Cyclic GMP modulating drugs in cardiovascular diseases: mechanism-based network pharmacology

Alexandra Petraina 1*, Cristian Nogales 1, Thomas Krahn 1, Hermann Mucke 2, Thomas F. Lüscher 3,4, Rodolphe Fischmeister 5, David A. Kass 6, John C. Burnett Jr 7, Adrian J. Hobbs 8†, and Harald H.H.W. Schmidt 1†

1Department of Pharmacology and Personalised Medicine, School for Mental Health and Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands; 2H.M. Pharma Consultancy, Enenkelstrasse 28/32, A-1160, Vienna, Austria; 3Royal Brompton & Harefield Hospitals, Heart Division and National Heart and Lung Institute, Guy Scadding Building, Imperial College, Dovehouse Street London SW3 6LY, United Kingdom; 4Center for Molecular Cardiology, Schlieren Campus, University of Zurich, Wagistrasse 12, CH-8092 Schlieren, Switzerland; 5INSERM UMR-S 1180, Faculty of Pharmacy, Université Paris-Saclay, F-92296 Châtenay-Malabry, France; 6Division of Cardiology, Department of Medicine, Ross Research Building, Rm 858, Johns Hopkins Medical Institutions, 720 Rutland Avenue, Baltimore, MD 21205, USA; 7Department of Cardiovascular Medicine, Mayo Clinic, 200 First St, SW, Rochester, MN 55905, USA; and 8William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, EC1M 6BQ, London, UK

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Abstract

Mechanism-based therapy centred on the molecular understanding of disease-causing pathways in a given patient is still the exception rather than the rule in medicine, even in cardiology. However, recent successful drug developments centred around the second messenger cyclic guanosine-3′-5′-monophosphate (cGMP), which is regulating a number of cardiovascular disease modulating pathways, are about to provide novel targets for such a personalized cardiovascular therapy. Whether cGMP breakdown is inhibited or cGMP synthesis is stimulated via guanylyl cyclases or their upstream regulators in different cardiovascular disease phenotypes, the outcomes seem to be so far uniformly protective. Thus, a network of cGMP-modulating drugs has evolved that act in a mechanism-based, possibly causal manner in a number of cardiac conditions. What remains a challenge is the detection of cGMPopathy endotypes amongst cardiovascular disease phenotypes. Here, we review the growing clinical relevance of cGMP and provide a glimpse into the future on how drugs interfering with this pathway may change how we treat and diagnose cardiovascular diseases altogether.

Keywords

Guanylate cyclase • Natriuretic peptides • Nitric oxide • Cyclic GMP • Biomarkers

1. Background

For decades, the number of approved drugs has been in decline, indicating fundamental problems with respect to the productivity and innovation in basic, translational, and industrial research.1 Potential reasons for this include, amongst others, the underlying concept of disease, which is mainly based on symptoms, an organ and its phenotypic function rather than on molecular pathways. Indeed, causal, mechanistic understanding of disease is still the exception and currently relevant primarily for monogenic diseases.2 Common and complex diseases are primarily treated based on their symptoms, on risk factors or markers; clearly, a low-precision approach evidenced by the high numbers needed to treat and low efficacy of currently available drugs.3–5 Cardiology is no exception to this, and given its many unmet needs, this represents one of the most important knowledge gaps in medicine.3

Therapeutic agents that modulate the second messenger cyclic guanosine-3′-5′-monophosphate (cGMP) seem to be one exception to this conceptual roadblock and may lead the way towards a different, mechanism-based approach to a variety of diseases using also the powers of big data, networks and systems medicine.6,7 cGMP modulators have
emerged as one of the most promising compounds in recent cardiovascular drug discovery. This may be because they do not act only symptomatically but, at least in a subset of suitable patients, target a disease mechanism rather than alleviating symptoms or modulating risk factors. In contrast, current cardiovascular treatments, such as renin–angiotensin–aldosterone system (RAAS) blockade do not follow a pathomechanistic approach. RAAS-blockers are not chosen as a therapy because in a patient up-regulation of RAAS has been measured, but solely symptomatically because RAAS blockade causes vasodilation. This is not a mechanism-based, causal therapy. The same holds true for other commonly used therapies, such as beta-blockers and calcium channel antagonists.

Cyclic GMP modulating drugs are used in a broad set of cardiovascular symptoms and conditions, such as angina, myocardial infarction, heart failure, pulmonary hypertension (PH), hypertensive crisis, and erectile dysfunction. Furthermore, preclinical evidence suggests benefit in ischaemic stroke. In addition, cGMP-related biomarkers, natriuretic peptides (NPs), are used to monitor heart failure patients.

In the cardiovascular system, the effects of cGMP are predominantly mediated by cGMP-dependent protein kinases and cGMP-regulated phosphodiesterases (PDEs) (Figure 1). Cyclic GMP appears to exert almost exclusively beneficial effects with a single overt limitation, vasodilation which in some patients may lead to hypotension and syncope, in particular in combination therapy. Therefore, cGMP increase leverages apparently only additional therapeutic gain, particularly in those cardiovascular conditions associated with a proven, i.e. mechanism-based, deficit in cGMP signalling. Clinically, this is achieved mainly by two approaches, either by (i) activating guanylyl cyclases to increase cGMP synthesis or by (ii) inhibiting relevant PDEs to inhibit cGMP breakdown. Future cGMP-centric strategies will most likely include combinations of different types of cGMP-modulating drugs and be increasingly guided by additional innovative plasma- or cell-based biomarker panels yielding powerful therapeutic and diagnostic (‘theranostic’) couples with cGMP-modulating drugs for cardiovascular precision medicine.

2. Cyclic GMP, a mechanism-based approach for cardiology

cGMP-modulating drugs are very promising in cardiovascular medicine and provide a broad clinical applicability. Indeed, cGMP-modulating drugs provide protective effects within the heart and vasculature by inhibiting vascular smooth muscle contraction and proliferation, suppressing

![Figure 1](Image)

**Figure 1** Classical, curated representation of cGMP signalling. Shown in green are the GCs and in yellow their positive regulators; NO produced by NOS for soluble GC and NPs (ANP, BNP, and CNP) for particulate GC. Negative regulators (cGMP metabolizing PDEs 1, 2, 5, 6, 9, 10, 11, and NPs degrading NEP) and pathophysiological conditions (oxidized/heme-free apo-sGC) are shown in pink. All clinically relevant cGMP-modulating drugs are shown in blue (bold for approved drugs and in italic for drugs under investigation): ARB, Angiotensin II receptor blockers; GC-As, GC-A stimulators; GC-A/Bs, GC-A/B stimulators; NEPi, neprilysin inhibitors; NOd, NO donors; NOSr, NOS recoupling nutraceuticals; sGCa, sGC activators; sGCS, sGC stimulators; PDE, PDE inhibitors. cGMP effector proteins (PKG and cyclic nucleotide-gated ion channels, CNG) and their substrates are shown in grey. cGMP can also inhibit some isoforms of the PDE enzyme family. In turn, this leads to an altered phosphoprotein profile, a decrease in intracellular calcium levels and sensitivity, and altered cGMP and cAMP levels (proffering crosstalk between cGMP and cAMP networks). GC-C is localized in intestines and GC-E and -F in the retina, thus not relevant to the context of this review.

Ang II, angiotensin II; Sta, heat-stable enterotoxin I STA.
platelet and leukocyte reactivity, and both anti-fibrotic and anti-hypertrophic actions. A second, probably even more promising aspect is the fact that dysfunctional cGMP formation and signalling appear to play direct pathomechanistic roles in cardiovascular disease. Genome-wide association studies have identified single nucleotide polymorphisms in genes encoding several components of this pathway to be correlated with cardiovascular diseases (Table 1). Thus, any up-regulation of cGMP has the potential to act and cure in a unique mechanism-based manner. Table 2 demonstrates cGMP-related drugs for therapeutic cardiovascular applications, either approved or under clinical investigation.

2.1 cGMPopathies

Cyclic GMP modulating drugs in cardiovascular diseases

| Gene          | Chromosome | Association with |
|--------------|------------|------------------|
| ANP (NPPA)   | 1          | A22, BP17, MetS24, VR178 |
| BNP (NPPB)   |            | BP17             |
| Furin (Pcsk3) | 15         | BP17, MI/CAD179   |
| eNOS (Nos3A) | 7          | BP180, CAD181, MetS19 |
| Npr1 (Npr1)  | 4           | BP12, VR178      |
| Pdeesa (Pde5A)| 4          | CAD18           |
| sgcx1 (Gucy1a3) | 4      | BP17,182, Pl183   |
| sgcx1 (Gucy1a3) | +        | MI/CAD21       |
| Cct7 (Cct7)  |            |                  |
| sgcx1 (Gucy1b3) | 4       | BP17,182        |

AF, atrial fibration; BP, blood pressure; CAD, coronary artery disease; CCT7, chaperonin containing TCP1 subunit 7; MetS, metabolic syndrome; MI, myocardial infarction; PH, pulmonary hypertension; VR, ventricular remodelling.

Table 2

| Gene | Chromosome | Association with |
|------|------------|------------------|
| ANP (Nppa) | 1 | A22, MetS24, VR178 |
| BNP (Nppb) | | BP17 |
| Furin (Pcsk3) | 15 | BP17, MI/CAD179 |
| eNOS (Nos3A) | 7 | BP180, CAD181, MetS19 |
| Npr1 (Npr1) | 4 | BP12, VR178 |
| Pdeesa (Pde5A) | 4 | CAD18 |
| Sgcx1 (Gucy1a3) | 4 | BP17,182, Pl183 |
| Sgcx1 (Gucy1a3) | + | MI/CAD21 |
| Cct7 (Cct7) | | |
| Sgcx1 (Gucy1b3) | 4 | BP17,182 |

AF, atrial fibration; BP, blood pressure; CAD, coronary artery disease; CCT7, chaperonin containing TCP1 subunit 7; MetS, metabolic syndrome; MI, myocardial infarction; PH, pulmonary hypertension; VR, ventricular remodelling.

Endothelial dysfunction characterized by NO dysregulation and inflammation coincides with reactive oxygen species (ROS) formation leading to cardiovascular disease states. Notably in heart failure, neurohumoral activation, secretion of inflammatory messengers, and altered shear stress lead to ROS generation that interferes with NO. The resulting endothelial dysfunction causes a further imbalance of NO and unphysiological ROS formation that worsens HF. Reduced PDK activity and cGMP concentrations, probably resulting from low NO bioavailability, are related to cardiomyocyte stiffness in the HF with preserved ejection fraction (HFpEF) myocardium. Higher levels of uncoupled eNOS and PDE9A were also shown in HFpEF myocardium. Elevated ANP levels have been associated with HF, but blunted responses to ANP infusion in HF patients indicate the possibility of down-regulation of ANP receptors or up-regulation of the NP-metabolizing receptor. In human failing hearts, guanylate cyclase (GC)-A in cardiomyocytes does not respond to ANP stimulation, and PDE1C and PDE5 levels are up-regulated.

Endothelial dysfunction, as a result of dysregulated ROS formation and inflammation, correlates with atherosclerosis. Increased activity of NADPH oxidase (as a source of superoxide) was associated with decreased endothelial vasorelaxations and increased atherosclerotic risk factors. In CAD patients, the oxidized form of sGC was increased and asymmetric-dimethyl-L-arginine (ADMA) levels associated with eNOS uncoupling. In hyperlipidaemia, cGMP modulators are unable to induce cardioprotective effects, suggesting a dysfunction downstream of the cGMP formation. In the same study, PKG activity is down-regulated in hyperlipidaemic rats as assessed by troponin I phosphorylation.

Nitric oxide is implicated in impaired vasodilation in hypertensive patients. Endothelial NO production by eNOS is decreased and systemic NO production by iNOS increased (resulting in hyperproduction of toxic NO levels) in patients with coronary heart disease (CHD) associated with hypertension; these effects are more expressed in CHD with hypertension compared to isolated CHD patients. In addition, diminished L-arginine transport has been proposed as a link from dysfunctional NO signalling to essential hypertension. In pulmonary arterial hypertension (PAH) patients, arginine levels in airway epithelial cells are inversely associated with pulmonary arterial pressures, while in pulmonary artery endothelial cells NO production is reduced and arginase activity higher.

Importantly, when moving from organ- and symptom-based to mechanistic disease definitions not all patients with a given clinical disease phenotype is expected to suffer from the same cause. cGMPopathy rather represents one endotype, and there will be others that could lead to a similar phenotype. NOX5-induced uncoupling of eNOS as a causal mechanism of age-related hypertension is a good example of this as it affects approximately only one in four or five patients with hypertension. Different endotypes of one phenotype may have different symptoms or comorbidities, which in multiscale modelling is used to identify the mechanism of unclear endotypes.

3. Drugs increasing cGMP generation

3.1 Nitric oxide and its receptors, GC-1 & GC-2

The traditionally defined ‘soluble guanylate cyclase (sGC)’, more recently termed guanylyl cyclases GC-1 and GC-2 (to differentiate them from the membrane-spanning, guanylyl cyclases, GC-A, GC-B, activated by
| Target | Mechanism | Compound | Indication | Status | Clinical trial identifier |
|---|---|---|---|---|---|
| Nitroglycerine | NO donor | Angina pectoris, Acute HF | Approved | NCT04155476 |
| | | Peripheral arterial disease | Phase I | NCT01864252 |
| | | Myocardial reperfusion injury | Phase III | NCT03813992 |
| | | Erectile dysfunction | Phase III | NCT03813992 |
| Isosorbide nitrates | NO | Angina pectoris, HF | Approved | NCT03451591 |
| SNP | | Lacunar stroke | Phase II/III | NCT03451591 |
| Molsidomine | | Angina pectoris, atherosclerosis | Phase IV | NCT01363661 |
| PETN | | Angina pectoris | Phase III | NCT03451591 |
| Nicorandil | | Angina | Approved | NCT01475123 |
| | | Coronary artery disease—end stage renal disease | Phase IV | NCT01475123 |
| | | Myocardial infarction | Phase IV | NCT02435797 |
| | | Myocardial infarction—percutaneous coronary intervention | Phase IV | NCT03445728, NCT03252665, NCT0465648, NCT04826497 |
| Sodium nitrite | | HFrEF | Phase II | NCT02918552 |
| | | HFpEF | Phase II | NCT02713126 |
| | | Pulmonary hypertension—HFpEF | Phase II | NCT03015402 |
| | | Metabolic syndrome—hypertension | Phase II | NCT01681810 |
| | | Hypertension | Phase I | NCT02527837 |
| | | Painful diabetic neuropathy | Phase II | NCT02412852 |
| Sodium nitrate | | Acute HF | Phase I/II | ACTRN12616000951459 |
| | | Pulmonary hypertension | Phase I | NCT03787082 |
| | | Pulmonary hypertension—HFpEF | Phase I | NCT02980068 |
| Potassium nitrate | | Angina | Phase II—negative | NCT02078921 |
| | | HFpEF | Phase II | NCT02840799 |
| | | HF | Phase I/II | NCT02797184 |
| | | Contrast-induced nephropathy | Phase II | NCT03627130 |
| | | Aortic valve stenosis | Phase II | NCT03483051 |
| Dietary nitrate | | Arrhythmia—HF | N/A | NCT03658174 |
| | | HF | Phase I/II | NCT01682356 |
| | | Obesity | N/A | NCT03545009 |
| | | Hypertension | N/A | NCT04755400 |
| | | Hypertension-induced target organ damage | Phase II | NCT03088514 |
| | | Peripheral arterial disease | Phase II | NCT02533733 |
| | | Chronic kidney disease | Phase IV | NCT03826147 |
| | | Angina | Phase II | NCT02529189 |
| | | Ischaemic stroke | Phase II | NCT02838901 |
| | | Erectile dysfunction | N/A | NCT04116060 |
| HNO donor | | HFpEF | Phase II | NCT03357731 |
| CXL-1427 | (Cimlanod) | HF | Phase II | NCT01096043 |
### Table 2  Continued

| Target | Mechanism | Compound | Indication | Status | Clinical trial identifier |
|--------|-----------|----------|------------|--------|--------------------------|
| Arginine | Recoupling | Preeclampsia | Hypertension | Phase III | NCT03363348 |
| Arginine | Recoupling | Reperfusion injury after lower limb bypass | Peripheral artery disease | N/A | NCT01682889 |
| NOS | Recoupling | Peri-operative cardiovascular complications | N/A | N/A | NCT0143815 |
| Arginine | Recoupling | Peripheral arterial occlusive disease | N/A | N/A | NCT04239482 |
| Arginine | Recoupling | Insulin sensitivity | N/A | N/A | NCT04239482 |
| Citrulline | | Sickle cell disease | Chronic kidney disease | Phase IV | NCT02363348 |
| Citrulline | | Cardiopulmonary bypass during heart surgery | Phase III | Phase IV | NCT03352444 |
| Citrulline | | Peripheral artery disease | N/A | N/A | NCT02521220 |
| H4Biopterin | Inhibition | Sickle cell disease | Phase II | NCT00445978 |
| H4Biopterin | Inhibition | Peripheral artery disease | Phase I | NCT04800692 |
| H4Biopterin | Inhibition | Chronic kidney disease | Phase II | NCT02947750 |
| H4Biopterin | Inhibition | HFrEF | Phase I | NCT03136029 |
| H4Biopterin | Inhibition | Hypertension | Phase I | NCT00208780 |
| GC-1/2 | Stimulation | Pralicigut | Type II diabetes-hypertension | Phase II | NCT03091920 |
| GC-1/2 | Stimulation | HFrEF | Phase II—negative | NCT03547583 |
| GC-1/2 | Stimulation | Diabetic nephropathy | Phase II—negative | NCT03217591 |
| GC-1/2 | Stimulation | Aortic valve calcification | Phase II | Phase II | NCT02481258 |
| Ataciguat | Activation | Peripheral arterial disease | Phase II—negative | NCT00443287 |
| Cinaciguat | | HF | Terminated-Phase II | NCT01064037 |
| Cinaciguat | | HF—renal impairment | Phase II | N/A | NCT04507061 |
| Cinciguat | | Diabetic retinopathy | Phase II—negative | NCT04722991 |
| Runcaciguat | | Chronic kidney disease | Phase II | NCT04507061 |
| HFrEF, HfPef | | LCZ696=Sacubitril + Valsartan | HFrEF, HfPef | Approved | NCT04637152 |
| HFrEF, HfPef | | Resistant hypertension | Phase II | NCT04173511 |
| HFrEF, HfPef | | Myocardial infarction | Phase IV | NCT04342351 |
| HFrEF, HfPef | | Type II diabetes | Phase II | NCT03744975 |
| HFrEF, HfPef | | Atrial fibrillation—cardiac remodelling | N/A | NCT03791723 |
| Carperitide | | Acute HF | Approved | NCT01661634 |
| Carperitide | | Acute HF | Phase III—negative | NCT01661634 |
| Carperitide | | Acute HF | Approved | NCT01661634 |
| Nesiritide | GC-A | Hypertension | Phase I/II | NCT02608996 |
| Nesiritide | GC-A | Diabetes | Phase I | NCT03234751 |
| ANX-042 | GC-A/B | Cardiorenal syndrome | Phase I | NCT03019653 |
| MANP | GC-A/B | Hypertension, metabolic syndrome | Phase I | NCT03781739 |
| MANP | GC-A/B | Hypertension | Phase I | NCT04542681 |
| Cenderitide | GC-A/B | HF | Phase II | NCT02359227 |
| Cenderitide | GC-A/B | HF—renal impairment | Phase II/III | NCT02603614 |
| Cenderitide | GC-A/B | Myocardial infarction | Phase I | NCT02071602 |
| Cenderitide | GC-A/B | HFrEF | Phase II | NCT04318145 |
| Vinpocetine | PDE1 | Ischaemic stroke | Phase II/III | NCT02878772 |
| Lenrispodun (ITI-214) | PDE1 | HF | Phase II/III | NCT03387215 |
| Lenrispodun (ITI-214) | PDE1 | Parkinson’s disease | Phase II/III | NCT03275046 |
NPs), is a heterodimeric haemoprotein comprised of one of two alpha subunits ($\alpha_1$ or $\alpha_2$) and a beta subunit ($\beta_1$). An N-terminal pocket binds Fe(II) haem via a proximal histidine and thereby confers sensitivity to NO. Binding of NO cleaves the proximal histidine-Fe(II) haem bond and induces a structural shift that activates the catalytic site converting GTP into cGMP. Inappropriate formation of ROS, in particular superoxide, can interfere with NO-cGMP signalling in at least three ways: (i) by chemically scavenging NO; (ii) by uncoupling NO synthase (NOS); or (iii) by oxidizing the haem group within GC-1/2 from Fe(II) to Fe(III) eventually resulting in heme-deficient apo-GC. The latter is not only insensitive to NO but also prone to rapid degradation.

Therapeutically, three avenues are clinically promising for reinstating or augmenting NO-GC-1/2 signalling: (i) repairing or replacing NO synthesis; (ii) sensitizing GC-1/2 to lower levels of NO by allosteric modulators, so-called sGC activators; or (iii) re-activating NO-insensitive, haem-free apo-GC by haem-mimetics, so-called sGC stimulators, which also prevent enzyme degradation.

### 3.1.1 Repairing or replacing NO synthesis

Recoupling NOS, by dietary supplementation of its redox-sensitive cofactor tetrahydrobiopterin or its substrate L-arginine, is pre-clinically effective. So far, there are, however, no clinical trials with positive outcomes to demonstrate the efficacy of such a nutraceutical approach. Therapeutically, NO substitution with so-called NO-donor or nitrovasodilator compounds has the longest history (e.g. in angina, heart failure), but also limitations, such as pharmacokinetic and pharmacodynamic tolerance, which requires therapy-free intervals to regain nitrate sensitivity. According to ESC/AHA guidelines, the use of sodium nitroprusside, chemically an NO donor, is limited to i.v. application in hypertensive emergencies e.g. as first-line treatment in acute cardiogenic pulmonary oedemas, and in acute HF as second-line therapy.

### Table 2

| Target | Mechanism | Compound | Indication | Status | Clinical trial identifier |
|--------|-----------|----------|------------|--------|----------------------------|
| Milrinone | HF | Pulmonary hypertension | Approved | NCT04484675 |
|  |  | Ischaemic heart disease | N/A | NCT04153383 |
|  |  | Anti-thrombotic | Approved | NCT01261234 |
|  |  | Restenosis | N/A | NCT02983214 |
|  |  | Ischaemic, atherosclerotic events in type II diabetes | Phase IV | NCT03248401 |
| Cilostazol | Inhibitor | Acute HF | Approved | NCT02933788 |
|  |  | Systolic HF | Phase IV | NCT03764722 |
|  |  | Pulmonary hypertension-HFpEF | Phase II | NCT03624010 |
|  |  | Aortic stenosis- HF | Phase IV | NCT04573049 |
|  |  | Myocardial infarction—acute HF | Phase IV | NCT03189901 |
|  |  | Cardiogenic shock | Phase III | NCT04020263 |
|  |  | Acute HF | Approved | NCT00051285 |
|  |  | HF | Terminated-Phase III | N/A |
|  |  | Erectile dysfunction, pulmonary hypertension | Approved | NCT03686306 |
|  |  | Peripheral artery disease | Phase III | NCT01616381 |
|  |  | HF—pulmonary hypertension | Phase III | NCT03855332 |
|  |  | Small vessel cerebrovascular disease | Phase II | NCT04356716 |
|  |  | Ischaemic retinal disorders | Phase II | NCT02061989 |
|  |  | Cardiomyopathy—renal impairment | Phase III | NCT02058095 |
|  |  | Cerebral small vessel disease | Phase II | NCT02801032 |
|  |  | Diabetic cardiomyopathy | Phase IV | NCT01803828 |
|  |  | Erectile dysfunction | Approved | NCT01649739 |
|  |  | Pulmonary hypertension | Phase IV | N/A |
and haemodynamic profile in acute decompensated HF. Nicorandil, a nicotinamide-nitrate ester and K+ channel opener, is suggested as a second-line antianginal drug for patients with chronic coronary syndromes in Europe but not approved in USA. In addition to nitrate tolerance, a general concern is that under conditions of elevated ROS levels, NO donors may lead to unwanted reactive nitrogen species and endothelial dysfunction.

Two more targeted and mechanism-based strategies circumvent these shortcomings and risks i.e. sGC stimulators and sGC activators. Despite their very similar sounding names, they have distinct targets, i.e. Fe(II)haem-containing GC-1/2 and apo-GC-1/2, respectively. Importantly, both enhance cGMP synthesis independently of modulating NO levels and are thus devoid of tolerance.

3.1.2 sGC stimulators
These compounds interact with an allosteric site to sensitize (FeII)haem containing GC-1/2 for NO. If tissue levels of NO are low, this will result in a mechanism-based ‘recovery’ of a physiological cGMP response. However, if levels of NO are high, these compounds have limited or no additional effect on cGMP.

Riociguat (BAY 63–2521) was the first registered sGC stimulator approved for use in PH, i.e. chronic thromboembolic pulmonary hypertension and PAH. No evidence-based first-line therapy is suggested for PH, but riociguat is one of the initial monotheories that can be chosen according to ESC/CHEST guidelines. However, following the early termination of the phase II RISE-IIP trial because of serious adverse events, riociguat is not suggested to patients with PH associated with idiopathic interstitial pneumonia. Riociguat was also evaluated in PH associated with left systolic heart failure, and, despite not meeting the primary endpoint of change in mean pulmonary artery pressure (mPAP), it had favourable effects on secondary outcomes. The DILATE-1 trial tested riociguat in patients with HFP EF and PH; stroke volume and cardiac index were increased, systolic blood pressure and right ventricular end-diastolic area decreased, but there was no significant change on peak decrease in mPAP. At the moment, riociguat is under investigation for its long-term treatment in PH associated with HFP EF (NCT02744339).

Vericiguat (BAY-1021189) reached the primary outcome in reducing cardiovascular mortality or hospitalization for HF in a Phase 3 clinical trial for HFrEF (VICTORIA) and recently received approval in USA. It was also further evaluated in a phase IIb HFP EF trial (VITALITY-HFPEF) where it failed to improve the quality of life (physical limitation score of the KCCQ), which was the previously suggested beneficial outcome in phase IIb SOCRATES-PRESERVED.

Another sGC stimulator with promising effects in an animal model of cardiorenal failure, praliciguat (IW-1973), showed favourable trends in metabolic and hemodynamic variables in patients with type 2 diabetes (T2D) and hypertension. However, it failed to reach the primary endpoints of improved peak rate of oxygen consumption and reduction in albuminuria in Phase 2 trials for HFP EF and diabetic nephropathy, respectively.

A shortcoming of all these trials still is that they stratified patients purely on clinical grounds and did not use biomarkers to identify HFP EF and HFrEF patients with a mechanistic endotype indicating cGMP dysregulation. By failing to do so, potential benefits in some patients may have been diluted through non-responders with different underlying pathomechanisms. Of note, the terms HFP EF and HFrEF are purely descriptive overarching terms, recently complemented by Heart Failure with mid-range or intermediate ejection fraction (HFmrEF or HFmEF).

3.1.3 sGC activators
These molecules specifically bind to the NO-insensitive, haem-free or oxidized apo-GC-1/2. Large molecules, such as cinaciguat (BAY58–2667), but not the smaller ataciguat (HMR1766) binding the oxidized form, occupy the empty haem site and prevent its ubiquitination and proteasomal degradation, thereby both activating and stabilizing apo-sGC. However, clinical phase II trials (COMPOSE programme) with cinaciguat in patients with acute heart failure had to be stopped prematurely due to severe hypotension. Moreover, the safety of ataciguat (HMR1766) has been evaluated in patients with moderate aortic valve stenosis (NCT02049203) and efficacy in patients with aortic valve calcification (NCT02481258) and peripheral arterial disease (NCT00443287); however, ataciguat’s development was discontinued. A novel compound with improved physicochemical and pharmacokinetic characteristics, runcaciguat, is now investigated in chronic kidney disease and diabetic retinopathy.

3.2 NPs and their GC-coupled receptors
The second cGMP forming family is plasma membrane-spanning GCS, often referred to as particulate GCS due to their subcellular localization in the particulate fraction. They comprise seven members (GC-A to GC-G), of which two, GC-D and GC-G, are pseudogenes and three, GC-C, GC-E, and GC-F, are—as far as we know—not relevant for the cardiovascular system. This organ- and function-based GCS compartmentalization is further confirmed in silico (Figure 2). Here, we make use of experimentally validated protein–protein interaction (PPI) data from the Integrative Interactive Database (IID). Starting from GC-coupled receptors, we look at their direct protein interactions in IID to build the first neighbour PPI network. After pruning the network from highly connected but non-relevant protein interactions, four different subnetworks or signalling modules are extracted: (i) sGC module, (ii) ANP receptors module, (iii) intestinal GC module, and (iv) retinal GCs module. GC-A and GC-B are homodimers containing an N-terminal extracellular ligand-binding domain for NPs. They are therefore also termed NP receptors NPR-A and NPR-B, respectively.

Humans express four types of NPs, atrial (ANP), brain (BNP), and C-type natriuretic peptide (CNP) and urodilatin. Each activate GC-A; CNP is the sole endogenous GC-B agonist. Via GC-A/B, NPs have a wide range of cardio- and vaso-protective effects, i.e. natriuresis, diuresis, inhibition of vasoconstriction, as well as anti-hypertrophic, anti-fibrotic and anti-proliferative effects and possibly also metabolic actions, such as lipolysis and browning of adipocytes. NPs bind to another NP receptor, natriuretic peptide clearance receptor (NPR-C), which has no GC activity and is responsible for NPs clearance from the circulation. However, NPR activation of NPR-C plays a crucial role in cardiac function and vascular homeostasis.

Elevated NP levels are also disease biomarkers, in particular in heart failure. Paradoxically though, increased expression and release of NPs does not necessarily translate into enhanced activation of the particulate GC-cGMP pathways. Instead, there appears to be a disconnect. In heart failure, proBNP, the precursor of mature BNP, is the predominant circulating form and lacks significant GC-A activating properties compared to BNP. More recently, studies have revealed the presence of glycosylation of ANP, resulting in a molecular form with reduced GC-A activation. The presence of altered molecular forms of ANP and BNP with...
reduced cGMP production supports the use of native and designer synthetic NPs to rescue these NP structural abnormalities. Such a hormone replacement strategy is also underscored by the presence of an ANP deficiency in human heart failure due to either reduced production and/or increased peptide degradation. Therefore, even when plasma levels of NPs are elevated in heart failure and other conditions, pharmacological GC-A/B stimulation may still be beneficial. Three therapeutic approaches to enhance NP signalling have entered the clinic; natural peptides, such as neseritide or carperitide, designer peptides, such as uralitide, and molecules that inhibit peptide breakdown via neutral endopeptidase (NEP), such as sacubitril.

3.2.1 Recombinant and designer NPs

The clinical utility of GC-A/B-cGMP stimulation was first examined with recombinant ANP, carperitide, in acute heart failure, but its impact on in-hospital mortality and length of hospitalization was inferior to nitrates. LASCAR-AHF now tests the long-term effects of carperitide in acute HF. Despite the lack of sufficient evidence, carperitide is used in Japanese practice as second-line treatment in acute HF. In the j-WIND trial, recombinant ANP decreased infarct size and improved ejection fraction in patients with myocardial infarction undergoing percutaneous coronary intervention but had no effect on in-hospital mortality.

Another NP, a synthetic form of urodilatin, ularitide, neither affected a clinical composite endpoint nor cardiovascular mortality in patients with acute HF. Similarly, the recombinant BNP, nesiritide, despite a small change in dyspnoea, neither improved all-cause death nor re-hospitalization for HF in patients with acute, decompensated HF. Even worse, a meta-analysis associated the use of nesiritide to an increase in the short-term risk of death in such patients. This perhaps provides a warning that excessive GC-A activation may be detrimental due to significant hypotension that may compromise renal function and lead to sympathetic activation, both unfavourable events in patients with heart failure. Currently, it is considered a second-line intravenous vasodilator for acute HF in Europe and USA.

An alternative strategy has been the development of ‘designer’ NPs, which aim to combine beneficial effects of different endogenous peptides. CD-NP, i.e. cenderitide (CD-NP), is a modified CNP with 15 additional amino acids at the C-terminal tail of DNP (a related peptide identified in the venom of the green mamba, Dendroaspis angusticeps). The rationale behind this combination is to promote the vasodilator and anti-fibrotic properties of C-type natriuretic peptide (at least in part via GC-B stimulation), with the natriuretic properties of DNP (which stimulates GC-A but is thought to avoid a dose-limiting hypotension). Both these pharmacodynamics as well as safety, i.e. absence of hypotension, were established in stable HF patients. ANX-042, a peptide designed based on an alternative spliced variant of BNP, is currently under investigation as a non-hypotensive drug in cardiorenal syndrome (NCT03019653). Beyond heart failure, the designer ANP-analogue (MANP) was engineered as a novel ANP mimetic whose biological properties of natriuresis, blood pressure-lowering, and aldosterone suppression are greater than ANP. This analogue retains the 28 amino acids of
3.2.2 NEP inhibitors

NEP (also known as neprilysin) is a membrane-bound metallopeptidase responsible for the breakdown of many vasoactive mediators, including NPs, but also glucagon, bradykinin, oxytocin, substance P, angiotensin II, endothelin, and beta-amyloid. Clinically, however, NEP inhibitors (NEP) have little or no effect on blood pressure despite significantly elevated plasma NEP concentrations. This paradox was attributed to the fact that NEP metabolizes both vasodilating (e.g., NPs, bradykinin) and vasoconstricting (e.g., angiotensin II and endothelin) peptides, thereby possibly outweighing any hemodynamic benefit.

As a result, drug development in this area focused on a combined blockade of NEP and angiotensin-converting enzyme (ACE) to prevent the accumulation of pro-hypertensive angiotensin II—leading to the so-called vasopeptidase inhibitors. However, in heart failure, the vasopeptidase inhibitor, omapatrilat, did not meet its primary endpoint of all-cause death or hospitalization for HF vs. enalapril but was associated with an increased incidence of angioedema (likely because both NEP and ACE are involved in the degradation of bradykinin). In hypertensive patients, the effect of omapatrilat on systolic blood pressure change and use of adjunctive antihypertensive therapy exceeded that of an ACE inhibitor alone, but again at the expense of a higher incidence of angioedema. Accordingly, omapatrilat did not make it to its clinical use.

Co-crystallizing the NEP1-sacubitril with the angiotensin II type 1 receptor blocker, valsartan, in a one-to-one molar ratio as LCZ696, jointly termed an angiotensin receptor-neprilysin inhibitor (ARNI), was more successful than valsartan in reducing diastolic blood pressure in hypertensive patients, with no reports of angioedema. The rationale was to avoid the double hit on bradykinin breakdown and angioedema by blocking angiotensin II type 1 receptors rather than inhibiting ACE. In HFrEF patients, sacubitril-valsartan reduced the risk of cardiovascular death and HF hospitalization more effectively than the ACE inhibitor, enalapril (PARADIGM-HF). PARAGON-HF compared sacubitril-valsartan vs. valsartan alone in HFrEF, but the primary outcome of total hospitalizations and death from cardiovascular causes did not differ. A high heterogeneity within the HFrEF population and the definition of HFrEF itself might be the underlying explanations of the failure of PARAGON-HF. Indeed, sacubitril-valsartan was beneficial in a subgroup with lower ejection fraction, a patient population more likely to represent early HFrEF rather than HFrEF. The protective effect in women remains unclear and warrants further investigation. The abovementioned studies have led LCZ696 to get FDA approval for HFrEF and also very recently for HFrEF patients with stronger evidence for those with below-normal LVEF. LCZ696 is recommended to replace ACE inhibitor as first-line treatment for HFrEF ambulatory symptomatic patients despite optimal therapy with ACE inhibitor, beta-blocker and a mineralocorticoid receptor antagonist according to ESC/AHA guidelines.

In addition, a meta-analysis showed a potent antihypertensive effect of sacubitril-valsartan vs. valsartan alone or olmesartan in elderly hypertensives.

4. Drugs preventing cGMP breakdown

In addition to enhancing cGMP production, PDE inhibitors can exert, in principle, similar effects by inhibiting cGMP degradation. However, therapeutic exploitation of PDE inhibition has not been as great as one might have anticipated. A total of 11 superfamilies of PDE isoforms are present at different subcellular localizations, thereby targeting different cGMP (or cAMP) enzymatic sources and pools. With respect to cGMP, especially PDE1, 2, 3, 5, and 9 have been implicated in cardiovascular disorders.

4.1 PDE5

Sildenafil and tadalafil are used in erectile dysfunction, as first-line treatments in Europe and USA, and in PH; among the initial treatments that can be chosen since there are not available head-to-head comparisons between compounds according to ESC/CHF guidelines. Sildenafil also improved peak oxygen uptake in PH due to HFrEF, and pulmonary pressure and right ventricular function in PH due to HFrEF. It showed beneficial effects on glycometabolic control and P-selectin in T2D. In HFrEF, sildenafil improves left ventricular (LV) diastolic function and cardiac geometry, while in diabetic cardiomyopathy benefits LV contractile function. In another use-extension trial in HFrEF, sildenafil showed no improvement in exercise capacity or clinical status. However, in HFrEF, cGMP concentrations are down-regulated due to low NO bioavailability, while sildenafil minimally increases plasma cGMP; thus, PDE5 inhibition would not be expected to represent an effective mechanism-based approach whilst the cGMP dysfunction most likely comes from a source different from the targeted one.

4.2 PDE3

The PDE3 inhibitor milrinone is licenced in Europe and USA for acute HF in its intravenous form as second-line treatment, while oral milrinone was associated with increased all-cause and cardiovascular mortality in severe chronic heart failure. Another PDE3 inhibitor, cilostazol, has antithrombotic properties and, as such, has been under investigation for its antiplatelet effects in T2D (NCT02983214, NCT03248401, NCT02933788). In T2D patients with symptomatic lower extremity artery disease, cilostazol reduced the incidence of acute ischaemic stroke/transient ischaemic attack, acute myocardial infarction, and vascular causes-associated death. Moreover, in T2D with carotid atherosclerotic plaques, it diminished the carotid plaque progression. This benefit can be explained mechanistically by a crosstalk between cGMP and cAMP, where cAMP-specific PDE3 is inhibited by cGMP through direct competition at the catalytic site. Thus, some effects of cGMP, e.g., in platelets, are likely to be mediated at least in part via the cAMP-PKA axis. More recently, PDE3 inhibition was explored in HFrEF, focusing on a new extended-release version of milrinone. This small pilot study showed a safe profile and improved quality of life in HFrEF patients.

The inotrope-dilator molecule levosimendan is used in 60 countries outside the USA as second-line treatment for acute HF and, in addition to its calcium-sensitizing properties, also inhibits PDE3, where cAMP-specific PDE3 is inhibited by cGMP through direct competition at the catalytic site. Thus, some effects of cGMP, e.g., in platelets, are likely to be mediated at least in part via the cAMP-PKA axis. More recently, PDE3 inhibition was explored in HFrEF, focusing on a new extended-release version of milrinone. This small pilot study showed a safe profile and improved quality of life in HFrEF patients. The inotrope-dilator molecule levosimendan is used in 60 countries outside the USA as second-line treatment for acute HF and, in addition to its calcium-sensitizing properties, also inhibits PDE3, where cAMP-specific PDE3 is inhibited by cGMP through direct competition at the catalytic site. Thus, some effects of cGMP, e.g., in platelets, are likely to be mediated at least in part via the cAMP-PKA axis. More recently, PDE3 inhibition was explored in HFrEF, focusing on a new extended-release version of milrinone. This small pilot study showed a safe profile and improved quality of life in HFrEF patients. The inotrope-dilator molecule levosimendan is used in 60 countries outside the USA as second-line treatment for acute HF and, in addition to its calcium-sensitizing properties, also inhibits PDE3, where cAMP-specific PDE3 is inhibited by cGMP through direct competition at the catalytic site. Thus, some effects of cGMP, e.g., in platelets, are likely to be mediated at least in part via the cAMP-PKA axis. More recently, PDE3 inhibition was explored in HFrEF, focusing on a new extended-release version of milrinone. This small pilot study showed a safe profile and improved quality of life in HFrEF patients.
4.3 PDE9A

PDE9A is the most selective cGMP-hydrolysing PDE of the superfamily. In 2015, a study performed in mice demonstrated a role in a model of cardiac pressure-overload, with both global genetic deletion and treatment with a selective PDE9 inhibitor reducing hypertrophy and fibrosis while improving cardiac function. The study established a close linkage of PDE9A with the regulation of cGMP generated by NP (rather than nitric oxide) signalling. In a recent study in mice, the PDE9 inhibitor CRD-733 improved HF characteristics; human trials in HF using CRD-733 are now underway.

4.5 PDE10A

PDE10A is a dual cAMP/cGMP PDE. In a recent study in mice, PDE10A inhibition with TP-10 improved pathological cardiac remodelling. PDE10A inhibition has been clinically tested in schizophrenias and Huntington’s disease, proving that it is a safe target for drug treatment and a potential therapeutic option for diseases related to cardiac remodelling.

5. Network pharmacology

As indicated above, cGMPopathies can emerge from different dysfunctions within cGMP formation, breakdown or signalling. Network medicine analysis, however, shows that the specific cGMP source matters. PPI networks of validated seed genes suggest that cGMP signalling is segregated into modules. These modules are likely to define the therapeutic (and diagnostic, see below) targets. Thus, NP analogues may not necessarily compensate for a loss of GC-1/2 function, while vice versa, sGC stimulators may not compensate for a loss of GC-A or GC-B-mediated cGMP production; a phenomenon exemplified in experimental heart failure.

A complex disease mechanism is comprised of a protein network rather than being definable by a single target protein. Within these network modules, the specific source of cGMP matters, as they are not interchangeable. Moreover, another important therapeutic option emerges from that i.e. network pharmacology. A dysfunctional multi-protein network is more likely to be remedied to a more physiological interchangeability. Moreover, another important therapeutic option emerges from that i.e. network pharmacology. Thus, PDE9A inhibition reduces hypertrophy and fibrosis while improving cardiac function.

6. How to diagnose cGMPopathies and stratify patients?

The missing link between cardiovascular phenotypes and cGMP-modulating treatments are mechanism-based biomarkers. Such tools would identify the patients that have a cGMPopathy and also the specific part of the pathway that should be targeted. Therefore, who would benefit from a cGMP therapy and which cGMP-targeting drug or drugs combination to choose remain unknown. Here, we review the current cGMP-related biomarkers and their applications. In principle, three approaches exist to assess endogenous cGMP signalling in patients: (i) cGMP itself, (ii) cGMP-PKG-dependent protein phosphorylation (e.g. of the vasodilator-stimulated phosphoprotein, VASP); or (iii) levels of endogenous GC stimulators (NO- or NP-related). Of clinical relevance, so far, are only circulating NPs and phospho-VASP (Figure 4).

6.1 cGMP

cGMP has been used to monitor drug-induced increase as proof of target engagement e.g. with ARNIs, designer NPs, and PDE-5 inhibitors. However, variation in cGMP concentrations between individuals has hindered its use as a biomarker for primary diagnosis.

6.2 P-VASP

P-VASP was introduced two decades ago as a new biomarker able to monitor the vascular NO/cGMP/PKG signalling. In principle, cell-based assays could be suited to detect defective endogenous cGMP signalling, e.g. via lower than normal phosphorylation of VASP or other PKG target proteins. However, both the phosphorylation and dephosphorylation kinetics would require extremely reproducible procedures with respect to blood collection, work-up and analysis. So far, this has prevented the establishment of basal P-VASP levels as a biomarker. In contrast, P-VASP assays are clinically established to assess drug
responses, e.g. to predict responders and non-responders to antiplatelet drugs to reduce major cardiovascular and cerebral events.\textsuperscript{151,152} P-VASP responses to sGC activators have been used to detect a higher apo-GC-1/2/Fe(II)GC-1/2 ratio in CAD patients,\textsuperscript{37} which could be used for mechanism-based therapy in patients with elevated apo-GC-1/2 levels.

### 6.3 NPs and soluble neprilysin

Each of the NPs has been proposed as a predictive biomarker for cardiovascular diseases or to guide cardiovascular therapy. The best characterized is BNP and its N-terminal fragment post-processing NT-proBNP. The lack of NT-proBNP degradation by NEP and analogous enzymes makes it superior to BNP for monitoring patients. Plasma levels of BNP, NT-proBNP, and mid-regional pro-atrial natriuretic peptide (MR-proANP) have been used to aid the heart failure diagnosis as ‘rule-out’ tests, excluding significant cardiac dysfunction.\textsuperscript{10} Of note, the 2016 ESC Guidelines on the Management of Acute and Chronic Heart Failure recommend the use of BNP and NT-proBNP for the diagnosis of HF.\textsuperscript{10} Furthermore, BNP levels are associated with poor prognosis in stroke,\textsuperscript{153} NT-proBNP levels in hypertrophic cardiomyopathy,\textsuperscript{154} while both peptides predict cardiovascular events in the general population,\textsuperscript{155} and poor outcome in heart failure.\textsuperscript{156,157} However, NPs do have some limitations as diagnostic markers since there are many confounding factors.\textsuperscript{10} Indeed, age, sex, renal function but also cardiovascular diseases, including volume expansion and possibly increased wall stress, ischaemia, and hypertension, all affect circulating NP concentrations.\textsuperscript{10,158}

N-Terminal pro C-Type Natriuretic Peptide predicts HFpEF outcome\textsuperscript{159} and is negatively associated with myocardial ageing.\textsuperscript{160} N-terminal pro ANP has been proposed as a prognostic biomarker in stable angina.\textsuperscript{161} MR-proANP, in heart failure.\textsuperscript{162} Finally, high levels of circulating soluble NEP (sNEP) predict outcome in HFpEF,\textsuperscript{163} but not HFpEF, diabetes and other cardiovascular diseases.\textsuperscript{164} Application of the above correlations for NP-guided therapy is less developed and its value uncertain: HF-related hospitalization may be reduced\textsuperscript{166} and patients with cardiovascular risk factors but not heart failure may benefit.\textsuperscript{167}

### 6.4 Nitrogen oxides (NO\textsubscript{x})

Many pathological conditions have been associated with altered levels of nitric oxide through measurement of more stable metabolites, nitrite and nitrate (collectively abbreviated NO\textsubscript{x}) or nitrotyrosine. Rapidly measured plasma nitrite rather than nitrate reflects endothelial nitric
Nitrotyrosine, either scavenging of nitric oxide through ROS or myeloperoxidase activity, is associated with increased inflammation. Lower levels of NOx are associated with a more severe outcome in stroke and with increased mortality in idiopathic PAH, while higher levels of NOx and nitrotyrosine correlate with increasing severity of chronic HF. Finally, higher levels of NOx correlate with cardiovascular mortality.

6.5 Asymmetric dimethylarginine (ADMA)
Endogenous ADMA and N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) attenuate L-arginine-dependent NO production by inhibiting and uncoupling NOS. Elevated levels of ADMA impair endothelial function and thus promote atherosclerosis. ADMA and L-NMMA are possibly strong and independent risk factors for cardiovascular disorders, such as hypertension, CAD, atherosclerosis, PH, atrial fibrillation, stroke, and peripheral artery disease. However, ADMA-guided interventional studies are missing.

7. Summary and outlook
Several cGMP-modulating drugs have entered the clinical arena with indications across a wide spectrum of cardiovascular disease states.
Based on genetic evidence, correcting dysfunctional cGMP signalling, i.e. cGMPopathies, has the potential to become one of the few mechanism-based, causal interventions in cardiovascular medicine. Whilst all necessary drugs seem to be available, the key challenge will be to identify those patients with the right indications that present not only a suitable phenotype but, importantly, also exhibit cGMP dysfunction, i.e. the mechanotype. Some of the recent failures in HFrEF drug development may have been preventable by mechanism-based patient stratification. PKG phosphoprotein panels in combination with markers, such as ADMA and nitrotyrosine, may be components of such a cGMPopathy diagnostic algorithm. Once this milestone is achieved, diagnostic-enabled cGMP precision therapy will be possible, most likely by network pharmacology i.e. using multiple cGMP-modulating drugs with different targets in a synergistic manner and in doses that are lower than single drug approaches and, consequently, lower side effects. Based on the compartmentalization of cGMP and unique functions, there is a rationale for further drug discovery on both sGC and pGC. Moving from reductionistic approaches of disease development to molecular network modules is vital to understand the underlying mechanism of a disease state and the connection with its comorbidities, which is one of the reasons preclinical research fails to be translated in the clinic. Clearly, we are in an era of increasing clinical relevance and high precision, mechanism-based and curative applications of cGMP-modulating drugs.

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