designer ANPs for HTN.

The newest designer ANP-like peptide currently entering clinical trials for resistant HTN is MANP (ZD100), which is a best-in-class pGC-A activator. ZD100 is currently in clinical development for resistant HTN. Subjects with resistant HTN do not respond to current BP-lowering agents and are known to have a markedly increased risk of adverse CV outcomes, including HF, stroke, myocardial infarction, and end-stage kidney disease (38). MANP recently completed a first-in-human study in stable HTN and in resistant-like subjects with HTN.

Based upon genomic insights, MANP was engineered as a 40 amino acid peptide with the structure of a mature 28 amino acid ANP fused to a novel 12 amino acid carboxyl terminal extension (Central Illustration) (64). This novel C-terminal extension renders MANP
highly resistant to degradation by NEP and thus represents an alternative to a nonspecific NEP enzyme inhibitor strategy (79). Ongoing studies support possible enhanced activation of pGC-A and reduced binding to NPR-C. Thus, MANP has the attractive biological properties of both resistance to NEP degradation with specific and direct ligand activation of pGC-A.

In vivo, MANP had more sustained natriuretic, aldosterone-suppressing and BP-lowering actions than native ANP (64). MANP markedly increased circulating and urinary cGMP and is highly effective in reducing BP in experimental HTN (80). Both experimental and first-in-human studies suggested that MANP is a potent aldosterone inhibitor that reduces the secretion of aldosterone (Table 1). Experimental studies with native ANP and its interaction with the mineralocorticoid receptor also suggested that MANP might have direct actions to inhibit the mineralocorticoid receptor (25). Furthermore, in a model of acute HF in the setting of HTN, MANP was more effective than nitroglycerine in augmenting sodium excretion and preserving GFR, unloading the heart, and suppressing aldosterone (81).

Studies are underway to develop novel strategies to deliver MANP long-term in a formulation that results in sustained delivery. Similar to antidiabetic drugs such as glucagon-like peptide 1 analogs, fatty acids can be linked to MANP to sustain delivery and prolong activation of cGMP. Preliminary studies have demonstrated the feasibility of sustained release formulation of ZD100, and preclinical studies are underway in experimental HTN. Ongoing studies also suggest that advanced peptide engineering can result in next generation ZD100 analogues with gain of function in the activation of pGC-A (82). Recently, we reported data from a first in human Phase 1 trial, in which single ascending doses of ZD100 were administered to HTN subjects followed by once daily injection for 3 days by SQ administration. Treatment with ZD100 was safe and well-tolerated, and was associated with cGMP generation, natriuresis, enhanced GFR, and suppression of aldosterone (Table 1) (83). Thus, in these exciting days of biotechnology, engineering peptides such as ZD100 provide potentially greater clinical efficacy in the treatment of human disease and yet retain highly specific receptor-mediated actions that contribute to both safety and enhanced efficacy.

FUTURE DIRECTIONS

NPs have always been regarded as attractive targets for the treatment of HF and HTN. Although use of NPs was initially hampered by limited clinical efficacy, which was mainly the result of limitations of endogenous NPs, such as rapid enzyme degradation or unwanted properties such as excessive hypotension, therapeutic use of NPs remains a highly sought after goal. Progress in this field was made by the introduction of recombinant NPs and was further advanced by the concept of designer NPs. As we presented in this review, several first-in-class designer NPs are being tested in clinical studies. These novel chimeras reflect technological advances that have been made in drug development over the years, incorporating new and innovative engineering strategies, including novel delivery platforms. Overall, current designer NPs are more efficacious in their actions than their (recombinant) predecessors, and because of their specific targeting, advance the novel concept of precision medicine.

Following the successful introduction of Entresto, a first-in-class angiotensin receptor NEP inhibitor, we believe that the concept of a drug with simultaneous NP-augmenting and RAAS-counteracting properties is highly attractive and deserves further attention. The morbidity and mortality of HF and the difficulty of controlling HTN, nevertheless, remains high, and we hypothesize that the engineering of future designer NPs should incorporate a multivariat strategy in an attempt to develop dual-receptor designer NPs. These NPs may even target receptor pathways beyond the pGC receptor family, so as to optimize organ protective properties. The era of the designer NP continues to evolve with the promise of exciting therapeutics for CV disease and beyond.

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REFERENCES

1. Sidney S, Quenestberry CP Jr., Jaffe MG, et al. Recent trends in cardiovascular mortality in the United States and public health goals. JAMA Cardiol 2016;1:594–9.
2. McMurray JJ, Packer M, Desai AS, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med 2014;371:993–1004.
3. Mangiafico S, Costello-Boerrigter LC, Andersen IA, Cataliotti A, Burnett JC Jr. Neutral endopeptidase inhibition and the natriuretic peptide system: an evolving strategy in cardiovascular therapeutics. Eur Heart J 2013;34:886–893c.
4. Ruilope LM, Dukat A, Bohm M, Lacourciere Y, Gong J, Leitkowitz MP. Blood-pressure reduction with LCZ696, a novel dual-acting inhibitor of the angiotensin II receptor and neprilysin: a
randomised, double-blind, placebo-controlled, active comparator study. Lancet 2010;375:1255-66.

5. Jay SM, Lee RT. Protein engineering for cardiovascular therapeutics: untapped potential for cardiac repair. Circ Res 2013;113:923-43.

6. Fogerson K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug Discov Today 2015;20:122-8.

7. Marso SP, Daniels GH, Brown-Frandsen K, et al. Linagliptide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2016;375:311-22.

8. Rizzuti M, Nizzardo M, Zanetta C, Ramirez A, et al. Chronic actions of a novel oral B-type natriuretic peptide conjugate in normal dogs and acute actions in angiotensin II-mediated hypertension. Circulation 2008;118:1729-36.

9. Cathalini A, Chen HH, Schirger JA, et al. Human adipocytes. J Clin Investig 2012;122:1430-41.

10. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med 1998;339:321-8.

11. Bordinchichia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Investig 2012;122:1032-6.

12. Cannone V, Boerrigter G, Cataliotti A, et al. A genetic variant of the atrial natriuretic peptide gene is associated with cardiometabolic protection in the general community. J Am Coll Cardiol 2011;58:629-36.

13. Wang TJ. The natriuretic peptides and fat metabolism. N Engl J Med 2012;367:377-87.

14. Martinez-Rumayor A, Richards AM, Burnett JC Jr., Granger JP, Opgenorth TJ. Endo-vascular homeostasis. J Clin Investig 2014;124:4039-51.

15. Kuhn M. Structure, regulation, and function of mammalian membrane guanylyl cyclase receptors, with a focus on guanylyl cyclase-A. Circ Res 2003;93:700-9.

16. Burnett JC Jr., Granger JP, Oppegren TJ. Effects of synthetic atrial natriuretic factor on renal function and renin release. Am J Physiol 1984;247:F863-6.

17. Cataliotti A, Chen HH, Schirger JA, et al. Chronic actions of a novel oral B-type natriuretic peptide conjugate in normal dogs and acute actions in angiotensin II-mediated hypertension. Circulation 2008;118:1729-36.

18. Scholz-Knapsche P, Forsmann K, Herbst F, Hock D, Pikpom R, Forsmann WG. Isolation and structural analysis of "urodilatin", a new peptide of the cardiodilatin-(ANP)-family, extracted from human urine. Klinische Wochenschrift 1988;66:752-9.

19. Ichiki T, Huntley BK, Sangaralingham SJ, Burnett JC Jr. Pro-atrial natriuretic peptide: a novel guanylyl cyclase-A receptor activator that goes beyond atrial and B-type natriuretic peptides. J Am Coll Cardiol HF 2015;3:715-23.

20. Mills RM, Hobbies RE, Young JB. "BNP" for heart failure: role of nesiritide in cardiovascular therapeutics. Congest Heart Fail 2006;2:270-3.

21. von Lueder TG, Sangaralingham SJ, Wang BH, et al. Renin-angiotensin blockade combined with natriuretic peptide system augmentation: novel therapeutic concepts to combat heart failure. Circ Heart Fail 2013;6:594-605.

22. Huntley BK, Sandberg SM, Heublein DM, Sangaralingham SJ, Burnett JC Jr., Ichiki T. Pro-B-type natriuretic peptide-1:108 processing and degradation in human heart failure. Circ Heart Fail 2015;8:85-97.

23. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161-7.

24. Januzzi JL, van Kimmenade R, Lainchbury J, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute decompensated heart failure: an international pooled analysis of 1256 patients: the International Collaborative of Heart Failure Studies. J Am Coll Cardiol 2010;55:210-7.

25. Sangaralingham SJ, Wang BH, Huang L, et al. Cardiorenal fibrosis and dysfunction in aging: imbalance in mediators and regulators of collagen. Peptides 2016;76:108-14.

26. Schweiz H, Vigne P, Moinier D, Frelin C, Lazdunski M. A new member of the natriuretic peptide family is present in the venom of the green mamba (Dendroaspis angusticeps). J Biol Chem 1992;267:13928-32.

27. van Jaarsveld CH, Ranvor AJ, Kempen GI, Cioxy JC, van Veldhuisen DJ, Sanderman R. Epidemiology of heart failure in a community-based study of subjects aged > or = 57 years: incidence and long-term survival. Eur J Heart Fail 2006;8:23-30.

28. Macheret F, Heublen D, Costello-Boerrigter LC, et al. Human hypertension is characterized by a lack of activation of the anti-hypertensive cardiac hormones ANP and BNP. J Am Coll Cardiol 2012;60:1558-65.

29. McKie PM, Ichilli T, Burnett JC Jr. M-atrial natriuretic peptide: a novel antihypertensive protein therapy. Curr Hypertens Rep 2012;14:62-9.

30. Hobbs RE, Miller LW, Bott-Silverman C, James KB, Rincon G, Grossbard EB. Hemodynamic effects of a single intravenous injection of synthetic human brain natriuretic peptide in patients with heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 1996;78:896-901.

31. Colucci WS, Elsayam U, Horton DP, et al. Intravenous nesiritide, a natriuretic peptide, in the treatment of decompensated congestive heart failure. Nesiritide Study Group. N Engl J Med 2000;343:246-53.

32. Sackler-Bernstein JD, Kowalski M, Fox M, Aaronson K. Short-term risk of death after treatment with nesiritide for decompensated heart failure: a pooled analysis of randomized controlled trials. JAMA 2005;293:1900-5.

33. Sackler-Bernstein JD, Skopicki HA, Aaronson KD. Risk of worsening renal function with nesiritide in patients with acutely decompensated heart failure. Circulation 2005;111:1487-91.

34. O'Connor CM, Starling RC, Hernandez AF, et al. Effect of nesiritide in patients with acute decompensated heart failure. N Engl J Med 2011;365:32-42.

35. van Deursen VM, Hernandez AF, Stebbins A, et al. Nesiritide, renal function, and associated endpoints during hospitalization for acute decompensated heart failure: results from the Acute Study of Clinical Effectiveness of Nesiritide and Decompensated Heart Failure (ASCEND-HF). Circulation 2014;130:958-65.

36. Chen HH, Anstrom KJ, Givertz MM, et al. Low-dose dopamine or low-dose nesiritide in acute heart failure with renal dysfunction: the ROSE acute heart failure randomized trial. JAMA 2013;310:535-43.

37. Cataliotti A, Tonne JM, Bellavia D, et al. Long-term cardioc pro-B-type natriuretic peptide gene delivery prevents the development of hypertensive heart disease in spontaneously hypertensive rats. Circulation 2011;123:1297-305.

38. McKie PM, Schirger JA, Bellake SL, et al. Chronic subcutaneous brain natriuretic peptide therapy in asymptomatic systolic heart failure. Eur J Heart Fail 2016;18:433-41.

39. Van SH, McKie PM, Schirger JA, et al. Chronic peptide therapy with B-Type Natriuretic peptide in
patients with pre-clinical diastolic dysfunction (stage B heart failure). J Am Coll Cardiol HF 2016; 4:539–47.

49. Chen HH, Gluckner JF, Schirger JA, Cataliotti A, Redfield MM, Burnett JC Jr. Novel protein therapeutics for systolic heart failure: chronic subcutaneous B-type natriuretic peptide. J Am Coll Cardiol 2012;60:2305–12.

50. Saito Y. Roles of atrial natriuretic peptide and its therapeutic use. J Cardiol 2010;56:262–70.

51. Suzuki M, Seino Y, Nomachi Y, Matsuki S, Funashiki K. Multicenter prospective investigation on efficacy and safety of carperitide for acute heart failure in the ‘real world’ of therapy. Circ J 2005;69:283–90.

52. Nomura F, Kurobe N, Mori Y, et al. Multicenter prospective investigation on efficacy and safety of carperitide as a first-line drug for acute heart failure syndrome with preserved blood pressure: COMPASS: Carperitide Effects Observed Through Monitoring Dyspnea in Acute Decompensated Heart Failure Study. Circ J 2008;72:1777–86.

53. Hata N, Seino Y, Tsutamoto T, et al. Effects of carperitide on the long-term prognosis of patients with acute decompenated chronic heart failure: the PROTECT multicenter randomized controlled study. Circ J 2008;72:1787–93.

54. Anker SD, Ponikowski P, Mitrovic V, Peacock WF, Filippatos G. Uradilite for the treatment of acute decompenated heart failure: from preclinical to clinical studies. Eur Heart J 2015;36: 715–23.

55. Abasi ZA, Powell JR, Golomb E, Keiser HR. Renal and systemic effects of urlodilatin in rats with high-output heart failure. Am J Physiol 1992;262:F16–21.

56. Chen HH, Cataliotti A, Schirger JA, Martin FL, Burnett JC Jr. Equimolar doses of atrial and brain natriuretic peptides and urlodilatin have differential renal actions in overt experimental heart failure. Am J Physiol Regul Integr Comp Physiol 2005; 288:R1093–7.

57. Kentsch M, Ludwig D, Drummer C, Gerzer R, Muller-Esch G. Haemodynamic and renal effects of urlodilatin bolus injections in patients with congestive heart failure. Eur J Clin Investig 1998;29:66–9.

58. Mitrovic V, Luss H, Nitsche K, et al. Effects of the renal natriuretic peptide urlodilatin (ularimate) in patients with decompenated chronic heart failure: a double-blind, placebo-controlled, ascending-dose trial. Am Heart J 2005;150:1239.

59. Luss H, Mitrovic V, Seferovic PM, et al. Renal effects of ularimate in patients with decompenated heart failure. Am Heart J 2008;155:1012.e1–8.

60. Patel JB, Valencik ML, Pritchett AM, Burnett JC Jr., McDonald JM, Redfield MM. Cardiac-specific attenuation of natriuretic peptide A receptor activity accentuates adverse cardiac remodeling and mortality in response to pressure overload. Am J Physiol Heart Circ Physiol 2006;288:H1777–84.

61. Lee CY, Huntley BK, McCormick DJ, et al. Cenderilide: structural requirements for the creation of a novel dual particulate guanylyl cyclase receptor agonist with renal-enhancing in vivo and ex vivo actions. Eur Heart J Cardiovasc Pharm 2016;2:98–105.

62. Dickey DM, Yoder AR, Potter LR. A familial mutation renders atrial natriuretic peptide resistant to proteolytic degradation. J Biol Chem 2009;284:19196–202.

63. Lisy O, Huntley BK, McCormick DJ, Kurlansky PA, Burnett JC Jr. Design, synthesis, and actions of a novel chimeric natriuretic peptide: CD-NP. J Am Coll Cardiol 2008;52:60–8.

64. Mckie PM, Cataliotti A, Huntley BK, Martin FL, Olson TM, Burnett JC Jr. A human atrial natriuretic peptide gene mutation reveals a novel peptide with enhanced blood pressure-lowering, renal-enhancing, and aldosterone-suppressing actions. J Am Coll Cardiol 2009;54:1024–32.

65. Pan S, Chen HH, Dickey DM, et al. Biodesign of a renale-protective peptide based on alternative splicing of B-type natriuretic peptide. Proc Natl Acad Sci U S A 2009;106:11282–7.

66. Wei CM, Kim CH, Miller VM, Burnett JC Jr. Vasoconstrictor peptide: a unique synthetic natriuretic and vasorelaxing peptide. J Clin Invest 1993;92: 2048–52.

67. Feng HS, Zang YM, Zhu MZ, et al. [Comparison of vasorelaxing actions of vasonatrin peptide, C-type natriuretic peptide and atrial natriuretic peptide]. Sheng li xue bao [Acta Physiol Sinica] 1999;51:15–20.

68. Yu J, Zhu MZ, Wei GZ, et al. [Vasorelaxing role of vasonatrin peptide in human intramammary artery in vitro]. Sheng li xue bao [Acta Physiol Sinica] 2003;55:187–90.

69. Dong MQ, Zhu MZ, Yu J, Shang LJ, Feng HS. [Comparison of inhibitory effects of three natriuretic peptides on the proliferation of pulmonary artery smooth muscle cells of rats]. Sheng li xue bao [Acta Physiol Sinica] 2000;52:252–4.

70. Dickey DM, Burnett JC Jr., Potter LR. Novel bifunctional natriuretic peptides as potential therapeutics. J Biol Chem 2008;283:35003–9.

71. Martin FL, Sangaralingham SJ, Huntley BK, et al. CD-NP, a novel engineered dual guanylyl cyclase activator with anti-fibrotic actions in the heart. PLoS One 2012;7:e52422.

72. Ichiki T, Schirger JA, Huntley BK, et al. Cardiac fibrosis in end-stage human heart failure and the cardiac natriuretic peptide guanylyl cyclase system: regulation and therapeutic implications. J Mol Cell Cardiol 2014;75:199–205.

73. Lee CY, Chen HH, Lisy O, et al. Pharmacodynamics of a novel designer natriuretic peptide, CD-NP, in a first-in-human clinical trial in healthy subjects. J Clin Pharmacol 2009;49:668–73.

74. Lim J, Clements MA, Dobson J. Delivery of short interfering ribonucleic acid-complexed magnetic nanoparticles in an oscillating field occurs via caveolae-mediated endocytosis. PloS One 2012;7:e51350.

75. Ng XW, Huang Y, Chen HH, Burnett JC Jr., Boey FY, Venkatraman SS. Cenderilide-eluting film for potential cardiac patch applications. PloS One 2013;8:e68346.

76. Le KQ, Prabhakar BS, Hong WJ, Li LC. Alternative splicing as a biomarker and potential target for drug discovery. Acta Pharmacol Sinica 2015;36: 1212–18.

77. Wright JT Jr., Williamson JD, Whelton PK, et al. A randomized trial of intensive versus standard blood-pressure control. N Engl J Med 2015;373:2103–15.

78. Bugliani A, Cannone V, Cataliotti A, et al. Circulating aldosterone and natriuretic peptides in the general community: relationship to cardioenrald and metabolic disease. Hypertension 2015;65: 45–53.

79. Dickey DM, Potter LR. Dendroasapis natriuretic peptide and the designer natriuretic peptide, CD-NP, are resistant to proteolytic inactivation. J Mol Cell Cardiol 2011;51:67–71.

80. Mckie PM, Cataliotti A, Boerigter G, et al. A novel atrial natriuretic peptide based therapeutic in experimental angiotensin II mediated acute hypertension. Hypertension 2010;56:1152–9.

81. Mckie PM, Cataliotti A, Ichiki T, Sangaralingham SJ, Chen HH, Burnett JC Jr. Atrial natriuretic peptide and nitroglycerin in a canine model of experimental acute hypertensive heart failure: differential actions of 2 cGMP-activating therapeutics. J Am Heart Assoc 2014;3:e00206.

82. Bugliani A, Scott CG, Bailey KR, Rodeheffer RJ, Sarzani R, Burnett JC. Aldosterone levels, atrial natriuretic peptide and resistant hypertension in the general community. J Am Soc Hypertens 2016; 10:e40.

83. Chen HH, Neutel J, Smith D, Heublein D, Sarzani R, Burnett JC Jr. Aldosterone levels, atrial natriuretic peptide and resistant hypertension in the general community. J Am Soc Hypertens 2016;10:e23.

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