

# Clinical Relevance of Cyclic GMP Modulators: A Translational Success Story of Network Pharmacology

Citation for published version (APA):

Oettrich, J. M., Dao, V. T., Frijhoff, J., Kleikers, P. W. M., Casas Guijarro, A., Hobbs, A. J., & Schmidt, H. H. W. (2016). Clinical Relevance of Cyclic GMP Modulators: A Translational Success Story of Network Pharmacology. *Clinical Pharmacology & Therapeutics*, 99(4), 360-362. <https://doi.org/10.1002/cpt.336>

## Document status and date:

Published: 01/04/2016

## DOI:

[10.1002/cpt.336](https://doi.org/10.1002/cpt.336)

## Document Version:

Publisher's PDF, also known as Version of record

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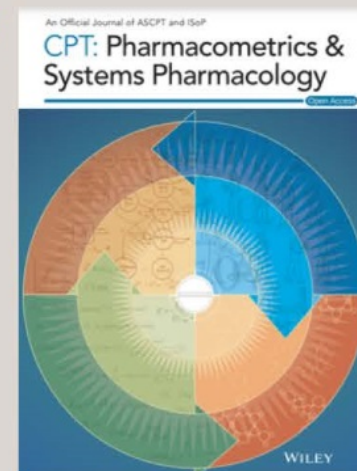
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# Clinical Relevance of Cyclic GMP Modulators: A Translational Success Story of Network Pharmacology

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Therapies that modulate cyclic guanosine-3'-5'-monophosphate (cGMP) have emerged as one of the most successful areas in recent drug discovery and clinical pharmacology. Historically, their focus has been on cardiovascular disease phenotypes; however, cGMP's relevance is likely to go beyond this rather limited organ-based set of indications. Moreover, the multitude of targets and their apparent interchangeability is a proof-of-concept of network pharmacology.

The key components of the cGMP signaling network (Figure 1) include cGMP-producing guanylyl cyclases (GC), cGMP-degrading phosphodiesterases (PDE), and cGMP target proteins (kinases, ion channels, and cGMP-regulated phosphodiesterases). We here provide an overview of the associated drugs and their potentially confusing terminology as well as an up-to-date snapshot of their clinical development and/or therapeutic relevance. In the future, this will most likely also include combinations of different types of cGMP-modulating drugs to increase overall disease specificity and safety. Finally, these approaches are likely to be increasingly guided by innovative biomarker panels yielding powerful therapeutic/diagnostic (theranostic) couples for precision medicine.

## CYCLIC GMP AND ITS CLINICAL PHARMACOLOGICAL RELEVANCE

Cyclic GMP signaling exerts an important protective influence within the vasculature by smooth muscle relaxation and inhibition of proliferation, fibrosis, and platelet aggregation. Cyclic GMP is synthesized from guanosine-3',5'-triphosphate (GTP) by soluble and particulate guanylyl cyclases, which function as receptors for nitric oxide (NO) and natriuretic peptides (NP), respectively. NO and NP signaling input is limited by oxidative stress and the metalloprotease neprilysin, which respectively hydrolyze NPs. NO- or NP-induced increases in cGMP activate cGMP-

dependent protein kinases (PKG), cyclic nucleotide-gated ion channels, and both cAMP and cGMP-hydrolyzing phosphodiesterases (PDEs; cGMP can also inhibit some isoforms of the PDE enzyme family). In turn, this leads to altered phospho-protein patterns, a decrease in intracellular calcium levels and sensitivity, and altered cAMP/cGMP levels. Cyclic GMP signaling is also physiologically limited by phosphodiesterases (PDEs) that hydrolyze cGMP.

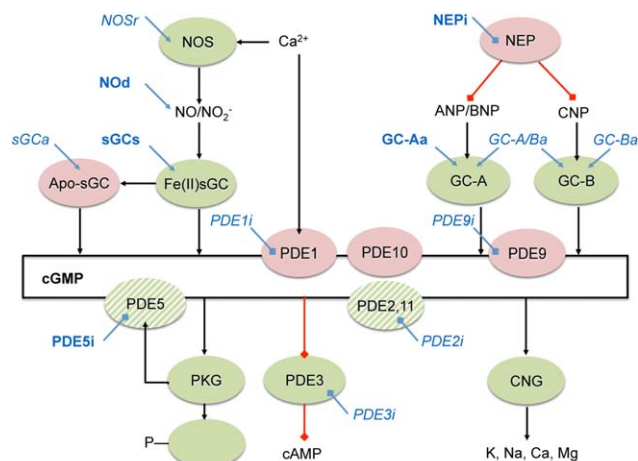
With respect to pathophysiology, mechanism-based dysregulation of cGMP signaling has been attributed to both reduced cGMP synthesis and/or enhanced cGMP breakdown. In this latter context, the underlying mechanisms include reduced levels of NO,<sup>1</sup> damaged NO-sensitive sGC,<sup>2</sup> and enhanced cGMP breakdown. In addition, and distinct from these mechanism-based alterations, cGMP modulating drugs have been employed in a symptom-based manner, i.e., without evidence of an underlying "cGMPopathy," in pulmonary hypertension<sup>3</sup> and chronic kidney disease.

## THE NO-RECEPTOR, SOLUBLE GC (SGC)

Soluble GC is a heterodimeric heme protein with two isoforms,  $\alpha_1/\beta_1$  or GC-1 and  $\alpha_2/\beta_1$  or GC-2. Both consist of three principal functional domains: an N-terminal Fe(II)heme-binding region that confers sensitivity to NO, a C-terminal catalytic functionality, and a central dimerization region. Binding of NO to the histidine-ligated Fe(II)heme induces a structural conformation that activates the enzyme's catalytic site. Inappropriate reactive oxygen species formation, in particular superoxide, can interfere with NO-cGMP signaling in at least three ways: by chemically scavenging NO; by uncoupling NO synthase (NOS); or by oxidizing sGC's Fe(II) to Fe(III)heme, resulting eventually in apo-sGC prone to proteasomal degradation. Recoupling NOS has been shown to be preclinically effective by supplying its redox sensitive cofactor, tetrahydrobiopterin, or L-arginine. Therapeutically, NO substitution with so-called

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**Figure 1** Schematic overview of the cGMP signaling pathway and its regulation by different guanylyl cyclases, PDEs, and enzyme modulating drugs (green = key agonistic regulator proteins and cGMP effector proteins; red = key antagonistic regulator proteins, striped green = hydrolyzing cGMP effector proteins; bold, blue letters = drugs approved in clinics and italic blue letters = drugs in clinical development).

NO donor compounds has the longest history but also limitations, including tolerance and lack of efficacy on apo-sGC, as it lacks the NO-binding heme. New targeted strategies circumvent some of these drawbacks. These so-called sGC stimulators or activators target either Fe(II)heme-containing sGC or apo-sGC, respectively, to enhance cGMP synthesis directly (**Figure 2**).

### sGC stimulators (sGCs)

The exact binding mechanism and site of sGCs are still unclear. They allosterically sensitize Fe(II)heme-containing sGC to NO. If levels of endogenous NO were pathologically diminished, this will result in a mechanism-based “repair” of a physiological cGMP response. If sGC is maximally activated by physiological NO levels, sGCs that sensitize sGC for NO have by definition no or only a very limited additional effect on cGMP. Importantly, two sGCs have shown therapeutic benefit in recent clinical trials. Riociguat (BAY 63-2521) was the first sGC stimulator to be approved for use in two different forms of pulmonary hypertension, i.e., pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Both are cardiovascular disorders with underlying endothelial dysfunction associated with decreased NO bioavailability as well as vascular remodeling. Riociguat is now being clinically evaluated in other forms of pulmonary hypertension, including that associated with hypoxia (NCT00640315) and left heart disease (NCT01065454), and also cystic fibrosis (NCT02170025) and systemic sclerosis (NCT02283762). A second sGC stimulator, vericiguat (BAY 1021189), has passed late-stage phase II (SOCRATRES program) to investigate its efficacy and safety in heart failure (HF) with either reduced or preserved ejection fraction (HFrEF and HFpEF). Both Bayer Healthcare (Berlin, Germany) compounds, riociguat and vericiguat, are based on the leading indazole structure of YC-1, which was the first described sGCs. However, novel classes of sGCs have been recently discovered by Ironwood Pharmaceuticals (Cambridge, MA) and are

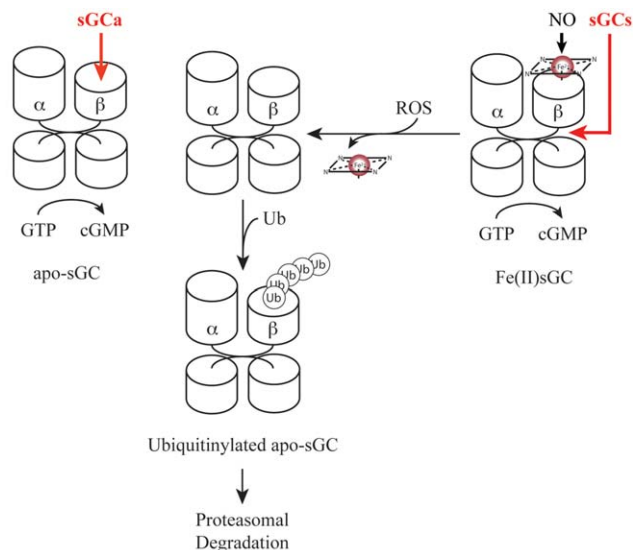
characterized by a pyrazole-pyrimidine structure. Preclinically, these compounds are antihypertensive and improve heart failure as well as biomarkers of fibrosis and inflammation. IW-1973 is currently in clinical phase Ib for later application in cardiovascular diseases.

### sGC activators (sGCa)

sGCa specifically bind to NO-insensitive, heme-free apo-sGC.<sup>4</sup> They thereby reactivate oxidized and NO-insensitive enzyme otherwise prone to degradation. Larger sGCa molecules such as cinaciguat (BAY58-2667), but not the smaller ataciguat (HMR1766), occupy the empty heme site and thereby prevent ubiquitination and proteasomal degradation. Apart from activation, this then leads to stabilization and enhanced sGC protein levels. The nomenclature takes no note of these profound differences: sGCs, sGCa, and stabilizing sGCa all have the same INN ending, -ciguat. sGCa have not yet entered late stage clinical development. A clinical phase II trial (COMPOSE program) with cinaciguat in patients with acute decompensated chronic congestive heart failure had to be abrogated due to severe hypotension. The anthranilic acid derivative ataciguat (HMR1766) is now in phase II (NCT02481258) to evaluate therapeutic efficiency in patients with aortic valve calcification.

### NATRIURETIC PEPTIDES AND THEIR GC RECEPTORS

The family of plasma membrane integrated “particulate” guanylate cyclases contains seven members (GC-A to GC-G), of which GC-D and GC-G only exist as pseudogenes in humans. GC-A and GC-B are receptors for different natriuretic peptides (NP).



**Figure 2** Mechanisms of action of sGC stimulators (sGCs) and activators (sGCa). Fe(II)heme containing sGC (Fe(II)sGC) is the nitric oxide (NO) receptor. sGCs allosterically sensitize Fe(II)sGC so that lower concentrations of NO exert maximal cGMP formation. Upon oxidative stress, sGC’s Fe(II) heme is oxidized to Fe(III) and eventually lost. Heme-free apo-sGC is ubiquitinated and enters proteasomal degradation. sGCa instead binds to the empty heme binding site, preventing ubiquitination and thus degradation, and activates apo-sGC to similar  $V_{max}$  levels as NO-Fe(II)sGC.

These homodimers consist of an extracellular ligand binding domain which is linked by a single transmembrane domain to an intracellular guanylyl cyclase domain similar to sGC. GC-A synthesizes cGMP upon binding of atrial (ANP) and brain (BNP), whereas GC-B is stimulated by C-type NP (CNP).

For therapeutic purposes, various synthetic peptide activators of GC-A and GC-B have been developed. The ANP analog ularitide has entered a phase III clinical trial (TRUE-AHF, NCT01661634) for acute decompensated heart failure; the recombinant human CNP BMN11, phase II (NCT02055157) for the treatment of achondroplasia in children (GC-B plays a key role in chondrogenesis and bone growth). In addition, the chimeric peptide CD-NP (cenderitide) is now in phase II (NCT02359227) for the treatment of acute heart failure. Besides the difficulties of short biological half-life and poor oral bioavailability, therapeutically optimized NPs, or small molecules targeted to GC-A and/or GC-B, may offer an alternative to sGCs/sGCa to elevate cGMP, most likely with different tissue selectivity and indications.

In addition to supplementing designer NP, compounds, such as sacubitril (AHU-377) block—among other enzymes—the NP-degrading enzyme, neprilysin. Such compounds could offer yet another therapeutic approach in elevating cGMP levels via NP-cGMP signaling. A phase III clinical evaluation (PARADIGM; NCT01035255) of LCZ696 vs. enalapril was halted prematurely following an almost 20% reduction in HF-related hospitalization and cardiovascular (CV) death in the active arm. LCZ696 was fast-tracked by the US Food and Drug Administration (FDA) and licensed for the treatment of heart failure recently.

### PDE INHIBITORS (PDEi)

In addition to enhancing cGMP production for therapeutic benefit, PDEi inhibit cGMP degradation to bring about similar effects. The first and most prominent drug in this class is the PDE5i sildenafil, now used clinically for the treatment of erectile dysfunction and PAH. Different PDE isoforms are present at different subcellular localizations, thereby targeting different cGMP pools and represent a platform for the search for new medical indications. Especially PDE1, PDE2, PDE3, PDE5, and PDE9 have been implicated in different cardiovascular disorders. Proof-of-concept has been obtained for PDE5i in erectile dysfunction and pulmonary hypertension, and the acute use of PDE3i in heart failure and thrombosis. The largest therapeutic benefit of PDEi may reside in combination therapies that enhance cGMP production, i.e., sGCs, sGCa, NP, and neprilysin inhibitors. Such

combinations would target the cGMP signaling network from different angles and form an example of network pharmacology.

### NETWORK PHARMACOLOGY: THE CASE OF cGMP

NP analogs may not compensate for a loss of sGC function, while *vice versa* sGC stimulators may not compensate for a loss of GC-A or GC-B-mediated cGMP production. On top of that, different PDE isoforms can be more specific for either NO- or NP-triggered cGMP production, or both. A logical consequence of this signaling complexity is that in patients with multiple defects in cGMP signaling, cGMP modulators should be combined rather than used as monotherapy. A major hurdle in applying such an approach rationally lies in the lack of biomarkers that are able to assess whether a specific defect in cGMP signaling occurs in a patient.

### BIOMARKERS TO IDENTIFY “cGMPOPATHIES” AND STRATIFY PATIENTS

Currently, the most direct readout of NO<sup>5</sup> and NP-cGMP signaling is PKG-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP). However, basal VASP phosphorylation *per se* is too low and variable to identify deregulated cGMP signaling. Filling this gap is necessary to enable patient stratification, and optimize dosing for higher efficacy and safety by providing effective therapeutic/diagnostic (theranostic) couples.

### CONFLICT OF INTEREST

AH acts on advisory boards of Bayer Healthcare and Novartis.

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