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SPOTLIGHT REVIEW

Natriuretic peptide pathways in heart failure: further therapeutic possibilities

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Abstract

The discovery of the heart as an endocrine organ resulted in a remarkable recognition of the natriuretic peptide system (NPS). Specifically, research has established the production of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) from the heart, which exert pleiotropic cardiovascular, endocrine, renal, and metabolic actions via the particulate guanylyl cyclase A receptor (GC-A) and the second messenger, cGMP. C-type natriuretic peptide (CNP) is produced in the endothelium and kidney and mediates important protective auto/paracrine actions via GC-B and cGMP. These actions, in part, participate in the efficacy of sacubitril/valsartan in heart failure (HF) due to the augmentation of the NPS. Here, we will review important insights into the biology of the NPS, the role of precision medicine, and focus on the phenotypes of human genetic variants of ANP and BNP in the general population and the relevance to HF. We will also provide an update of the existence of NP deficiency states, including in HF, which provide the rationale for further therapeutics for the NPS. Finally, we will review the field of peptide engineering and the development of novel designer NPs for the treatment of HF. Notably, the recent discovery of a first-in-class small molecule GC-A enhancer, which is orally deliverable, will be highlighted. These innovative designer NPs and small molecule possess enhanced and novel properties for the treatment of HF and cardiovascular diseases.

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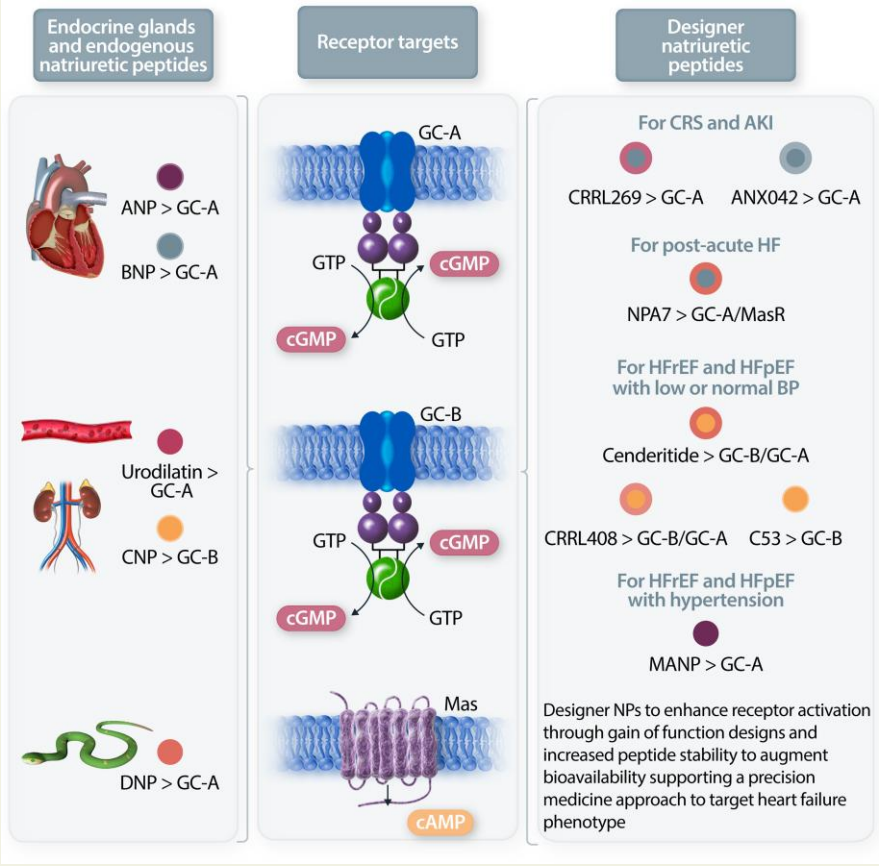
Graphical Abstract



Endocrine organs producing endogenous NPs, which activate GC receptors, include the heart, kidney, endothelium, and venomous glands of the green mamba snake. Specifically, ANP and BNP are produced in the heart and activate GC-A. URO is produced in the kidney and activates GC-A. CNP is produced in the kidney and endothelium and activates GC-B. DNP is produced in the venomous glands of the green mamba snake and activates GC-A. Designer NPs are enhanced engineered NPs which activate GC-A, GC-B, both GC-A/GC-B and GC-A/MasR. These designer NPs are being developed to target cardiorenal syndrome with acute kidney injury, post-acute HF, HFrEF, and HFpEF.

Keywords Heart failure • Natriuretic peptides • Guanylyl cyclase drug discovery • Small molecules

This article is part of the Spotlight Issue on Heart Failure

1. Introduction

Forty-one years ago, de Bold et al.[1] first reported that the atrial myocardium produced a substance which, when injected into rodents, reduced blood pressure (BP) and increased sodium excretion. Further groundbreaking research elucidated that this substance was a peptide, known today as atrial natriuretic peptide (ANP).[2] Following this discovery, the amino acid sequence was elucidated and work by Waldman and Murad established that ANP activates the particulate guanylyl cyclase receptor A [GC-A; also known as NP receptor 1(NPR1)] resulting in the production of the second-messenger cyclic guanosine monophosphate (cGMP), which mediates the diverse and beneficial biological actions of ANP.[3–5] Further research revealed that a second cardiac peptide exists, named B-type NP (BNP), whose actions are also mediated through the GC-A/cGMP pathway.[6] Extensive investigations have documented that ANP and BNP, via GC-A and cGMP, exert therapeutically relevant biological effects such as natriuresis, vasodilatation, suppression of the renin–angiotensin–aldosterone system (RAAS), inhibition of cardiac myocyte hypertrophy and apoptosis, stimulation of vascular regeneration, and inhibition of organ fibrosis.[7] More recently, studies have also revealed favourable metabolic actions of GC-A/cGMP activation that include lipolysis, browning of white adipocytes, release of adiponectin, and regulation of insulin secretion and sensitivity, facilitating glucose uptake and boosting HDL formation.[8–11] A third member of the NP system (NPS), C-type NP (CNP), was subsequently discovered and within the cardiovascular system is synthesized principally in the endothelium and the kidney. CNP is the endogenous ligand of the particulate guanylyl cyclase receptor B [GC-B; also known as NP receptor 2 (NPR2)], and activation GC-B also generates cGMP as its second messenger.[7] While both GC-A and GC-B activation results in cGMP generation, the production of cGMP by GC-A and GC-B

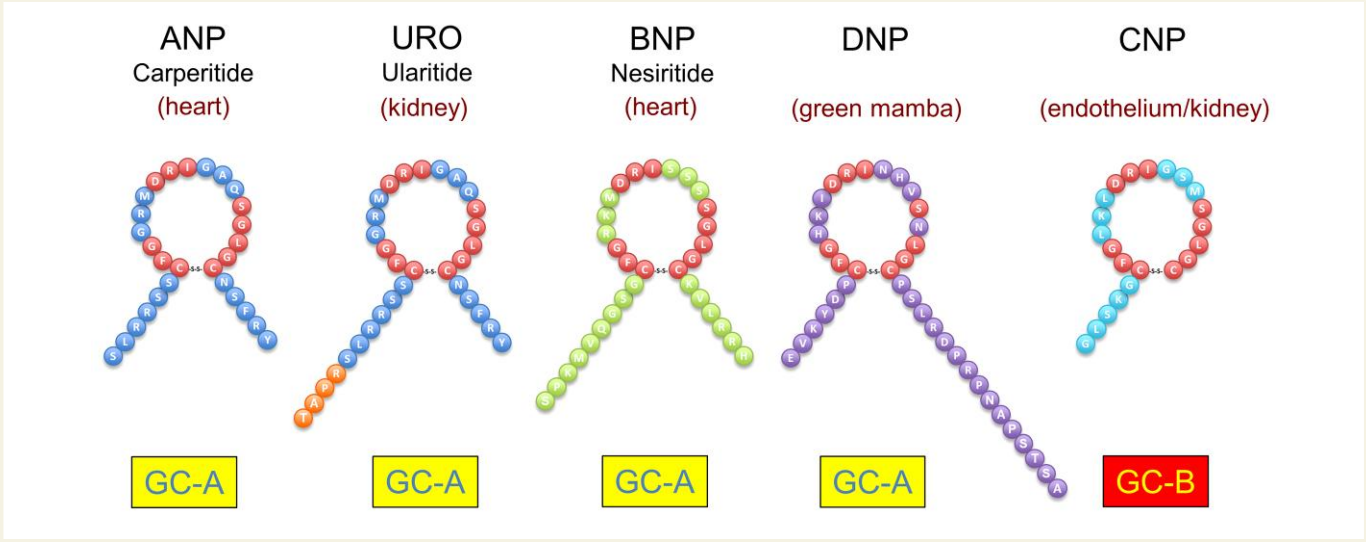


Figure 1 Amino acid structures of ANP, URO, BNP, DNP, CNP, and their respective receptor targets, GC-A and GC-B.

may be localized to different cellular compartments, thereby mediating distinct effects of ANP and CNP, respectively.[7,12] In particular, the CNP/GC-B/cGMP pathway has favourable anti-remodelling properties that include the suppression of fibrosis and smooth muscle cell proliferation as well as the promotion of angiogenesis. Moreover, this pathway has also anti-inflammatory and veno- and microcirculatory vasodilatory actions.[7] Thus, these distinct biological actions of the NPS, which are not reproduced by other therapeutics employed in heart failure (HF), render this system as a high-value therapeutic target.

A hallmark of HF is the activation of the NPS. Indeed, ANP and BNP are increased in the circulation of HF patients, and therefore, plasma BNP and NT-proBNP levels are widely considered the gold standard HF biomarkers.[13–16] Moreover, urinary and plasma CNP levels are emerging as additional HF biomarkers that will be discussed below. Further, harnessing the pleiotropic and protective properties of the NPS for the development of novel and therapeutically beneficial drugs in HF remains an unmet need. Indeed, the full potential of NP therapeutics remains both an opportunity in drug development and a challenge.

The goal of this review is to address opportunities for natriuretic peptide system (NPS) therapeutics in HF both in terms of drug discovery and clinical targeting in HF. First, however, we will establish the rationale followed by an update on innovative NP therapeutics in eight sections. These sections will provide concise overviews of: (i) the endogenous NPs and their respective receptor targets and biological actions; (ii) recent key preclinical studies employing novel mouse models in which the NP genes or their receptors have been genetically modified; (iii) key human studies of ANP and BNP genetic variants and the clinical phenotypes in both the general population and in Stage A and B HF which support novel GC-A activating strategies; (iv) new studies which report ANP and BNP deficiencies and the emerging prognostic role of urinary and plasma CNP in acute decompensated HF (ADHF); (v) the current state of the art of designer NPs for HF and employing a precision medicine approach to specific subclasses of HF; (vi) the discovery of a small molecule that is orally bioavailable and enhances GC-A receptor responsiveness to endogenous ANP and BNP; (vii) technologies for oral delivery of a designer NPs; and finally, (viii) we will provide a contemporary perspective of the challenges of harnessing the therapeutic potential of NPs in HF that need to be addressed for future success.

2. Biology of the natriuretic peptides and the guanylyl cyclase receptor system

Figure 1 illustrates five peptide structures which represent the NP landscape. All five NPs are structurally similar, and four are human peptides encoded by three distinct human genes. ANP is a 28 amino acid (AA) peptide that is encoded by the NP precursor A (*NPPA*) gene. Urodilatin (URO) is a 32 AA proANP-derived peptide with a 4 AA extension on the N-terminus which is the result of the processing of the 126 AA ProANP to URO in the kidney, thus URO is also encoded by the *NPPA* gene. BNP is a 32 AA peptide encoded by the NP precursor B (*NPPB*) gene, while CNP is a 22 AA peptide encoded by the NP precursor C (*NPPC*) gene. Dendroaspis NP (DNP) is a 38 AA peptide that was discovered and isolated from the venom of the green mamba snake. ANP, URO, BNP, and DNP are all GC-A activators, with ANP and DNP having the highest potency for activation of GC-A and BNP the least potency. CNP is both a potent and selective GC-B activator. Further, ANP and CNP are the most susceptible to degradation by neprilysin, while DNP and BNP are highly resistant.[17] In addition, insulin-degrading enzyme (IDE) is also an important peptidase for which NPs may be a substrate.[18]

Figure 2 illustrates selected organs and cell types that express the GC receptors. *Figure 3* illustrates the distinct properties of GC-A and GC-B activation. Our concept for the NPS as HF therapeutics is based upon knowledge of the fundamental biological properties of this system. We view the NPS as possessing multiple organ and cellular protective properties, especially in the kidney, heart, and vasculature. Moreover, the NPS uniquely suppresses and antagonizes aldosterone and possesses favourable metabolic properties which are not only efficacious in HF, but also in cardiometabolic diseases.

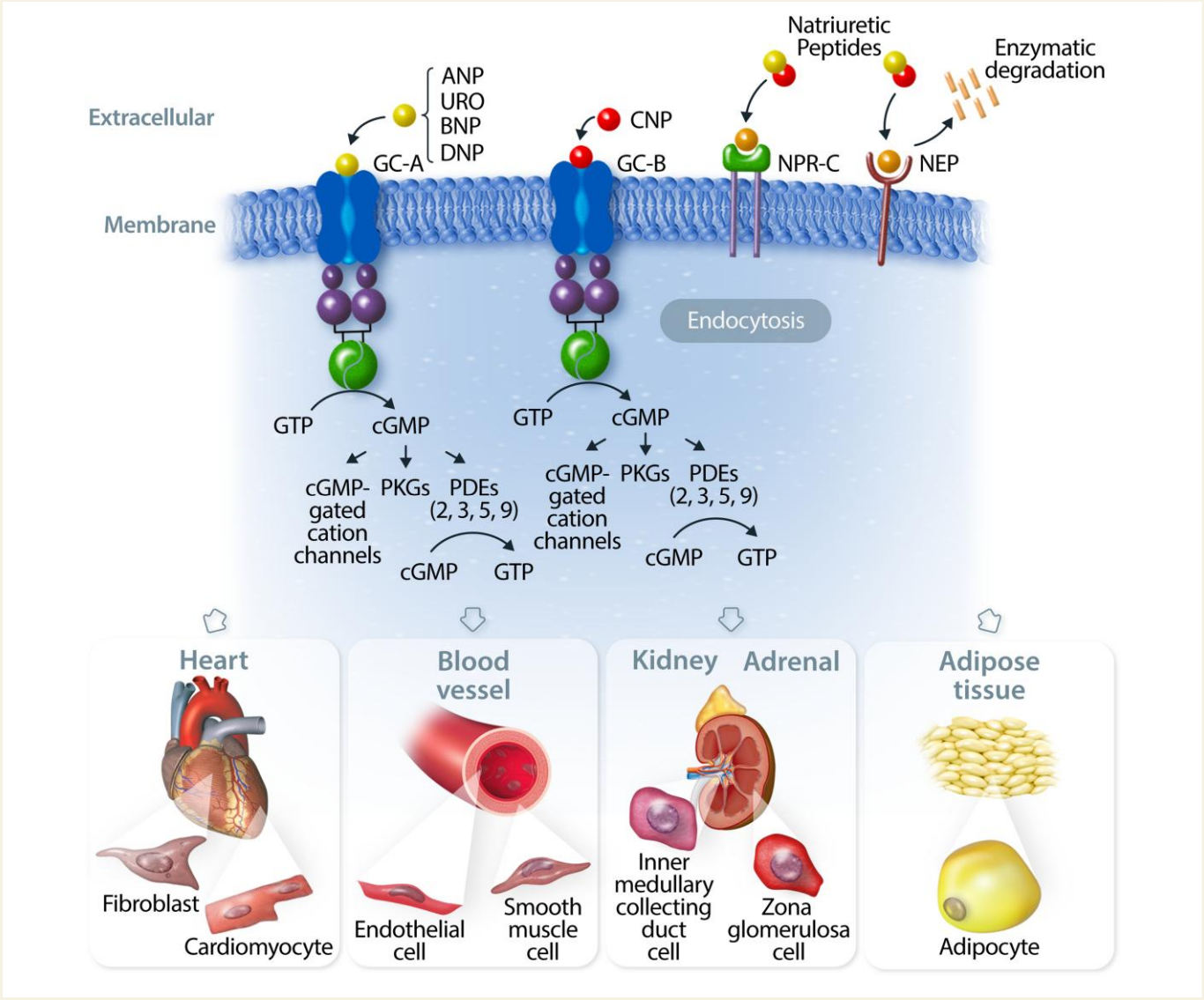


Figure 2 Organ and cell types in which NPs bind to GC-A or GC-B and activate the signalling pathways of cGMP. Once the intracellular concentration of cGMP increases, cGMP-gated cation channels, cGMP-dependent protein kinases, and phosphodiesterases generate important biological responses in different organs and cell types. GTP, guanosine triphosphate; PDEs, phosphodiesterases; PKGs, cGMP-dependent protein kinases G.

3. Lessons from genetic mouse models

Studies of mouse models with global or cell-restricted deletions of the genes encoding ANP, BNP and CNP, their receptors GC-A and GC-B, or downstream signalling pathways revealed that the protective effects of these hormones extend beyond the regulation of arterial BP. This section will focus on the data emphasizing their beneficial effects in the heart and microcirculation.

3.1 ANP counter-regulates the adverse cardiovascular actions of aldosterone

HF patients often present with high aldosterone levels, which impairs prognosis. Plasma levels can be up to 20 times higher in untreated patients with HF compared with healthy controls due to pronounced activation of the RAAS and reduced hepatic clearance.[19] The adverse impact of high aldosterone is not only related to renal sodium and water retention, but also to direct proinflammatory and profibrotic effects on the heart, vasculature, and kidneys.[19] Accordingly, treatment with mineralocorticoid receptor (MR) antagonists decreases mortality in patients with heart and kidney diseases.[19] However, many experimental studies emphasized that MR-independent non-genomic effects of aldosterone contribute to organ damage such as cardiac inflammation and fibrosis.[20] Therefore, aldosterone-synthase (CYP11B2) inhibitors have been in Phase II clinical trials, notably showing promising results.[21]

Physiologically, ANP and BNP are secreted from atrial granules into the circulation in response to acute or chronic atrial stretch to oppose the vascular and renal actions of the RAAS.[1,2] In chronic haemodynamic overload, there is a significant increase in ANP and BNP expression in the ventricular myocardium. Studies in different genetic mouse models indicated that in this situation NPs exert not only endocrine but also local anti-hypertrophic and anti-fibrotic actions.[7] Hence, mice with global genetic disruption of the GC-A gene exhibit arterial hypertension and

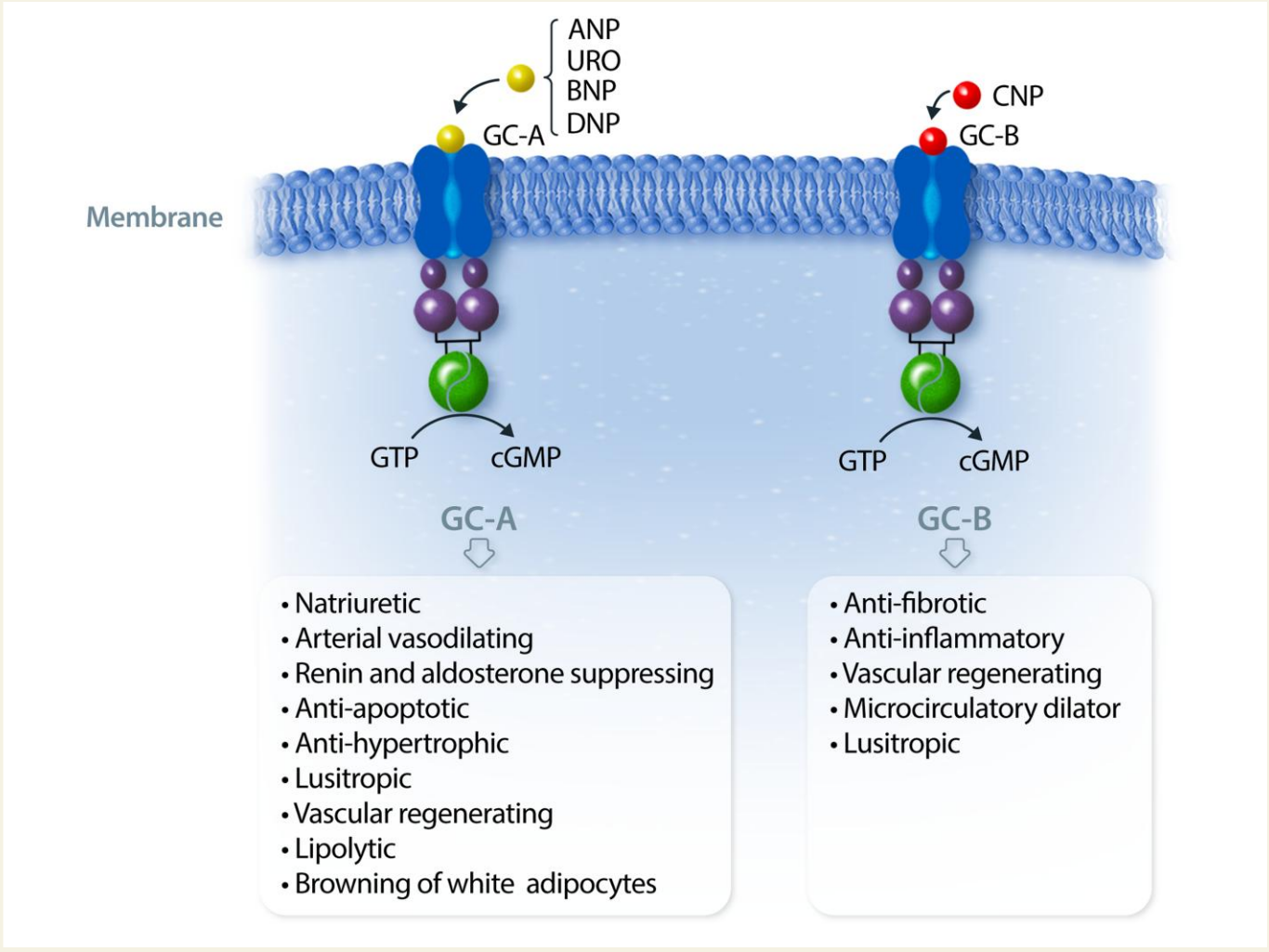


Figure 3 Specific biological properties of GC-A and GC-B.

marked cardiac hypertrophy that is disproportionate to their increased BP and resistant to antihypertensive medication.[22] Mice with cardiomyocyte-restricted GC-A deletion have mild cardiac hypertrophy despite low to normal BP.[23] Furthermore, their cardiac hypertrophic response to aortic constriction was enhanced and accompanied by marked ventricular dysfunction. Consistently, mice with ablation of the corin-dependent cardiomyocyte processing of proANP to active ANP had enhanced risk of cardiac hypertrophy.[24] Conversely, mice expressing a GC-A receptor which is resistant to dephosphorylation/desensitization have unaltered arterial BP but are protected from cardiac hypertrophy.[25] Moreover, amplification of ANP signalling by inhibition of PDE9A (the phosphodiesterase degrading ANP/GC-A-derived cGMP in cardiomyocytes) prevented cardiac hypertrophy.[26] Together, these phenotypes of different monogenetic mouse models indicate that local ventricular NP/GC-A/cGMP signalling may moderate pathological cardiac remodelling.

The inhibition of aldosterone/MR signalling markedly suppressed cardiac hypertrophy and fibrosis in mice with cardiomyocyte-restricted GC-A disruption.[27] This suggested that cardiac remodelling of GC-A-deficient mice is partly derived from augmented cardiac aldosterone signalling and/or that NPs, via GC-A, inhibit aldosterone-mediated excessive remodelling. Indeed, ANP not only counter-acts the renal and cardiovascular actions of aldosterone but also inhibits its synthesis by adrenal zona glomerulosa (ZG) cells. Very low concentrations of synthetic ANP (IC 50s between 0.15 and 0.20 nM) inhibited the stimulation of ZG cell aldosterone secretion by ANG II, ACTH, or hyperkalaemia.[28] Mechanistically the ANP/GC-A/cGMP pathway activates a cyclic AMP-hydrolysing phosphodiesterase, PDE2A, which is highly expressed in these cells. Thereby, ANP decreases cAMP levels and the activity of cAMP-dependent protein kinase (PKA), which ultimately inhibits the activity of the aldosterone synthase.[29] Such inhibitory effects of exogenous ANP were reproduced *in vivo*. In conscious unrestrained rats, ANP infusion prevented the increases of plasma aldosterone levels in response to sodium depletion (a manoeuvre activating the RAAS) or infusion of ACTH.[30] There were no significant effects of ANP on plasma renin activity and corticosterone concentration, indicating that ANP may directly moderate ANG II- as well as ACTH-stimulated aldosterone secretion. Conversely, mice with global, systemic deletion of GC-A exhibited increased adrenal and plasma aldosterone levels.[31] Taken together, these observations support the notion that ANP forms an endocrine axis between the heart and the adrenal gland to regulate aldosterone secretion. Hence, any NP deficiency and impairment which accompanies HF might contribute to the elevated aldosterone levels of these patients. Understanding the ANP pathways and mechanisms regulating adrenal

aldosterone secretion can foster the development of ANP analogues as aldosterone-inhibiting drugs for cardiovascular and renal protection.

3.2 Natriuretic peptides improve microcirculatory perfusion through vasodilating and proangiogenic actions

Apart from neurohumoral imbalance, another mechanism implicated in the progression of hypertensive hypertrophy to HF is coronary microvascular dysfunction.[32] Accompanying LV hypertrophy, the myocardial microcirculation also undergoes structural remodelling. In the early phase, myocardial microvessels may proliferate to meet the needs of the hypertrophic muscle.[32] However, over time, perivascular fibrosis and medial thickening reduce the lumen of arterioles. This is accompanied by a decrease in capillary density and endothelial dysfunction, with endothelial cell inflammation, decreased production of vasodilatory nitric oxide (NO), and increased levels of the vasoconstrictor endothelin-1.[32] Such pathological changes of the microcirculation may interfere with adequate blood supply at times of increased demand and thereby contribute to the progression of pathological cardiac remodelling.

Coronary and peripheral microvascular dysfunctions have a special pathogenic role in HF with preserved ejection fraction (HFpEF).[33] The fact that comorbidities highly prevalent in HFpEF, such as hypertension, diabetes mellitus, and obesity, are also associated with microcirculatory changes, complicates the discussion of whether microvascular dysfunction is a cause of HFpEF or a bystander.[33] However, the mutual influence of cardiac wall biomechanics and haemodynamics creates an insidious positive feedback loop that merits early therapeutical intervention.

Notably, preclinical studies demonstrated that NPs have important protective effects on the microcirculation by diminishing microvascular tone, improving endothelial regeneration, and augmenting the anti-inflammatory and anti-thrombotic properties of endothelial cells.[7] Complementing the endocrine effects of cardiac ANP and BNP, CNP is constitutively released at low levels by endothelial cells and exerts local autocrine/paracrine actions.[7] Mice with endothelial-specific disruption of CNP have mild chronic arterial hypertension and vascular inflammation, with an enhanced risk of macrovascular complications such as atherosclerosis and aortic aneurysms.[34] These phenotypes emphasize the physiological relevance of paracrine vascular CNP signalling.

To study the microvascular roles of CNP, Spiranec *et al.*[35] visualized the microcirculation of the murine cremaster muscle by intravital microscopy. Notably, the vasodilatory effects of CNP were weak in arteries and increased towards precapillary arterioles and capillaries.[35] CNP consistently did not prevent endothelin-1-induced acute constrictions of proximal arterioles, but fully reversed endothelin effects in fine distal arterioles and capillaries, where pericytes gradually replace vascular smooth muscle cells (VSMCs). Pericytes are contractile cells embedded within the endothelial basement membrane.[36] They have multiple thin cytoplasmic processes encircling the endothelial cells, with which they maintain an intimate cross-talk, both through direct interaction and via paracrine factors. This communication is essential for the control of microcirculatory resistance and blood flow.[36] Studies with cultured pericytes revealed that CNP, via GC-B, increases cGMP levels and thereby prevents the calcium and contractile responses to endothelin.[35] Moreover, mice with genetic deletion of the GC-B receptor in pericytes had chronic increases in peripheral resistance and arterial BP, despite unaltered renal function.[35] Together, these results indicate that CNP participates in the local, paracrine communication between endothelial cells and pericytes, and thereby in chronic BP regulation. In addition, CNP stabilizes resident perivascular mast cells at baseline and prevents their excessive activation and degranulation under pathological conditions, for instance, in response to ischaemia or endothelin.[37] This paracrine CNP effect may contribute to the maintenance of vascular barrier integrity and to the anti-inflammatory and anti-thrombotic characteristics of endothelial cells.

Whereas microvascular pericytes and immune cells, such as mast cells, mainly express GC-B receptors, microvascular endothelial cells express high levels of GC-A, and much lower levels of GC-B.[38] Preclinical and clinical studies suggested that the NP/GC-A/cGMP system might improve vascular regeneration. ANP and BNP stimulated the proliferation and migration of cultured human macrovascular and microvascular endothelial cells.[39] An increase in circulating BNP levels resulting from targeted overexpression of the BNP gene in the liver accelerated vascular regeneration in limb ischaemia experimentally generated in mice by femoral artery ligation.[40] Moreover, a clinical study showed the therapeutic potential of intravenously administered recombinant human ANP, carperitide, in patients with peripheral arterial diseases to increase limb perfusion and walking distance.[41] To decipher whether the endogenous NP/GC-A system modulates ischaemic vascular regeneration, Kuhn *et al.*[42] studied angiogenesis after critical hind limb ischaemia, a model of peripheral arterial disease, and after cardiac pressure overload in mice with endothelial cell-specific GC-A deletion (EC GC-A KO). The results demonstrated that BNP, produced by activated satellite cells within ischaemic skeletal muscle or by cardiomyocytes in response to pressure load, improves the regeneration of neighbouring endothelia via GC-A. This paracrine, BNP/GC-A-mediated communication might be critically involved in coordinating muscle regeneration/hypertrophy and angiogenesis.[42]

In summary, these data in genetic mouse models emphasized the antagonistic relation between the vasoconstrictor, proinflammatory pathways (endothelin, oxidative stress) and the vasodilatory, anti-inflammatory, and pro-regenerative NP pathways in the microcirculation. The imbalance between these pathways may contribute to microvascular dysfunction in HFpEF patients, which often have low levels of ANP and BNP. Therapeutic restoration of this imbalance might slow the progression of the cardiac and systemic alterations.

3.3 C-type natriuretic peptide complements the local cardioprotective actions of ANP and BNP

Over the past decade, a new paradigm for HFpEF development proposes that a systemic proinflammatory state, derived from comorbidities such as hypertension, obesity, and diabetes mellitus, drives the myocardial structural and functional alterations.[43] As already mentioned above, this proinflammatory state contributes to coronary microvascular endothelial dysfunction, impairing NO bioavailability. Diminished effects of endothelial NO on adjacent cardiac myocytes and fibroblasts reduces their cGMP levels and the activity of cyclic GMP-dependent protein kinase I (PKG I). Low PKG I activity favours hypertrophy and fibrosis development and myocyte stiffening because of hypophosphorylation of titin.[43] Both stiff cardiomyocytes and interstitial fibrosis contribute to high diastolic left ventricular (LV) stiffness and HF development. Accordingly, this HFpEF paradigm proposes that therapeutic strategies restoring myocardial cGMP/PKG I activity may attenuate LV stiffness.[43]

Cardiomyocyte cGMP levels and PKGI activity are modulated not only by NO but also by the NPs, which have distinct signalling and action

Table 1 Genetic studies on rs5068 and rs198389

SNP	Functional effect	Study	Population size	Specific phenotype associated with minor allele
rs5068	Located in the 3′ untranslated region of NPPA gene Rs5068 minor allele blocks micro-RNA 425 inhibitory effect resulting in higher production of ANP	Newton-Cheh *et al.*[52]	Framingham Heart Study cohort (n = 1456) Malmö Diet and Cancer study cohort (n = 5196) Finrisk97 study cohort (n = 7091)	Higher ANP circulating levels Lower blood pressure Reduced risk of new-onset hypertension
		Cannone *et al.*[11]	Olmsted County, MN, USA (n = 1608)	Higher ANP circulating levels Lower blood pressure Lower prevalence of hypertension Lower body mass index Smaller waist circumference Higher HDL cholesterol plasma levels Lower prevalence of obesity Lower prevalence of metabolic syndrome
		Cannone *et al.*[53]	Sicily, Italy (n = 812)	Lower systolic blood pressure Lower prevalence of hypertension Lower body mass index Lower prevalence of males with HDL cholesterol plasma levels below 40 mg/dl Lower prevalence of obesity Lower prevalence of metabolic syndrome
		Cannone *et al.*[54]	African Americans from MESA cohort (n = 1631)	Lower triglycerides plasma levels Lower insulin plasma levels Higher HDL cholesterol plasma levels Lower prevalence of metabolic syndrome Lower prevalence of diabetes mellitus
rs198389	Located in the promoter region of *NPPB* gene, rs198389 minor allele disrupts a putative binding site for transcription factor. Further studies are still needed to determine transcription factors implicated in this allele specific activity difference.	Seidelmann *et al.*[55]	African Americans and whites from the ARIC study cohort (African Americans, n = 1860; Whites, n = 1705)	Higher BNP plasma levels Lower blood pressure Reduced use of antihypertensive medications Lower prevalence of hypertension Lower risk of cardiovascular mortality Increased lifespan
		Cannone *et al.*[56]	STOP-HF follow-up study cohort (n = 971)	Higher BNP circulating levels Lower risk of hypertension Lower risk of new-onset LVD Lower prevalence of major adverse cardiovascular events

NPPA, natriuretic peptide precursor A; ANP, atrial natriuretic peptide; BP, blood pressure; MESA, Multi-Ethnic Study of Atherosclerosis; NPPB, natriuretic peptide precursor B; ARIC, Atherosclerosis Risk in Communities; BNP, B-type natriuretic peptide; Stop-HF, St. Vincent Screening to Prevent Heart Failure; LVD, left ventricular dysfunction.

patterns in cardiomyocytes.[44] The GC-A receptors for ANP and BNP are confined to transverse tubules (T-tubules) and mediate small cGMP increases in local T-tubular microdomains.[44] As referred to above, even minimal submembrane increases of cGMP can mediate significant anti-hypertrophic effects, for instance, via PKGI-dependent inhibitory phosphorylation of TRPC6 channels and diminished pathological calcium entry.[45] The GC-B receptors are more densely and uniformly distributed throughout the sarcolemma.[44] Their activation by CNP generates greater cGMP signals spreading through the sarcoplasm and stimulating PKGI-mediated phosphorylation of regulatory proteins at the sarcoplasmic reticulum and the sarcomere, such as phospholamban and troponin I.[44] Moreover, recent pharmacological and genetic studies in mice demonstrated that CNP, via GC-B/cGMP signalling, augments PKGI-mediated phosphorylation of the titin springs and diminishes the expression of proinflammatory myokines such as IL-6.[46] These effects did not impact LV function under physiological

conditions but prevented myocardial inflammatory infiltration, myocyte stiffening and diastolic dysfunction during early stages of mild pressure overload.[47] Moreover, other studies observed an inverse relationship between CNP levels and cardiac fibrosis.[48] Together these findings support NP therapeutics in early HFpEF especially targeting GC-B.

Neuroendocrine imbalance, with increased activity of the RAAS and diminished activity of the NPs, has a major role in the transition of cardiac hypertrophy to HF. Blockade of the RAAS together with the inhibition of neprilysin-mediated degradation of the NPs by sacubitril/valsartan has improved the morbidity and prognosis of HF with reduced ejection fraction (HFrEF) patients.[49] Because neprilysin degrades not only ANP and but also CNP, augmented endogenous CNP signalling in the microcirculation and heart may contribute to the clinical benefits of neprilysin inhibitors. Indeed, in healthy subjects, sacubitril/valsartan increased circulating CNP levels.[50] Despite recent setbacks in ongoing studies of sacubitril/valsartan in patients with overt HFpEF,[51] the results of experimental studies suggest that correction or augmentation of CNP/GC-B/cGMP signalling may moderate pathological myocardial wall stiffening and improve diastolic function at earlier or less severe disease stages. Because ANP and BNP also exert myocardial protective actions, a novel strategy in HF could be to co-activate both the GC-A and GC-B receptors for synergistic cardioprotection.

4. Precision medicine and the natriuretic peptides in the general population and in heart failure

NPPA and *NPPB* genes are both located on chromosome 1, and they encode for the pre-pro-hormones from which ANP and BNP are obtained after corin-mediated enzymatic cleavage, respectively. Genetic studies performed over the last two decades have highlighted the importance of investigating NP single nucleotide polymorphisms (SNPs). The clinical phenotypes associated with ANP and BNP genetic variants represent a unique 'human model' that demonstrated the key role played by NPs in cardiovascular biology, physiology, disease, and eventually therapeutics (*Table 1*).

In 2009, Newton-Cheh *et al.*[52] revealed that the minor G allele of the ANP SNP rs5068, which is associated with ~10% increase in ANP circulating levels, is related to a cardiovascular phenotype characterized by lower BP and reduced risk of new-onset hypertension in general populations of whites from Northern America and Scandinavia. Importantly, these genetic findings are consistent with the BP-lowering properties of ANP including vasodilation and natriuresis. A few years later, based on NP properties of inducing lipolysis, increasing insulin sensitivity, and browning of white adipose tissue, Cannone *et al.*[11] investigated the metabolic phenotype in the carriers of this rs5068 minor allele. Interestingly, in a community-based cohort from Minnesota, USA, that included mostly whites with northern European ancestries, the G allele was associated with lower body mass index (BMI) and waist circumference along with higher plasma levels of the protective HDL cholesterol. Importantly, obesity and metabolic syndrome were less prevalent among rs5068 carriers. Associations between the G allele and lower BP as well as lower risk of hypertension were also confirmed. Replication studies were performed by Cannone *et al.*[53] in a general population from the Mediterranean island of Sicily. In this cohort including whites of Southern Europe, the minor G allele was also consistently associated with a 'favourable' cardiometabolic phenotype characterized by lower BMI and lower systolic BP. Males with HDL cholesterol levels below 40 mg/dL, which represents one of the criteria for the diagnosis of metabolic syndrome, were less prevalent in the AG + GG genotypes. Notably, hypertension, obesity, and metabolic syndrome were less prevalent among the G allele carriers.

To investigate the cardiometabolic profile related to rs5068 in a different ethnicity, Cannone *et al.*[54] analysed the clinical phenotype associated with rs5068 genotypes in African Americans enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA). Carriers of the minor G allele had lower triglycerides and insulin plasma levels, whereas the 'protective' HDL cholesterol levels were higher in these subjects. Accordingly, metabolic syndrome along with diabetes were less prevalent among G allele carriers. Surprisingly, the minor allele did not protect against hypertension in African Americans who are known to have lower NP levels than non-African Americans.[57]

Additional studies focusing on BNP genetic variants also have enhanced our understanding of the role of NPs in the physiology and pathophysiology of the cardiovascular system highlighting potential ANP and BNP therapeutic actions.

More specifically, the BNP SNP rs198389 is a functional variant in the promoter region of the *NPPB* gene and its minor allele has been consistently associated with higher circulating levels of BNP. Such association provides the opportunity to investigate the phenotype related to lifetime exposure to slightly higher levels of this cardiac hormone. Seidelmann *et al.*[55] showed that in African Americans and whites from the Atherosclerosis Risk in Communities (ARIC) study, the rs198389 minor allele is associated with lower systolic and diastolic BP, reduced use of antihypertensive medications, as well as lower prevalence of hypertension. Over a median follow-up of >20 years, the risk of cardiovascular mortality was lower in the carriers of the minor allele and lifespan was increased.

While the study by Seidelmann *et al.* was conducted in community-based cohorts, Cannone *et al.*[56] studied the cardiovascular phenotype associated with rs198389 in subjects at risk of HF from the cohort of the St Vincent Screening to Prevent Heart Failure (STOP-HF) Follow-Up Study, which originated from the STOP-HF Study. The STOP-HF Study, a seminal randomized controlled prospective trial, demonstrated that clinical collaborative care between primary care physicians and cardiovascular specialists along with BNP-based screening decreases the risk of developing LV dysfunction and HF.[58]

In this genetic study by Cannone *et al.*,[56] Stage A and B HF subjects from the St Vincent Screening to Prevent Heart Failure (STOP-HF) Follow-up Study were genotyped for the BNP gene variant rs198389. The analysis revealed that rs198389 minor allele is associated with higher circulating levels of BNP and it also correlates with lower risk of hypertension and new onset of LV dysfunction. Over 5 years, the prevalence of major adverse cardiovascular events was lower among the carriers of the minor allele.

Importantly, the clinical phenotypes associated with ANP and BNP genetic variants support the protective properties of the two cardiac hormones and, together with the aforementioned experimental studies, lay the foundation for GC-A enhancing therapeutics in HF, hypertension, and metabolic disease. In addition, as recently emphasized by Lanfear and Luzum[59] regarding the genetic findings of the genetic STOP-HF Follow-Up Study, combining various genetic markers may improve risk stratification and identify subjects at greater cardiometabolic risk who might benefit the most from an NP-based therapy. It will be important to replicate this genetic study from STOP-HF in another population.

5. Differential regulation of ANP and BNP in heart failure and emerging role for CNP as a biomarker

A hallmark of human HF is the elevation of ANP and BNP which has established BNP and NT-proBNP as gold standard biomarkers in HF.[13–16] Research described in the previous sections supports the concept that the elevation of ANP and BNP in HF may serve as a beneficial compensatory response. As also mentioned, ANP is the most biologically active of the two cardiac-derived peptides in activating the GC-A receptor.[60] Consistent with the protective role of ANP in human HF, recent clinical studies showed that treatment of symptomatic HFrEF patients with sacubitril/valsartan also results in an increase in ANP, in addition to BNP, thus supporting a key biological role for ANP.[61,62]

However, despite elevated plasma levels of ANP and BNP, HF is in fact a state of NP deficiency. The circulating peptides are mostly unprocessed and inactive. Moreover, the GC-A receptor is desensitized in HF patients. NP deficiency extends to other cardiometabolic diseases: studies have demonstrated low levels of ANP and BNP in subsets of patients with hypertension, obesity, and African Americans.[57,63] Thus, the heart, like the pancreas in diabetes or the thyroid gland in hypothyroidism, may demonstrate an inadequate endocrine response to stress, such as that induced by acute and/or chronic HF. In support of this concept, Reginauld *et al.*[64] recently reported that there is a lack of elevation of circulating ANP in a subpopulation of HFrEF and HFpEF patients hospitalized for ADHF. First, he confirmed the general finding of the elevation of BNP in 95% of ADHF patients, with 5% of patients having unaltered BNP levels. Moreover, Bachmann *et al.*[65] also observed that 5% of patients hospitalized for HF had low BNP. Further a lack of ANP elevation (or normal levels) was found in 26% of ADHF patients.[64] These findings support the existence of a relative deficiency of ANP in ADHF, possibly due to reduced production, altered release, and/or enhanced degradation. We speculate that augmented neprilysin-mediated degradation may be a key mechanism of this ANP deficiency as ANP is more susceptible than BNP.[17]

There are specific mechanisms that may reduce ANP production in HF. Micro-RNA 425 and/or 155 (mir425 and/or mir155), which downregulate ANP gene expression and production, may be elevated in HF.[66,67] Moreover, Celik *et al.*[68] reported a *cis*-acting ANP antisense transcript (ANP-AS), negatively regulating ANP expression in human cardiomyocytes, which was increased in heart tissue of patients with advanced HF. Thus, in HF with elevated ANP-AS and an ANP deficiency, ANP-AS might be a contributing mechanism.

The presence of molecular forms of ANP and BNP with reduced biological activity also contributes to NP deficiency in HF. Liang *et al.*[69] reported that a major circulating form of BNP in these patients is unprocessed proBNP, a weak activator of GC-A.[70] In contrast, proANP remained an active agonist of GC-A.[71] However, Abell *et al.*[72] reported that ANP is cleaved into an open ring molecular form with antagonistic properties at GC-A and that such a cleaved ANP may be increased in plasma of HF patients. These studies taken together underscore the importance of measuring not only BNP but also ANP plasma levels in HF. They also indicate that the precise measurement of the circulating molecular NP forms could improve diagnostics. The relative deficiency and altered potency of ANP and BNP in HF supports therapeutic targeting of GC-A. ANP-based therapeutics might be superior because ANP is a greater activator of the GC-A receptor compared with BNP.

While conventionally CNP has not been considered an important HF biomarker, Ma *et al.*[73] measured NT-proBNP and plasma and urinary CNP in healthy subjects and in patients with ADHF. They reported significantly elevated concentrations of urinary and plasma CNP in ADHF patients compared with healthy subjects. Moreover, the levels of urinary CNP exhibited poor correlation with plasma CNP, thus suggesting a unique role for each in ADHF pathophysiology. Notably, ADHF patients with combined elevations of urinary and plasma CNP levels had greater disease severity and worse prognosis. Specifically, elevations of both urinary and plasma CNP conveyed higher risk for rehospitalization and rehospitalization/death. Further the addition of urinary and plasma CNP to established risk factors, including plasma NT-proBNP, enhanced the prediction of adverse outcomes. This recent finding supports advancing the potential clinical utility of determinations of urinary and plasma CNP, together with established markers, as a personalized medicine approach to identify high-risk ADHF patients which could especially profit from NP/GC/cGMP enhancing therapeutics.

6. Designer natriuretic peptides: particulate guanylyl cyclase receptor activators for heart failure

The approval of sacubitril/valsartan for HF, which reduces the risk of death and of hospitalization and augments ANP and BNP (principally ANP) levels through inhibition of their degradation by neprilysin via sacubitril, has refocused attention on the therapeutic opportunities to enhance the NPS.[61,62] Indeed, for over two decades, the use of low-dose ANP (carperitide) for ADHF, at non-hypotensive doses, has demonstrated overall efficacy and safety when administered often over 72 h. Nogi *et al.*[74] recently reported that low-dose carperitide in ADHF was associated with lower cardiovascular and all-cause mortality within 1 year after admission. The use of recombinant BNP (nesiritide) in ADHF in early studies demonstrated improved symptoms and haemodynamic unloading of the heart, yet hypotensive dosing often was associated with worsening renal perfusion and impaired renal function, resulting in limited use of nesiritide.[75]

Notably, Chen *et al.* provided a paradigm shift in NP therapeutics. These investigators focused on cardiac remodelling, preservation of renal function, and the RAAS. A randomized double-blind placebo-controlled study comparing 8 weeks of subcutaneous (SQ) injections of BNP, twice daily, compared with placebo was performed in 40 patients with HFrEF (New York Heart Association functional Class II–III HF and EF <35).[76] Eight weeks of SQ BNP resulted in a greater reduction of LV systolic and diastolic volume index and LV mass index when compared with placebo. There was also a significantly greater improvement of Minnesota Living with HF score, and reductions in LV filling pressure as demonstrated by the reductions of *E*/*e*′ ratio and decrease in left atrial volume index when compared with placebo. Glomerular filtration rate (GFR) was preserved with chronic SQ BNP, as was the ability to elevate plasma cGMP levels and suppress plasma renin activity. Thus, chronic SQ BNP proved to be a novel, safe, and efficacious peptide therapeutic strategy in human HF.

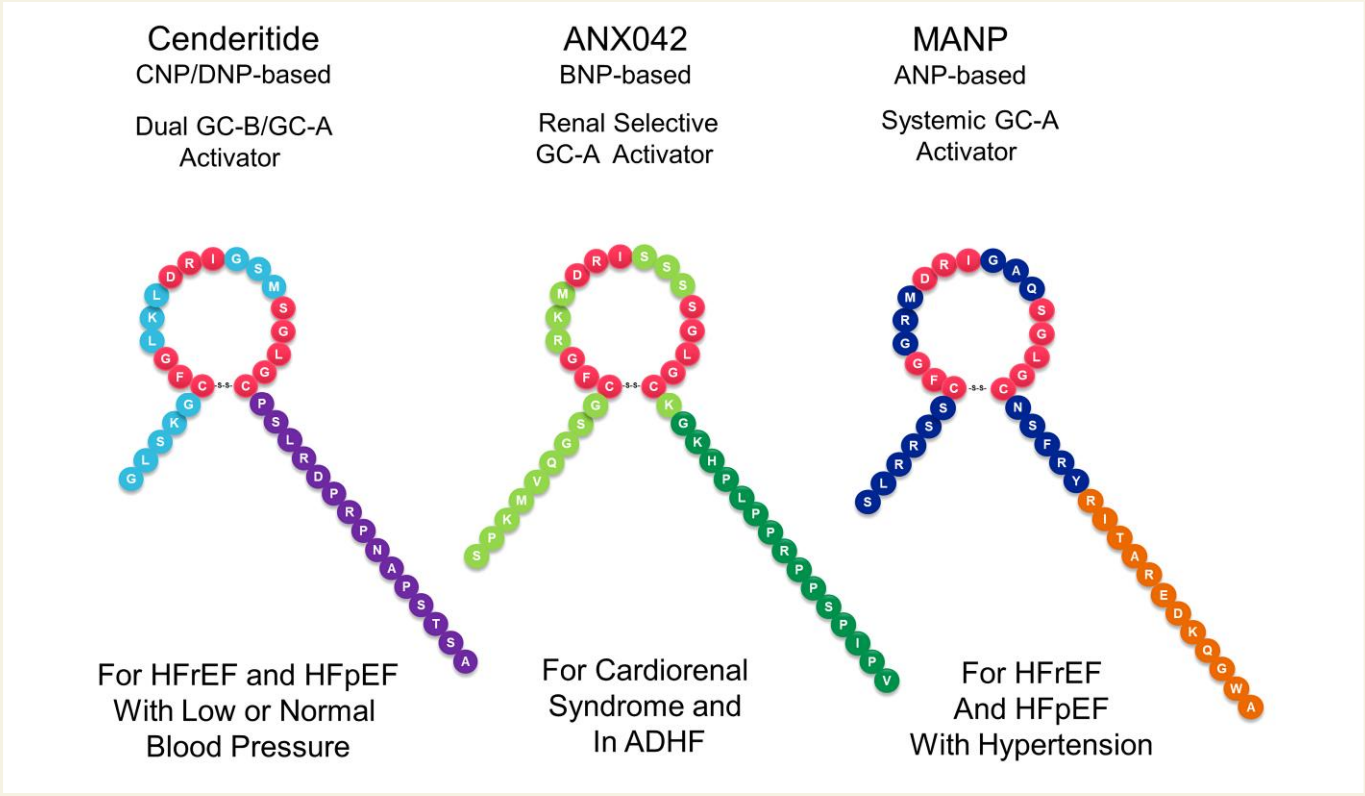


Figure 4 Amino acid sequences of the designer natriuretic peptides cenderitide, ANX042 and MANP.

6.1 Engineering designer natriuretic peptides

Based upon the growing knowledge about the biology of the NPS, the beneficial properties of chronic elevations of ANP and BNP in individuals with *NPPA* and *NPPB* gene variants as well as the exploding field of peptide therapeutics, the development of designer NPs has recently emerged. It was realized that the surface areas of peptides, compared with small molecules, are larger and more optimal to activate receptors. Peptides are also highly specific for their target receptors, and therefore possess minimal off-target effects. While inhibitors of enzymatic degradation of peptides are a mechanism for boosting endogenous peptides, such an approach may be inadequate to increase their levels to a pharmacological concentration for optimal receptor stimulation. Thus, designer peptides that possess efficacious biological properties may be an innovative strategy to overcome limitations of existing therapeutic options, as well as go beyond the capabilities of endogenous peptides.

There are five goals of designer NP engineering.[77] First, the safety of a designer peptide is of the highest priority and is carefully evaluated from a toxicology and experimental *in vivo* perspective. The second is enhancing receptor activation. This may be achieved through defining which amino acids in the NP structure are key mediators of receptor binding and/or limit full receptor activation. This knowledge can be used to design synthetic peptides with targeted sequence changes. A third goal is to design a peptide that is more stable and resistant to enzymatic degradation, thus improving pharmacokinetics and bioavailability. Fourth takes on a precision medicine approach, which is to engineer a peptide that possesses unique biological properties for specific disease syndromes such as a renal function enhancing peptide for HF without excessive hypotension or a peptide with potent BP lowering and natriuretic actions for HF in the setting of hypertension. The fifth goal is chronic delivery with a focus on oral peptide delivery as recently achieved with semaglutide, a glucagon-like peptide 1 (GLP-1) receptor activator.[78]

6.2 A precision medicine approach to designer natriuretic peptides

HF is a heterogeneous disease syndrome with multiorgan involvement. Hence, an optimal therapeutic approach may benefit from personalizing a drug for a specific subset of HF patients. A precision medicine approach is a major strategy in the engineering of designer NPs and targets important subsets of HF which includes the cardiorenal syndrome (CRS) with acute kidney injury (AKI) in ADHF, cardiorenal impairment in post-acute HF and chronic HFrEF, as well as HFpEF, in the presence of low, normal, or high BP. All these subsets of HF patients have unmet needs which are of high clinical importance. Below we discuss three designer NPs that have been or are in clinical trials and three which are in the pre-clinical stage of drug development.

6.3 Designer natriuretic peptides in clinical studies

Three designer NPs (*Figure 4*) have been bioengineered, tested in cell-based assays and *in vivo* animal models of HF, undergone pharmacotoxicology studies, received Investigational New Drug (IND) approval from the Food and Drug Administration (FDA) and moved into clinical studies (*Table 2*).

6.3.1 Cenderitide

The first designer NP to receive IND approval from the FDA for HF is cenderitide (also known as CD-NP, *Figure 4*) and was first reported by Lisy *et al.*[79] Cenderitide is a dual activator of GC-A and GC-B which does not exist in nature and takes advantage of the unique properties of both receptors. Specifically, cenderitide is a 37 AA peptide that was created by fusing the 22 AA of human CNP, with the 15 AA carboxyl terminus (C-terminus) of DNP. Importantly, CNP, a GC-B activator, possesses anti-fibrotic, anti-inflammatory, and favourable microcirculatory actions in preclinical studies.[7] Additionally, CNP is a more selective venodilator and less hypotensive than ANP or BNP.[80] However, CNP lacks overt natriuretic actions.[81] DNP is a potent natriuretic and diuretic peptide which functions via GC-A and is highly resistant to enzymatic degradation.[82] Therefore for dual GC-A and GC-B activation, we fused the 15 AA C-terminus of DNP to the vacant C-terminus of CNP in which full-length CNP remained intact.[83] *In vitro*, cenderitide stimulates cGMP production in human cardiac fibroblasts to a greater extent than BNP or DNP, exerts anti-proliferative actions in human cardiac fibroblasts as measured by BrdU uptake, and potently suppresses collagen I production.[79] Moreover, cenderitide generates cGMP in human embryonic kidney (HEK) 293 cells overexpressing either the human GC-A or GC-B receptor, it activates cGMP production in freshly isolated glomeruli and is natriuretic.[81,83] Together, these studies established that cenderitide represents the first designer NP working as a dual-acting GC-A and GC-B agonist. Preclinical *in vivo* studies in healthy animals and models of HF and renal disease demonstrated cardiac unloading, minimal hypotension, natriuresis, enhancement of GFR, and suppression of aldosterone and cardiac fibrosis together with increased plasma and urinary cGMP levels.[79,84] Because cenderitide is markedly more resistant to enzymatic degradation than ANP, BNP, and CNP, its *in vivo* half-life and biological actions are extended.

With promising preclinical findings, a first-in-human (FIH) clinical trial of cenderitide was performed as a two-stage randomized, placebo-controlled trial to assess safety and efficacy in healthy human volunteers.[85] Subjects that acutely received cenderitide had a significant increase in plasma and urinary cGMP compared with placebo. Moreover, while GFR was preserved in both groups, a significant diuretic and natriuretic response was observed in the cenderitide group. Furthermore, plasma aldosterone suppression and a modest (<5 mmHg) reduction in BP were also seen in the cenderitide group. These favourable cardiorenal actions were not associated with adverse events.

From 2006 to the present, cenderitide has been investigated inpatients with ADHF, stable HFrEF, or LV assist device (LVAD). Most recently, a prospective, randomized, placebo-controlled trial was performed to evaluate the safety, tolerability, cGMP generation, and effect on GFR of cenderitide in patients with stable chronic HF with GFR above and below 60 mL/min.[86] Specifically, 18 chronic HF patients with NYHA functional Class I–III and LVEF of ≤ 40% were randomized in a 2:1 ratio to cenderitide vs. placebo. The experimental protocol included a 1 h baseline and 4 h IV infusion of cenderitide. A 4 h infusion of cenderitide was safely tolerated in HF patients and importantly, elevated plasma and urinary cGMP, without hypotensive or adverse effects. Importantly, cenderitide increased GFR in those HF patients who had GFR at baseline below 60 mL/min, which is consistent with preclinical studies.

The clinical application of cenderitide is now based on the emerging biology of co-activation of GC-A and GC-B which includes cGMP-mediated natriuresis, suppression of cardiac fibrosis and possible enhancement of myocardial relaxation, favourable microcirculatory and anti-inflammatory actions, aldosterone suppression, as well as enhancement of GFR without hypotension. These protective cardiorenal actions support chronic HFrEF and HFpEF as clinical indications, especially in the setting of low or normal BP.

Capitalizing on the encouraging experimental and clinical findings of cenderitide and advances in bioengineering of peptides, a next-generation dual-acting GC peptide agonist has been designed which builds on the core structure of cenderitide. This novel dual activator, a 45 AA peptide, is named CRRL408. It was engineered to have enhanced pharmacokinetics compared with cenderitide and may be amendable to oral delivery. A preclinical IND-enabling programme is underway which is specially designed to advance CRRL408 in models of chronic HF.

6.3.2 ANX042

Genomic medicine provides an additional avenue to pursue novel drug discovery. We recognized that alternative splicing of genes account for extensive diversity of the human proteome. Thus, a strategy in drug discovery is to identify spliced variants of NP genes which could lead to the biodesign of innovative NP therapeutics based on unique structure and function. Further, the discovery of a novel endogenous alternatively spliced peptide could also lead to important diagnostics.

Using genome-wide analyses, Pan *et al.*[87] identified an alternative spliced form of BNP (AS-BNP) in human failing LV tissue and observed that the expression of AS-BNP was significantly reduced in the setting of LVAD therapy. The structure of AS-BNP includes the same AA sequence in the amino terminus (N-terminus) and ring structure of human BNP but a unique additional 30 AA cassette on its C-terminus resulting in a previously unknown 60 AA peptide. Interestingly, AS-BNP lacked the ability to stimulate cGMP in vascular endothelial cells and to relax preconstricted arterial rings compared with BNP. To make AS-BNP more amendable as a therapeutic, peptide engineering to optimize AS-BNP resulted in a 42 AA designer peptide that was named ANX042 (AS-BNP.1; *Figure 4*). *In vitro*, ANX042 was a weak activator of GC-A, however activated cGMP in isolated glomeruli and mesangial cells, with minimal cGMP production in VSMCs or endothelial cells. As ANX042 possessed renal but not vascular cGMP properties, the potential for a renal specific BNP analogue, without hypotension, became possible. Indeed, studies with ANX042 in a canine model of HF demonstrated no change in BP but produced an increase in GFR, natriuresis, together with increases in plasma and urinary cGMP and the inhibition of plasma renin activity. The renal specific mechanism of action for ANX042 has been attributed to activation of renal GC-A. However, ongoing research is also investigating the role of active renal metabolites of ANX042 and unique renal receptor subtypes which may also mediate the actions of this designer NP. Thus, the enhancement of renal function without altered systemic haemodynamics makes ANX042 a potential therapeutic agent for HF specifically targeting the CRS.

ANX042 received an IND and an FIH study in normal subjects demonstrated activation of cGMP production, safety, and tolerability with intravenous infusion.[88] Currently, studies of the potential of enhancement of renal function in subjects with stable HF and GFR below 70 mL/min are underway at the Mayo Clinic, supported by the National Heart, Lung, and Blood Institute (NHLBI) (ClinicalTrials.gov Identifier: NCT03019653). In such studies, ANX042 is administered as an acute IV infusion followed by in-depth cardiorenal phenotyping. Based on the outcome of this ongoing study, a Phase 2 study is planned targeting ADHF with CRS.

6.3.3 MANP

MANP (also referred to as 'frameshift ANP' or mutant ANP) is a selective and potent GC-A activator consisting of human ANP with

Table 2 Summary of designer natriuretic peptides in preclinical or clinical studies

Designer peptide	Stage	Receptor target(s)	Biological actions	Clinical target(s)
Cenderitide and CRRL408	Phase 2 and proof-of-concept clinical studies	GC-A and GC-B	Natriuretic GFR enhancing Vasodilating Aldosterone inhibiting Cardiac unloading Anti-fibrotic and anti-hypertrophic Minimal hypotension	HFrEF and HFpEF with low or normal BP
ANX042	Phase 1 and 1b clinical studies	Attributed to renal GC-A with continuing investigations on specific receptor targets	Natriuretic GFR enhancing Aldosterone inhibiting No hypotension	Cardiorenal syndrome
MANP	Phase 1 and 1b clinical studies	GC-A	Natriuretic GFR enhancing Vasodilating Aldosterone inhibiting Hypotensive	Uncontrolled hypertension and HFpEF with hypertension
CRRL269	Preclinical studies	Renal selective GC-A	Natriuretic GFR enhancing Renal vasodilating Anti-apoptotic Angiotensin II inhibiting Minimal hypotension	Cardiorenal syndrome with acute kidney injury
NPA7	Preclinical studies	GC-A and MasR	Natriuretic GFR enhancing Systemic and renal vasodilating Aldosterone inhibiting	Post-acute heart failure
C53	Preclinical studies	GC-B	Anti-fibrotic Anti-inflammatory Veno- and microcirculatory vasodilatory Minimal hypotension	HFrEF and HFpEF with low or normal BP

GC-A, guanylyl cyclase receptor A; GC-B, guanylyl cyclase receptor B; MasR, Mas receptor.

a 12 AA extension on the C-terminus, thus forming a 40 AA peptide (*Figure 4*).[89] Initial preclinical investigations demonstrated that MANP is a potent GC-A activator and is highly resistant to neprilysin and IDE degradation. This may contribute to its enhanced *in vivo* biological actions compared with ANP.[18,90] Indeed, MANP-mediated natriuresis, aldosterone suppression, and BP reductions in healthy canines were greater than the actions of ANP.[89] In canine and rodent models of hypertension and in a model of HF in hypertensive canines, the natriuretic, aldosterone suppressing and BP reductions were replicated as was a greater natriuresis than compared with BNP.[91–93] These cardiorenal properties were associated with activation of cGMP production *in vitro*, in GC-A overexpressing HEK293 cells, and elevations of urinary and plasma cGMP levels in healthy, hypertensive, and HF animals.

A clinical programme for the development of MANP is underway and IND from the FDA was issued. Three clinical indications are under investigation all characterized as relative ANP deficiency states. These include resistant (uncontrolled) hypertension, hypertension with metabolic syndrome and HFpEF in the setting of hypertension. Notably, Chen *et al.*[94] recently reported the results of an FIH study to define the BP, renal, aldosterone, and cGMP responses in a single ascending dose (SAD) study via SQ injection in hypertensive subjects in whom standard-of-care antihypertensive medications were discontinued for 14 days. A single SQ MANP injection resulted in an increase in plasma cGMP, which supports GC-A target engagement. Notably, both systolic and diastolic BP were reduced, and a dose-dependent natriuretic effect was also observed. A trend for a reduction of aldosterone was also reported.

Hypertension is a major mechanism for HF.[95] Studies have established that subsets of patients with HFrEF and HFpEF have low levels of ANP and BNP, especially in the setting of metabolic disease and hypertension.[63] In a preclinical model of HFrEF, we compared the activation of GC-A by MANP with the soluble guanylyl cyclase activator nitroglycerin (NTG). Both MANP and NTG decreased BP, cardiac filling pressures and activated cGMP production, yet only MANP increased GFR and

sodium excretion and reduced aldosterone.[92] Studies also recently reported that MANP is an inhibitor of aldosterone synthase further supporting this unique property of GC-A activation.[96] Currently, a study of safety, tolerability, cardiovascular, renal, aldosterone, and cGMP responses to a single dose of MANP is underway in HFpEF patients. This exploratory HFpEF study is supported by the NHLBI (ClinicalTrials.gov Identifier: NCT05279742). MANP's clinical indication(s) are uncontrolled hypertension and HFpEF in the setting of hypertension.

6.4 Designer natriuretic peptides in preclinical discovery

Advances in peptide engineering and increasing knowledge into the biology and subsets of HF pathophysiology have led to new designer NPs which target such subsets of HF. Here, we review three peptides which are in preclinical investigations for targeting three subsets of HF namely the CRS with AKI, post-acute HF, and chronic HFrEF and HFpEF with low or normal BP (*Table 2*).

6.4.1 CRRL269

CRRL269 is a 32 AA peptide that was created to be GC-A specific, but with less BP-lowering actions compared with ANP, BNP, or URO.[97] CRRL269 possesses renoprotective and enhancing properties such as diuresis, natriuresis, and increased GFR, while retaining the RAAS-suppressing properties with less hypotension. The mechanisms for its enhanced renal actions are thought to be related to increased resistance to degradation by renal proteases and RAAS suppression with enhanced sensitization of GC-A in the kidney. The mechanism for less hypotension is attributed to reduced arterial vasorelaxation and greater cardiomyocyte cAMP generation secondary to CRRL269 greater inhibition of cAMP hydrolyzing phosphodiesterases.[97]

A recent American Heart Association report stated that AKI with sustained functional and structural changes within the kidney is a key mechanism contributing to the CRS.[98] Importantly, the mechanism of AKI is increasingly recognized as involving cell death in the kidney, with a key role played by ANGII.[99,100] Despite the increasing prevalence of AKI with high mortality and high risk for HF, there are no FDA-approved drugs for AKI.

Given the renoprotective properties of CRRL269, Chen *et al.*[101] investigated the renocardiac protective and RAAS-suppressing actions of CRRL269 in a large animal model of ischaemic AKI which has high relevance to ADHF and the CRS. IV treatment with CRRL 269 increased plasma, urinary, and renal tissue cGMP, and enhanced renal blood flow, natriuresis, and diuresis, while preserving GFR *in vivo* in the absence of hypotension. A key mechanism of the more preserved BP found in this study was at least partially mediated by reduced intracellular Ca^{2+} suppression by CRRL269, compared to ANP and BNP, in VSMCs stimulated by ANGII.

RAAS activation in AKI may induce pathological renal and cardiac cellular remodelling and dysfunction, resulting in the development of CKD.[102] Studies reported that plasma ANGII was significantly increased in ischaemic AKI.[101] Notably, CRRL269 reduced plasma ANGII levels, supporting the potential of renal protection in AKI.

Further, studies have established that apoptosis is a central feature of AKI.[100] Recognizing the critical role of ischaemic–reperfusion (I/R)-induced apoptosis in renal tubular cells in AKI, an *in vitro* model of hypoxia/reoxygenation-mediated apoptosis in renal tubular cells was used to investigate the molecular mechanism of CRRL269's renal protective action. CRRL269 showed robust anti-apoptotic actions compared with ANP and BNP and this property was mediated by cGMP and cGMP-dependent protein kinase I (PKGI).[101] Furthermore, in renal tubular cells CRRL269 suppressed apoptotic pathway genes (e.g. caspase 7).

6.4.2 NPA7

In cancer and diabetes, a strategy in drug discovery is the development of dual receptor-acting therapeutics, in which a dual-acting peptide engages two independent signalling pathways resulting in superiority to single signalling pathway activation. This concept has been highly successful in HF with the small molecule, sacubitril/valsartan.

As previously stated, it is well established that GC-A mediates cardiorenal protection via its second-messenger cGMP.[7] There is also growing evidence that the Mas receptor (MasR) mediates anti-apoptotic, anti-inflammatory, vasodilatory, anti-thrombotic, and AT_1R antagonizing actions by activation by Ang 1–7 and its second-messenger cAMP.[103,104] The Ang 1–7/MasR axis also has cardiorenal protective actions in models of HF and diabetic nephropathy.[105,106] However, the development of MasR activating drugs has been challenging due to the rapid *in vivo* degradation of Ang 1–7.[107] NPA7 represents the first-in-class designer peptide that co-targets the MasR and GC-A, which was synthesized by replacing the 9 AA N-terminus of BNP with the MasR agonist Ang 1–7.[108] The goal was to bioengineer a bispecific drug that would possess greater renal vasodilating, natriuretic, diuretic, and cardiac unloading properties compared with either Ang 1–7 or BNP alone. Such as drug may have beneficial efficacy for the treatment of post-acute HF which is an unmet need as deficiencies in the endogenous levels of ANP and BNP (GC-A activators) and ANG1–7 (MasR activator) may exist. Indeed *in vitro* studies demonstrated that NPA7 increased the second messengers of both GC-A and MasR, cGMP, and cAMP, respectively. Moreover, in healthy dogs, NPA7 was biologically active and exerted sustained and potent cardiorenal actions beyond MasR or GC-A stimulation alone.[108] Blockade of the MasR attenuated the haemodynamic, natriuretic, and diuretic *in vivo* responses to NPA7, underscoring the favourable actions of NPA7 are partly mediated by the MasR.

Post-acute HF is associated with a high rate of rehospitalization and high mortality. To date, there is no approved drug for post-acute HF, although the cGMP producing soluble GC activator, vericiguat, reduced hospitalization in patients with chronic HF.[109] In contrast, in such a patient population, sacubitril/valsartan was not superior to valsartan alone.[110] As previously described, HF is characterized by a neurohumoral imbalance caused by a relative NP deficiency with excessive RAAS activation. Notably, a survival benefit has been reported in HF patients with higher levels of ANG1–7 compared with ANGII, underscoring the protective role of alternative RAS.[111] Thus, NPA7 takes a complementary approach to restore this neurohumoral imbalance by engaging the NPS together with the protective RAS, by direct pharmacological stimulation with a ligand that specifically targets both receptors, GC-A and MasR. Currently, studies are underway to define the renoprotective models in a large animal model of HF which mimics post-acute HF. Furthermore, studies are also ongoing to define the actions of SQ administration of NPA7 which would provide a chronic delivery strategy in post-acute HF trials.

6.4.3 C53

The development of novel therapies for the treatment of chronic HFrEF and HFpEF without reducing BP is also a high unmet need. As described above, experimental studies unravelled important biological roles of the CNP/GC-B/cGMP pathway in maintaining cardiac, renal, and vascular

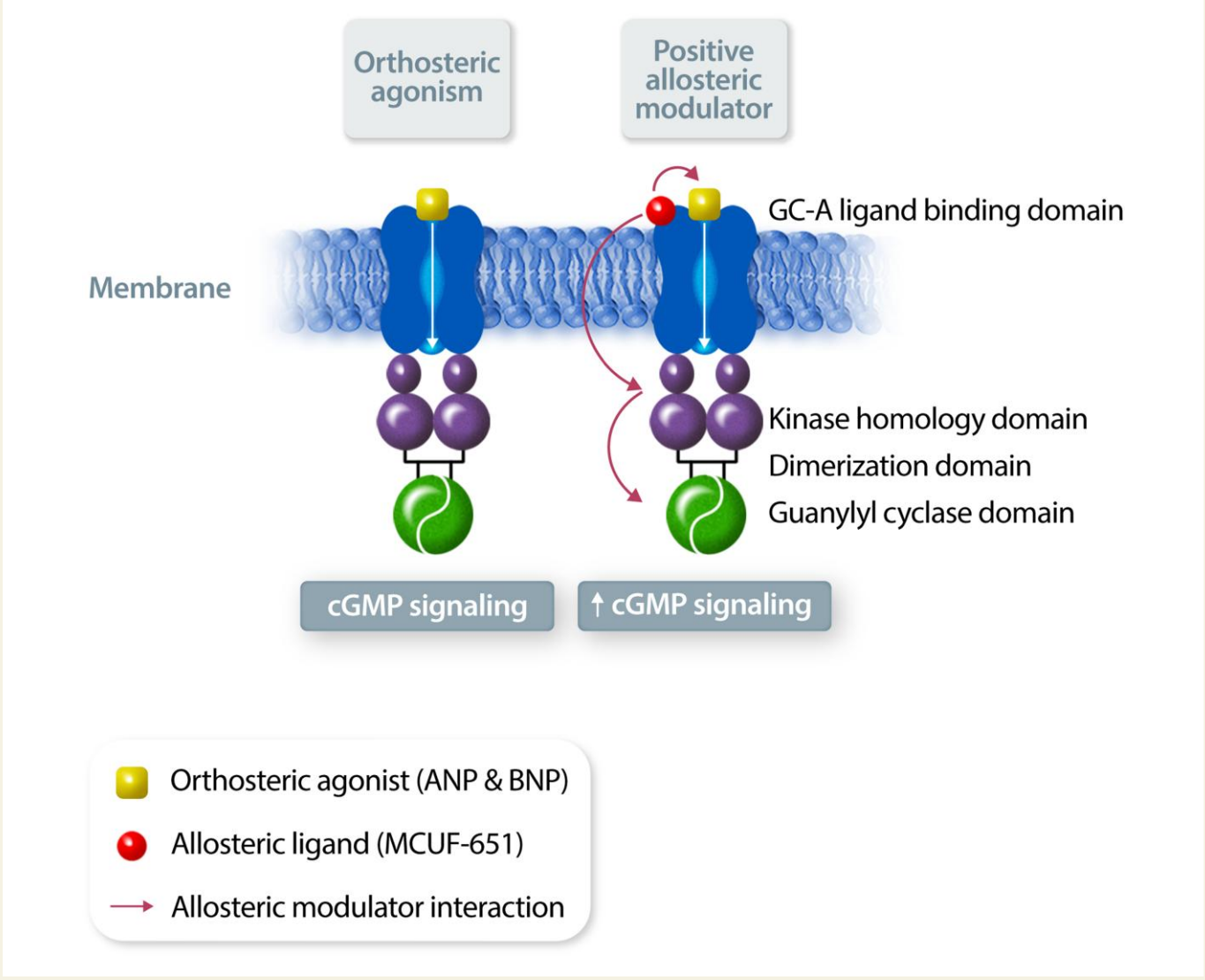


Figure 5 Orthosteric and positive allosteric modulation. NPs (square), such as ANP and BNP, would bind to the orthosteric site of GC-A inducing a conformational change that would lead to cGMP generation. GC-A-positive allosteric modulator (PAM), such as MCUF-651, would bind to topographically distinct site(s) on the receptor to increase the affinity and/or efficacy of orthosteric ligands to which enhances cGMP signalling.

structure and function, properties which are critical to HF drugs. Despite favourable experimental studies, the translation of CNP to clinical HF has been limited because of its rapid catabolism and low bioavailability.

C53 is a CNP analogue with a 31 AA extension on the N-terminus and was designed from our understanding of proCNP processing.[112] *In vitro* studies demonstrated that C53 attenuates TGF-β-stimulated proliferation of human cardiac and renal fibroblasts and suppresses the differentiation to myofibroblasts. In rats, acute infusion of C53 markedly elevated both plasma and urinary cGMP levels, when compared with CNP, consistent with an extended circulating half-life. These findings were supported by *in vitro* studies which demonstrated that the extended N-terminus of C53 markedly improved its resistance to degradation by neprilysin and reduced its interaction with the NP clearance receptor, NPR-C.[112] Moreover, in rats despite the elevation of plasma and urinary cGMP levels, C53 had no significant effect on GFR, natriuresis, diuresis, or BP, which is consistent with selective GC-B activation.

Currently, chronic administration of C53 is under preclinical investigation in experimental models of HFrEF and HFpEF. Based upon the protective anti-inflammatory, anti-fibrotic, myocardial, and microvascular properties of GC-B activation, both HFrEF and HFpEF may be ideal targets for chronic C53 administration, especially in HF patients with normal or reduced BP. Further additional areas of exploratory research that may benefit from the potent anti-fibrotic properties of C53, because of the presence of excessive pathological fibrosis, also include hypertrophic cardiomyopathy (HCM) and aortic stenosis.[113]

7. Small molecule GC-A enhancer for heart failure

7.1 MCUF-651

To date, designer NPs have advanced the furthest in terms of bioengineering and clinical development of NP therapeutics. Recently, Sangaralingham *et al.*[114] reported the discovery of MCUF-651, a small

molecule positive allosteric modulator (PAM), of the GC-A receptor. This discovery represents a new paradigm in GC/cGMP therapeutics, which is not only based on enhancing the GC-receptor with small molecules but also on receptor allostery. Here, the intracellular signalling of the endogenous or designer NP agonists is augmented by the binding of a second ligand (MCUF-651) to a topographically distinct (allosteric) site within GC-A (*Figure 5*).[115]

Accordingly, *in vitro* studies demonstrated that MCUF-651 potentiated ANP-mediated cGMP generation in HEK293 cells overexpressing the GC-A receptor and was devoid of cGMP generating activity in the absence of ANP. Moreover, MCUF-651 did not generate cGMP in HEK293 cells overexpressing the GC-B receptor in the presence or absence of CNP, thus supporting that MCUF-651 is a selective GC-A PAM. Binding analyses demonstrated that MCUF-651 enhanced the binding of ANP and BNP to GC-A and did not alter the binding of CNP to GC-B. In cultured human cardiomyocytes, renal proximal tubular cells, and adipocytes, cGMP levels increased dose-dependently with MCUF-651 in the presence of ANP. The biological, functional relevance was tested in cultured human cardiomyocytes. A 96 h exposure to TGF-β resulted in a significant increase in cardiomyocyte size and this hypertrophy was inhibited by treatment with ANP (100 pM) in the presence of MCUF-651, but not by treatment with ANP (100 pM) alone. Notably, *in vivo* pharmacokinetic studies demonstrated that MCUF-651 is bioavailable after oral administration and enhances the generation of cGMP by GC-A.[114] These findings confirm the druggability of the NPS with a small molecule and serves as a foundation to design and develop non-peptide molecules targeting the GC receptors for therapeutic benefit in patients with HF or with risk factors that contribute to the development of HF such as hypertension.

8. Oral delivery of designer natriuretic peptides

To date, chronic administration of peptides has relied on either SQ injections or use of infusion pumps. During the last few years, there have been significant advances in the oral delivery of peptides, as highlighted by Drucker in a recent review. These advances resulted in the approval of oral semaglutide, a glucagon-like peptide 1 (GLP-1) receptor activator, by the FDA.[78]

The feasibility of oral delivery of NPs was first demonstrated by studies of Cataliotti *et al.*[116] where a short, amphiphilic oligomer was attached to BNP. In healthy dogs, this modified peptide, CONJ-BNP, was rapidly absorbed after oral administration, increased plasma BNP and cGMP levels over 60 min, and reduced BP. In a follow-up study, a more advanced conjugated BNP, CONJ-BNP54, was orally administered once daily over 6 days in a canine model of ANGII-induced hypertension.[117] Here, CONJ-BNP54's BP-lowering action remained intact over the 6 days in association with cGMP generation, thus further demonstrating the feasibility and efficacy of oral NP delivery. Such pioneering studies support the strategy to employ more advanced oral peptide delivery technologies to administer designer NPs for HF.[78]

9. Challenges for natriuretic peptide therapeutics in heart failure

The short-term use of the new class of designer NPs in clinical studies has demonstrated safety and efficacy in cardiorenal protection. In addition, advances in solid-state peptide synthesis have provided an effective manufacturing of peptides which in the past has been met with high cost. Three major challenges however remain. The first is immunogenicity which must be followed closely especially as one moves into chronic studies. To date, no immune responses have been reported in toxicology studies of designer NPs, nor in human studies. However, surveillance for immunogenicity is a part of all clinical investigations with NP therapeutics. The high unmet need, yet a challenge for designer NP therapeutics, is the development of oral delivery for both compliance and safety. The pioneering work of Cataliotti *et al.* with oral modified BNP, and the recent successful development of an oral GLP-1 analogue by Novo Nordisk for patients with diabetes, establish that such a challenge for oral delivery of a designer NP can be overcome. Another important challenge is defining the interactions with other drugs often used in HF such as diuretics, beta-blockers, RAAS inhibitors, sGC activators, and sacubitril/valsartan. As HF therapeutics has taken a polypharmacy approach, the potential drug interactions may augment or attenuate the actions of a designer NP or of small molecules targeting the NPS, and thereby may alter the effective dose. Therefore, in an era of precision medicine, such interactions should be carefully evaluated and understood.

10. Summary and the future

The NP/GC/cGMP signalling pathway has emerged as possessing pleiotropic protective cardiorenal, endocrine, and metabolic properties. In HF, the endogenous cardiac NPS may be activated to counter-act the deleterious actions of RAAS. However, the compensatory response by the heart in HF is inadequate, with impaired production and/or the release of endogenous NPs with reduced biological activity. Thus, there exist therapeutic opportunities for the NPS in HF, especially in defined subsets of patients such as CRS with AKI, post-acute HF, and chronic HFrEF and HFpEF with reduced, normal, or elevated BP. Advances in NP engineering, small molecule discovery, and oral delivery technologies are paving the way towards laying the rationale, in specific HF groups or phenotypes, for novel precision medicine therapeutic opportunities targeting the protective NPS to reduce the growing health burden of human HF.

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Conflict of interests: S.J.S. is listed as an inventor on pending patents related to designer NP therapeutics, including CRRL408, filed by the Mayo Foundation for Medical Education and Research. H.H.C. is listed as an inventor on issued and/or patents related to designer NP therapeutics, including ANX042 (ASBNP.1), filed by the Mayo Foundation for Medical Education and Research. J.C.B. is listed as an inventor on issued and/or pending patents related to designer NP therapeutics, including cenderitide, ANX042 (ASBNP.1) and MANP, CRRL408, CRRL269 and NPA7, filed by the Mayo Foundation for Medical Education and Research. S.J.S. and J.C.B. are listed as inventors on pending patents related to small molecule GC-A enhancers, including MCUF-651, filed by the Mayo Foundation for Medical Education and Research and Sanford Burnham Prebys Medical Discovery Institute. S.J.S. and J.C.B. are listed as inventors on issued or pending patents, related to the use of CNP as a

potential biomarker, filed by the Mayo Foundation for Medical Education and Research. S.J.S. and J.C.B. are listed as inventors on a pending patent, related the *ex vivo* human therapeutic potency assay, filed by the Mayo Foundation for Medical Education and Research. MANP and MCUF-651 has been licensed to E-Star Biotech and AlloRock, respectively. S.J.S., H.H.C., and J.C.B. serve on AlloRock's scientific advisory board. All research is being conducted in compliance with Mayo Clinic conflict of interest policies. All other authors declared no conflict of interest.

Data availability

All data are from published papers and available in the papers cited.

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