The 11th International Conference on cGMP 2024: recent trends and developments in cGMP research —meeting report

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MEETING REPORT

The 11th International Conference on cGMP 2024: recent trends and developments in cGMP research —meeting report

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Abbreviations

ACEi	Angiotensin-converting-enzyme inhibitor
ANP	Atrial natriuretic peptide
ARB	Angiotensin II receptor blocker
BNP	B-type natriuretic peptide
BAT	Brown adipose tissue
BH_4	Tetrahydrobiopterin
BP	Blood pressure
cAMP	3',5'-CAMP: cyclic adenosine monophosphate
cCMP	3',5'-CCMP: cyclic cytidine monophosphate
cGK	CGMP-dependent protein kinase, aka PKG
cGMP	3',5'-CGMP: cyclic guanosine monophosphate
CKD	Chronic kidney disease
CNG	Cyclic nucleotide-gated
CNP	C-type natriuretic peptide
cUMP	3',5'-CUMP: cyclic uridine monophosphate
ED	Erectile dysfunction
GC	Guanylyl cyclase
GC-A	Guanylyl cyclase A
GC-B	Guanylyl cyclase B
GMP	Guanosine monophosphate
GTP	5'-GTP: guanosine triphosphate
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
KO	Knockout
MetS	Metabolic syndrome
NO	Nitric oxide
NO-GC	NO-sensitive guanylyl cyclase
NOS	Nitric oxide synthase
NP	Natriuretic peptide
PAH	Pulmonary arterial hypertension
PDE	Phosphodiesterase
PH	Pulmonary hypertension
QD	Quaque die = once daily
RBC	Red blood cell
sGC	Synonym of NO-GC
SGLT2i	Sodium-glucose cotransporter-2 inhibitor
SMC	Smooth muscle cell
TID	Ter in die = three times a day
T2D	Type 2 diabetes
tmGC	Transmembrane guanylyl cyclase, also called pGC
uACR	Urinary albumin-to-creatinine ratio
WT	Wild type

History of cGMP meetings

The cGMP research community was excited to reunite after the last conference held in 2022 in Augsburg 2 years ago. Over 100 researchers attended the meeting in Lübeck in 2024, which is an historical city located in northern

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Germany. The meeting took place at the stunning 'Media Docks' in the Lübeck's harbor, offering a productive and engaging setting for scientific discussions and presentations (Fig. 1).

Held every 2 years since 2003, the International Conference on cGMP: Generators, Effectors, and Therapeutic Implications, has grown into a key event in cGMP research. It covers basic aspects of cGMP signaling such as cGMP enzymatic formation, degradation and downstream targets, as well as translational and clinical applications, including late-stage drug development. It was established in 2003 in Leipzig. Subsequent meetings were held in Potsdam (2005), Dresden (2007), Regensburg (2009), Halle (Saale) (2011), Erfurt (2013), Trier (2015), Bamberg (2017), Mainz (2019), Augsburg (2022), and most recently in Lübeck (2024). The meeting is held biannually, except in 2021 due to the COVID-19 pandemic.

cGMP signaling pathways

The cGMP is a second messenger that plays a crucial role in maintaining cellular and tissue homeostasis. Disruptions in this pathway can result in various diseases, including erectile dysfunction (ED), heart failure (HF), pulmonary hypertension (PH), ischemic heart disease, retinal degenerative disorders, neurodegenerative diseases such as Alzheimer's and Parkinson's disease, and certain types of cancer (e.g., Stehle et al. 2023; Triposkiadis et al. 2022)).

The synthesis of cGMP is catalyzed by two distinct types of guanylyl cyclases (GC): nitric oxide (NO)-sensitive guanylyl cyclase (NO-GC or sGC) and transmembrane guanylyl cyclase (tmGC).

NO is produced enzymatically by three independent nitric oxide synthase (NOS) isoforms, including the neuronal NOS (nNOS, NOS1), the inducible NOS (iNOS, NOS2), and the endothelial NOS (eNOS, NOS3). NO can also be produced in a NOS-independent fashion from nitrite in reactions catalyzed by hemoglobin, myoglobin, and other globins during hypoxic conditions, by xanthine oxidoreductase or by the microbiota.

In the vessel wall and other tissues, NOS-derived NO activates its primary receptor, NO-GC, which subsequently generates the secondary messenger cGMP. Recent discoveries have highlighted the involvement of cellular heme metabolism in the regulation of apo-sGC (heme free) activation. It has been demonstrated that free heme is transferred into apo-sGC and contributes to its activation. Interestingly,

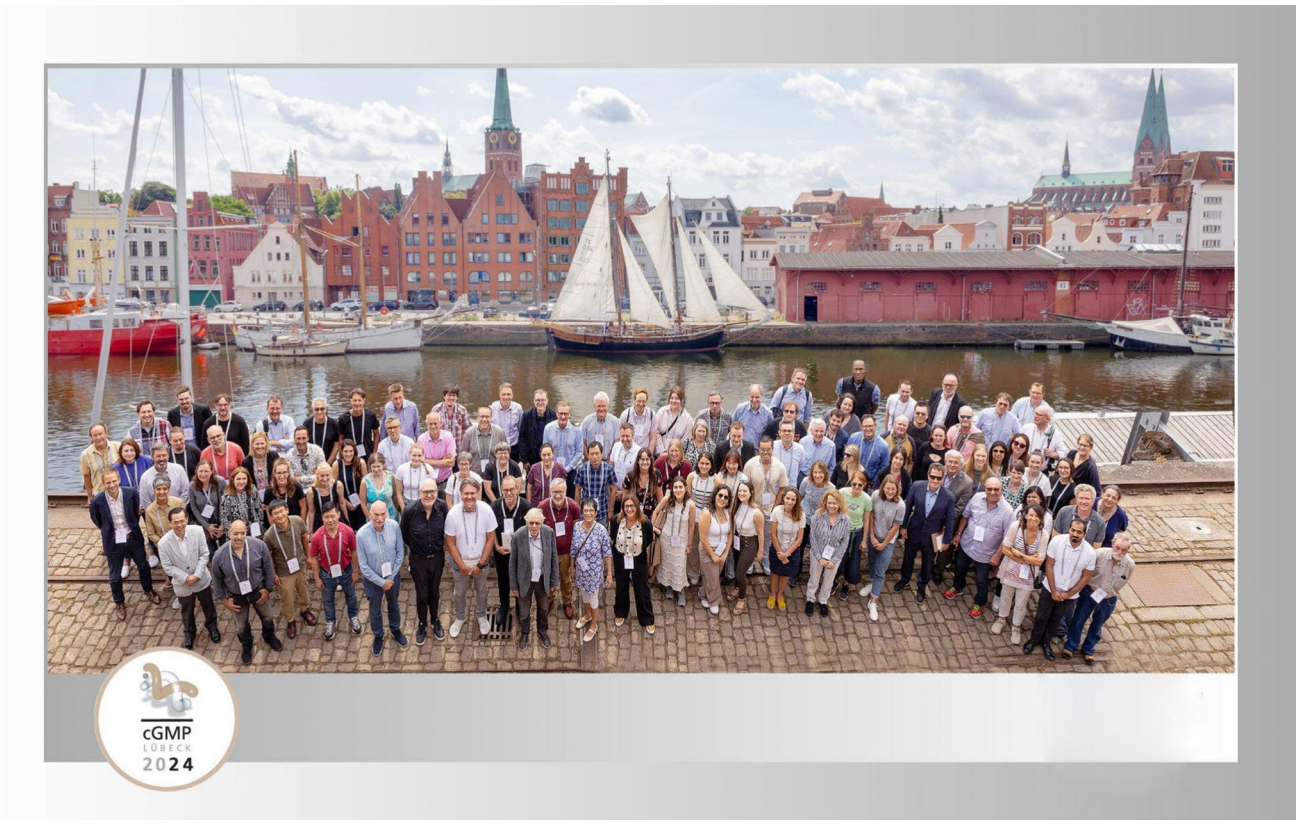

Fig. 1 Participants of the 11th International Conference on cGMP, held in Lübeck, Germany, from June 28th-30th, 2024. For program, abstracts, and meeting information seehttps://www.cyclicgmp.net/index.html

NO bound to free heme (heme-NO) may directly activate the enzyme. Additionally, there is accumulating evidence suggesting a significant role of sGC in hematopoiesis, further expanding the physiological relevance of the NO-sGC pathway.

The cellular effects of cGMP are mediated by three types of cGMP-binding proteins: cyclic nucleotide-gated (CNG) cation channels, cGMP-regulated phosphodiesterases (PDEs), and cGMP-dependent protein kinases (cGKs) (Fig. 2).

The interplay between cGMP and cAMP signaling occurs through cGMP- or cAMP-regulated PDEs, as well as through cAMP-dependent protein kinases, cGKs, and hyperpolarization-activated CNG channels.

In the cardiovascular system, one of the most critical actions of cGMP is mediated through cGMP-dependent protein kinase I (cGKI), which induces smooth muscle cell (SMC) relaxation and subsequent lowering of arterial pressure via phosphorylation of multiple substrates.

Besides NO-driven cGMP synthesis, seven tmGCs (GC-A to GC-G) produce cGMP when activated by natriuretic peptides (NPs) and other ligands.

The processes of cGMP production via NO-GC and tmGC, cGMP signaling through downstream targets, and cGMP degradation have been extensively investigated. Numerous high-quality manuscripts and review articles provide comprehensive overviews of these mechanisms.

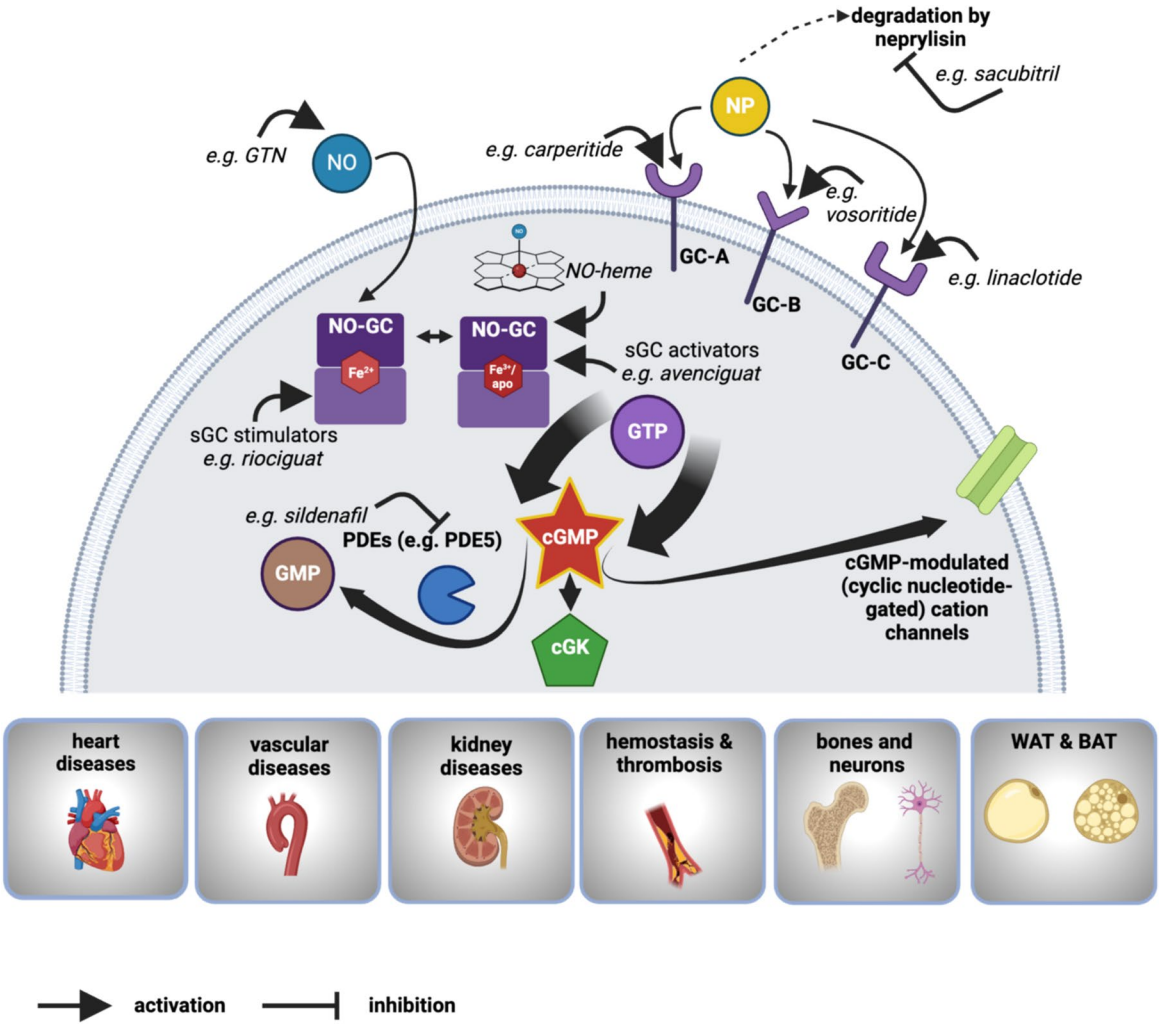

Fig. 2 Overview of signaling pathways in which cGMP is involved, including pharmacological targets. Abbreviations: BAT: brown adipose tissue; cAMP: cyclic adenosine monophosphate; cGK: cGMP-dependent protein kinase; cGMP: cyclic guanosine monophosphate; GC-A, -B, -C: guanylyl cyclase A, B, C; GMP: guanosine monophosphate; GTN: glyceryl trinitrate; 5'-GTP: guanosine triphosphate; NO: nitric oxide; NO-GC: NO-sensitive (soluble) guanylyl cyclase (also sGC); NP: natriuretic peptide(s); PDE: phosphodiesterase; WAT: white adipose tissue (adapted from Friebe et al. 2023; created with BioRender.com)

Welcoming session

Harald H. H. W. Schmidt from Maastricht University (Maastricht, Netherlands) welcomed all participants to Lübeck on behalf of the organization committee.

The city has been the leader of the hanseatic league in the middle age with its vicinity to the Baltic Sea but remained a relevant German city afterwards due to its unique architecture and three noble prize winners, the author Thomas Mann, who wrote his first novel 'The Buddenbrooks' in Lübeck in the early twentieth century, the politician Willy Brandt and the author and painter Guenter Grass.

Harald Schmidt closed his welcome statement by citing the inscription on the "Holstentor," the landmark of Lübeck:»*Concordia domi foris pax*«, meaning»harmony within, peace outside« and transformed it slightly to our motto for the three-day meeting: "Innovation inside, patient benefits outside".

Meeting Highlights

Overview

Table 1

Clinical translation

As in earlier meetings, the 2024 meeting started with a session in which recent clinical developments and applications of cGMP-increasing drugs were discussed. In addition to the benefit for patients suffering from devastating diseases, these results are also important to constantly check and refine the preclinical concepts, the assays, as well as in vivo models, and to adjust them. Since cGMP-increasing drugs are available for patients, back-translation from the patients to the preclinical models has become an important part in this meeting .

Avenciguat and runcaciguat in chronic kidney disease

The results of the clinical studies of the sGC activators avenciguat and runcaciguat (Fig. 3) in patients with chronic kidney disease (CKD) were presented by Dominik Steubl (Boehringer-Ingelheim, Ingelheim, Germany) in the speech entitled "Efficacy and safety of avenciguat in diabetic and non-diabetic kidney disease: Pooled analysis from two Phase II randomized controlled clinical" and Karen Paraschin (Bayer AG, Wuppertal, Germany) in the talk "The sGC activator runcaciguatreduces albuminuria in patients with chronic kidney disease (CKD): results from the phase 2CONCORD study" respectively.

Table 1 Key findings presented at the cGMP meeting 2024 in Lübeck

Session	Key Findings
Clinical Translation	- sGC activators avenciguat and runcaciguat reduce albuminuria in CKD - Combination therapy of L-citrulline, folate, and vericiguat shows potential in HFpEF
cGMP in the Cardiovascular System	- cGMP plays a role in erythropoiesis - Protective function of CNP in sepsis - NO-GC stimulation improves limb reperfusion in diabetic PAD - Targeted biosensors reveal cGMP compartmentalization
cGMP in Cancer Biology and Metabolism	- cGMP-PKG pathway suppresses metastasis - NO-GC modulates tumor vasculature - Cyclic nucleotides exhibit anti-proliferative effects in glioblastoma and neuroblastoma
NO-GC Structure, Modulators, Function	- Cryo-EM elucidates sGC activation - NO-ferroheme acts as a novel signaling molecule - Genetic studies link NO-GC activity to atherosclerosis risk
Emerging Fields	- Cytoglobin regulates NO signaling in organ development - Isoform-specific sGC activators identified - Structural insights into PKG1Î ± activation
Crosstalk	- Erythrocytes release cGMP to protect cardiomyocytes - PDE1 inhibition improves cardiomyocyte relaxation - PDE2 upregulation impairs endothelial barrier function in ischemia
Novel Aspects of Natriuretic Peptide Signaling	- Natriuretic Peptides, their Receptors and beyond - NP-cGMP pathway influences cardiovascular aging - Long-acting CNP analog developed for achondroplasia treatment
Technological Advances & New Applications	- Optogenetics enables cyclic nucleotide manipulation - PDE10A inhibition relieves pain - PfPKG inhibitors show promise for malaria prevention - Heme mimetics extend beyond sGC modulation

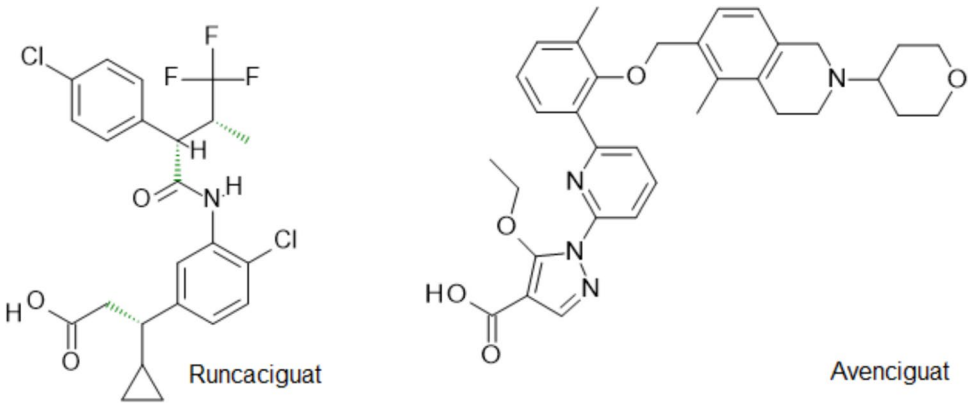

Fig. 3 Chemical structures of runcaciguat (BAY 1101042) and avenciguat (BI 685509).

Oxidative stress impairs NO-binding to sGC and can result in the loss of the heme-group, preventing the formation of cGMP (Annuk et al. 2001; Fritz et al. 2011; Stasch et al. 2015), contributing to CKD progression and accelerating cardiovascular (CV) disease in patients with CKD. Two novel potent sGC activators, avenciguat (BI 685509) and runcaciguat (BAY 1101042), are in development for the treatment of CKD, and have been the focus of recent clinical trials due to their potential to restore the impaired NO-pathway and potentially prevent CKD progression.

Avenciguat has been investigated in two phase II double-blind, placebo-controlled trials of identical design, in patients with diabetic (NCT04750577) or non-diabetic (NCT04736628) CKD (PMID: 38,795,055). Adults aged ≥ 18 years with CKD were randomized to receive 20 weeks of placebo or avenciguat as adjunct to maximum tolerated angiotensin-converting-enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARBs). Avenciguat reduced urinary albumin-to-creatinine ratio (uACR) in 10-h urine compared with placebo throughout the treatment period. At week 20, the placebo-corrected geometric mean percent changes from baseline in uACR in 10-h urine with avenciguat at 3 mg TID were −21.5%. Avenciguat was well tolerated, with hypotension reported in a small percentage of patients treated with any avenciguat dose. Avenciguat was effective in lowering albuminuria and was well tolerated in patients with diabetic and non-diabetic CKD.

The efficacy, safety, and tolerability of runcaciguat (Hahn et al. 2021) in CKD patients with and without type 2 diabetes (T2D) were assessed in the multicenter, randomized, double-blind, placebo-controlled, parallel-group, individual-titration, phase II trial (Chronic Kidney Disease Outcomes with Runcaciguat: Clinical Outcomes and Renal Disease, CONCORD, NCT04507061). Runcaciguat was dosed between 30 and 120 mg QD with ~ 80% of runcaciguat-treated patients reaching the highest dose. The primary efficacy endpoint was the reduction in uACR from baseline to the average of days 22, 29, and 57. An average reduction in uACR of 44% from baseline was observed from day 22 until end of treatment (day 57) in the runcaciguat treatment arm. Statistically significant reductions in uACR with runcaciguat versus placebo were observed on top of ACEi/ARB, with or without concomitant sodium-glucose cotransporter-2 inhibitor (SGLT2i) treatment. Runcaciguat was well tolerated with no safety concerns. The results of the CONCORD study suggest that sGC activation may represent a promising approach for the management of patients with CKD with or without T2D.

In conclusion, both avenciguat and runcaciguat show promise in the treatment of CKD, demonstrating the potential of sGC activators in managing this condition. These findings represent significant advancements in the field and provide hope for improved treatment options for patients with CKD.

Example of a combination therapy for HFpEF

The NO/NO-CG/cGMP pathway can not only be hampered by the oxidation of the heme-group in sGC, but even earlier by NOS uncoupling. The enzyme uses L-arginine and oxygen as substrates and requires several cofactors (e.g., BH_4 and heme) for its function. NOS uncoupling refers to a state where the transfer of electrons to the substrates is impaired, leading to the production of superoxide ($^{\bullet}O_2^-$) instead of NO (Forstermann & Sessa 2012; Janaszak-Jasiecka et al. 2023). This can further enhance oxidative stress and contribute to various pathological conditions.

Rafael de la Espriella (Valencia, Spain) presented the results from "NOS recoupling with L-citrulline, folate, and vericiguat in a NOX5-positive subgroup of HFpEF patients: A Phase IIa proof-of-concept trial" an EU-funded phase IIa trial with the aim to evaluate if recoupling of NOS could be achieved by supplementation with the vitamin folate and the amino acid L-citrulline in combination with the sCG stimulator vericiguat (Verquvo®), a drug already approved for the therapy of HF (Armstrong et al. 2020). High doses of folate can reverse the oxidation of tetrahydrobiopterin (BH_4) to dihydrobiopterin (BH_2) and, hence, support the function of

NOS (McCarty 2017). To apply L-citrulline to support the function of NOS seems to be counterintuitive if L-arginine is converted to L-citrulline and NO by NOS, but since the bioavailability of L-citrulline is higher than for L-arginine and L-citrulline is converted to L-arginine, the supplementation of L-citrulline is seen to be superior to L-arginine (Allerton et al. 2018). See Fig. 4.

The REPO-HFpEF phase IIa study was carried out to investigate a specific cause of HFpEF related to NO synthase and apo-sGC. This trial was single-centered, prospective, randomized, and controlled by standard treatment. It was also open-label and in phase IIa, serving as a proof-of-concept.

In this trial, 21 HFpEF patients were randomly selected and treated for 12 weeks. They were either given the standard care or a combination of standard care and a triple mix of vericiguat, L-citrulline, and folate. The patients were chosen based on whether they tested positive for the plasma NOX5 biomarker (≥ 105 ng/ml), were apo-sGC-positive (apo-sGC/sGC ratio > 1.05), or both.

The total number of adverse events between the treatment groups was 22 for 14 patients in the REPO group and 6 for 7 patients in the SOC group. The median peak VO_2 at screening for the REPO and SOC groups was 13.1 ml/min/kg and 10.6 ml/min/kg, respectively.

A linear mixed regression analysis showed that the least square mean difference for peak VO_2 at the end of treatment was $+0.27$ for the REPO group and -0.23 for the SOC group. The treatment's effect on Kansas City Cardiomyopathy Questionnaire (KCCQ), peak VO_2, and NT-pro-BNP did not significantly vary across NOX5 and sGC.

However, a numerical trend towards improvement in KCCQ, peak VO_2, and NT-pro-BNP was observed in REPO-treated NOX5-positive patients. This differential effect was not found in apo-sGC-positive vs. negative patients.

In conclusion, using network pharmacology with vericiguat, L-citrulline, and folate in a subgroup of NOX5-positive HFpEF patients did not increase the overall risk of adverse events. There were some indications of improvement in HF-related quality of life, exercise capacity, and NT-pro-BNP reductions. More extensive studies are needed to confirm these findings.

A stable form of ANP to treat cardiometabolic disease

Hypertension and metabolic syndrome (MetS) are often co-occurring conditions that significantly increase the risk of severe cardiovascular diseases. The heart hormone known as atrial natriuretic peptide (ANP) is crucial in regulating blood pressure (BP) and metabolism. It does so by activating the GC receptor A, which in turn produces cGMP as a secondary messenger.

Valentina Cannone (Parma, Italy) presented the study titled "MANP: A natriuretic peptide-based therapy for cardiometabolic diseases," aimed to evaluate the cardiovascular, metabolic, and cGMP effects of MANP, a new ANP analog, in individuals diagnosed with hypertension and MetS (NCT03781739) (Ma et al. 2024).

The investigators carried out a double-blind, placebo-controlled trial involving 22 patients (17 of whom received MANP) with hypertension and MetS. The trial involved a single subcutaneous injection of either MANP (2.5 µg/kg) or a placebo.

Relative to baseline measurements, MANP led to an increase in plasma cGMP at 30 min ($+4.8 \pm 2.0$ pmol/mL,

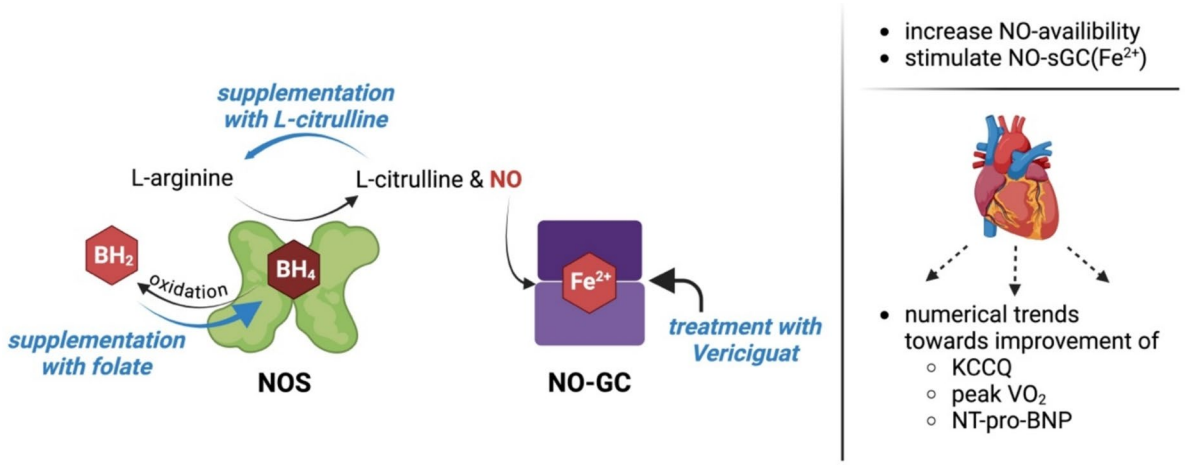

Fig. 4 Schematic for the mode of action of the triple therapy with folate, L-citrulline, and vericiguat. [BH2, dihydrobiopterin; BH4 tetrahydrobiopterin; Fe2+, ferrous iron; KCCQ, Kansas City Cardiomyopathy Questionnaire; NO, nitric oxide; NOS, nitric oxide synthase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; VO2 max, maximal oxygen consumption]

$P = 0.02$) and 1 h (+ 2.9 ± 1.3 pmol/mL, $P = 0.03$) after injection. Six hours post-injection, systolic BP in the MANP group decreased by 5.7 ± 2.9 mmHg ($P = 0.06$), while no BP reduction was observed in the placebo group. Following the injection of MANP, glucose levels dropped at 2 (- 4.7 ± 2.1 mg/mL, $P = 0.04$) and 4 h (−13.1 ± 3.9 mg/mL, $P = 0.003$) compared to baseline, while insulin levels remained unchanged. In the 4 h after injection, a trend towards increased insulin sensitivity index (HOMA2-IS) and decreased insulin resistance index (HOMA2-IR) in the MANP group, with the opposite trends observed in the placebo group. One hour after administering MANP, circulating non-esterified fatty acids increased by 108.2 ± 37.4 µM compared to baseline ($P = 0.01$).

The results suggest that MANP has promising cardiovascular and metabolic effects, making it a potential therapeutic option for treating hypertension in patients with MetS.

cGMP in the cardiovascular system

The session about the cardiovascular system covered a broad field including cGMP in erythrocytes, specifically its effects on erythropoiesis, the protective role of C-type natriuretic peptide (CNP) on microcirculation and cardiac function in sepsis, the improvement of limb reperfusion by stimulators of NO-GC in diabetic peripheral artery disease and the application of targeted cGMP biosensors and cGMP scavengers to analyze spatial cGMP signaling in cardiac myocytes.

In the first talk, "The role of NO/sGC signaling in erythron-poiesis: lessons from erythroid-specific knock-out and knock-in mice," Miriam Cortese-Krott (Duesseldorf, Germany) set out to analyze how NO-GC stimulators (vericiguat) might cause anemia as observed in the VICTORIA trial with anemia in 7.6% of patients in the vericiguat vs. 5.7% in the placebo groups (Ezekowitz et al. 2021). In red blood cell (RBC)-specific eNOS-deficient mice, a reduced NO-heme content in RBC was observed compared to unchanged NO-heme content in endothelial cell-specific eNOS-deficient mice. RBC-specific knock out (KO) mice of NO-sensitive guanylyl cyclase 1 (NO-GC1) displayed smaller colonies and a lower number of colony-forming units of erythroid progenitor cells and a concomitant reduction of proerythroblasts in bone marrow. In contrast, erythropoiesis took place in spleen leading to splenomegaly. The stress erythropoiesis in the spleen was able to compensate for reduced erythropoiesis in the bone marrow leading to preserved number of RBC, hemoglobin content as well as transferrin and erythropoietin levels. Interestingly, this phenotype was only found in RBC-specific KO mice but not in global KO of NO-GC1. In sum, reduced bone marrow erythropoiesis compensated by erythropoiesis in the spleen in RBC-specific KOs argues in favor of a stimulatory role of NO-GC1 in erythropoiesis contrary to the slightly increased prevalence of anemia in vericiguat-treated patients in the VICTORIA trial.

Because plasma levels of CNP are known to be elevated in sepsis, Amie Moyes (London, UK) during the talk entitled "Endogenous C-type natriuretic peptide mitigates vascular dysfunction, inflammation and cardiomyopathy during endotoxemia and polymicrobial sepsis" analyzed the function of CNP in sepsis by using mice devoid of CNP in endothelial cells (ecCNP-KO) and mice lacking the natriuretic peptide C receptor (NPR-C-KO). Two sepsis models were applied, LPS and cecal ligation and puncture model. In ecCNP-KO mice, LPS and cecal puncture elicited a reduced drop in BP and endothelial-dependent relaxation compared to wild type (WT). Furthermore, ear and hind limb blood flow was reduced. Similar results were obtained in NPR-C-KOs. However, cardiac parameters such as heart rate and ejection fraction as well as left ventricular wall thickness were more severely disturbed in NPR-C-KO than in ecCNP-KO. In the NPR-C-KO, tight (ZO-1) and gap (Cx43) junctions were reduced in the heart and more macrophage infiltration was observed. In sum, endogenous CNP on the one hand increased blood flow but on the other hand reduced BP in sepsis. Furthermore, CNP improved cardiac function and dampened inflammation. Thus, it was concluded that CNP via NPR-C played a protective role in sepsis.

Macroangiopathy presenting e.g. as peripheral artery disease is one of the major long-term complications of T2D. Because endothelial dysfunction caused by impaired NO/cGMP signaling plays an important role in the pathogenesis of peripheral artery disease, Marie-Ange Renault (Bordeaux, France) during the talk "sGC is a promising therapeutic target for the management of lower limb ischemia" asked whether the stimulator of NO-GC praliciguat might improve reperfusion of ischemic legs. Leptin receptor-deficient mice develop a rather strong hyperglycemia by two months of age and are used as a mouse model of T2D. Three to four months old mice were treated with praliciguat orally starting three days before surgically inducing hind limb ischemia by femoral artery excision. Two to four weeks later, praliciguat-treated Leptin receptor-deficient mice displayed increased perfusion assessed as ratio of ischemic leg/control compared to untreated receptor-deficient mice. In contrast, non-deficient mice (heterozygotes) did not benefit from praliciguat and displayed ratios comparable to receptor-deficient, praliciguat-treated animals. Apparently, the effect of praliciguat was not mediated by metabolic effects (e.g., weight, triglycerides, insulin). Capillary densities in muscle cross sections were not changed by praliciguat treatment and were unaffected by genotype (leptin receptor deficiency), but praliciguat treatment increased the diameter of arterioles. In diabetic, i.e., leptin receptor-deficient, mice, praliciguat treatment decreased ICAM1 (intercellular adhesion molecule 1) expression. In sum, praliciguat improved ischemic

leg reperfusion in a T2D model probably by increasing arteriole diameter and decreasing ICAM1 responsible for endothelial adhesion properties.

Mette Ovensen (Oslo, Norway) had a talk on "Combining cGMP biosensors and scavengers to decipher cardiac compartmented signaling" and reported on the application of targeted FRET-based cGMP biosensors and targeted cGMP scavengers to decipher cGMP signaling in cardiac H9c2 cells. As analyzed with an evenly distributed biosensor, CNP elicited 20% changes of the ratiometric signals which were abolished by an evenly distributed cGMP scavenger. Scavengers targeted to the outer mitochondrial membranes, lipid rafts, non-raft membrane or outer mitochondrial membranes reduced the signals by 50%. ANP-induced smaller 5% signals that were reduced by membrane targeted scavengers only (rafts and non-rafts). With a sensor targeted to the outer mitochondria membrane, smaller signals (12%) were (3%); again, only the scavenger targeted to the outer mitochondrial membrane reduced these signals. Thus, GCA and GC-B differentially regulate cGMP in different cellular compartments.

cGMP in cancer biology and metabolism

Another area of highly relevant and translational potential for pharmacological modulators of intracellular cGMP levels is the cancer field. The cGMP-PKG signaling axis functions as an anti-apoptotic pathway particularly in certain subtypes of cancer (Browning et al. 2010; Fajardo et al. 2014; Fallahian et al. 2011; Kumazoe et al. 2013; Mujoo et al. 2010; Wen et al. 2015). Drugs that increase cellular cGMP levels have already received broad approval for the treatment of diseases such as ED (Wright 2006), acute vasodilation (Hottinger et al. 2014) or PH (Cannon & Pepke-Zaba 2014); thus, their application for cancer therapies would require repurposing that would facilitate the approval procedure.

The first talk in the session entitled "cGMP in cancer biology" was given by Donald McDonnell from the Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, North Carolina, USA. During the talk entitled "Manipulation of cancer cell intrinsic and extrinsic cGMP signaling as a therapeutic approach in cancer" he unveiled a novel calcium-dependent signaling axis via activation of calcium/calmodulin kinase kinase-2 he unveiled a novel calcium-dependent signaling axis via activation of calcium/calmodulin kinase kinase-2 (CAMKK2) and phosphodiesterase-1 (PDE1) that cancer cells and tumor-associated macrophages (TAMs) use to drive their metastatic potential. Important was the observation that pharmacological or genetic inhibition of CAMKK2 activity reduced the metastatic potential of cancer cells and this was mediated by decreased abundance of phosphodiesterase 1A (PDE1A) decreasing cGMP hydrolysis and in consequence enhanced PKG activity. Strikingly, enhanced PKG-activity led to a dramatic increase in the phosphorylation of the bona fide PKG substrate vasodilator stimulated phosphoprotein (VASP). As a potent actin polymerase, VASP not only bundles actin filaments but also promotes their elongation and prevents capping proteins from binding to filament ends, thus enhancing dynamic actin assembly. Phosphorylation by PKGI at S239, however, inhibits the association of VASP with filamentous F-actin, thereby modulating actin filament interactions and cellular motility. Phosphorylated VASP reduces F-actin polymerization, a prerequisite for metastatic migration and conveys polarization of functionally active cytotoxic T cells within the tumors. A similar observation was made when PDE1 activity was inhibited providing two novel druggable targets for future cancer therapies.

Next, Robert Feil from the Institute of Biochemistry at University Tübingen (Germany) presented intriguing results on the important role of the cGMP-PKG signaling axis in the tumor vasculature during the talk entitled "NO/cGMP signaling in the tumor microenvironment." The prominent role of cGMP-PKG in maintaining vascular homeostasis and BP control is well established in the field, with its disease-impacting role on the tumor microvasculature is only starting to emerge (Dhayade et al. 2016; Kemp-Harper & Schmidt 2009; Stehle et al. 2023). The tumor vasculature represents distinct features compared to the physiological system that selectively impairs immune cell infiltration and the efficacy of anti-cancer drugs (Chen et al. 2003; Fukumura & Jain 2007). Feil and colleagues located protein expression in the tumor vasculature predominantly to the pericytes, multi-functional mural cells of the microcirculation that wrap around endothelial cells that line the capillaries. They investigated the role of pharmacological and genetic modulation of NO-GC, the cGMP-generator, on tumor growth in murine melanoma and breast cancer models using sophisticated imaging techniques to visualize cGMP fluxes in real-time. Here, they demonstrated that genetic NO-GC ablation in pericytes decreased tumor growth, while pharmacological activation of NO-GC stimulator increased growth and substantiated this observation convincingly in an in vitro human vascular organoid model.

The session was concluded by the third speaker, Emelie Janke from the Institute of Pharmacology Hannover Medical School (Germany) who introduced the "Anti-proliferative effect by cyclic nucleotides in human glioblastoma and neuroblastoma cell lines." They widened their investigations and analyzed the impact of a broad range of cyclic nucleotides cGMP, cAMP, cCMP, cUMP on proliferation of HEK293 cells as well as of a glioblastoma U373 and a neuroblastoma SK-N-BE(2) cell line and observed a time- and concentration-dependent anti-proliferative effect conveyed by all tested cyclic nucleotides. Janke and colleagues pointed out that the metabolism of cyclic nucleotides within the

cancer cells is of utmost importance, representing a target for cancer-therapy. They demonstrated that multi-drug-resistant proteins (MRPs), "energy-dependent drug-pumps" with broad substrate specificity within tumor cells are responsible for the efflux of cyclic nucleotides as when inhibited with probenecid, a lower number of tumor cells was observed.

The data presented in this session unveiled important novel targets for cancer therapies and highlighted important aspects that require a deeper understanding and individualized approaches. In essence, they demonstrated the highly heterogenous cell-type specific actions of cGMP in immune and tumor cells where enhancing of its signaling conveys therapeutically beneficial anti-metastatic effects compared to its role in pericytes of the tumor microvasculature where NO-GC-generated cGMP reshaped the vessel characteristics to enhance tumor growth. Whether this has relevance in defining therapeutic approaches needs to be further investigated.

Ferid Murad Memorial Lecture

Professor Alexander Pfeifer from the University of Bonn (Germany) gave an invited talk entitled "Regulation of metabolism by cGMP: focus on adipose tissue and obesity" as Ferid Murad memorial lecture. He discussed his research on the role of cGMP signaling in adipose tissue metabolism and its implications for obesity. Professor Pfeifer has strongly contributed to the cGMP field with a number of studies demonstrating that cGMP is a key second messenger involved in cell metabolism (Hoffmann et al. 2016; Pfeifer et al. 2013; Sanyal et al. 2017). His research revealed the crucial role of cGMP in adipose tissue function, promoting brown adipose tissue (BAT) differentiation and activation, enhancing energy expenditure through non-shivering thermogenesis in BAT, and affecting white adipose tissue (WAT) metabolism.

The lecture highlighted observations that obesity leads to dysregulation of the cGMP pathway, particularly in visceral fat. Murine studies showed cGMP cascade disruption in gonadal (visceral) but not inguinal (subcutaneous) fat of obese mice, and human studies confirmed defective cGMP signaling in visceral fat of obese subjects. Professor Pfeifer emphasized the significant role of obesity-induced inflammation in cGMP dysregulation, noting that TNF-α suppresses key components of the cGMP pathway in white adipocytes, with NF-κB mediating TNF-α-induced suppression of sGCβ1, and JNK signaling repressing PKG and suppressing PPARγ and aP2. The lecture also presented novel findings on the role of cGMP in glucose metabolism, revealing that NPs promote glucose uptake in adipocytes via cGMP-dependent mechanisms, independently of insulin and involving mTORC1/2 and Akt signaling. These findings are consistent with recent research of the Collins group showing that PDE9 KO mice resist diet-induced obesity through increased energy expenditure and enhanced fat tissue browning. Taken together these data demonstrate the importance of cGMP signaling in adipose tissue metabolism and its potential as a therapeutic target for obesity and related metabolic disorders. The speaker concluded the lecture by discussing the therapeutic implications of these findings, suggesting that targeting the cGMP signaling pathway could be a promising approach for regulating energy expenditure and potentially improving insulin sensitivity and glucose metabolism in obesity treatment.

NO-GC structure, modulators, function

The following Session 4 "NO-GC Structure, Modulators, Function" was chaired by Miriam Cortese-Krott (Düsseldorf, Germany), Andreas Friebe (Wuerzburg, Germany). This session focused on novel or very recently published cutting-edge research on structure and novel modulators of NO-GC and its role in vascular physiology and therapeutic implications.

The session opened with a presentation by Michael Marletta (Berkeley, USA), entitled "Molecular aspects of sGC activation and stimulator function." In his talk, Marletta provided an in-depth analysis of the molecular architecture and regulatory mechanisms of NO-GC (Horst et al. 2019; Wittenborn et al. 2023). Specifically, Marletta reported on the molecular mechanisms of activators and stimulators of NO-sensitive guanylyl cyclase. The heterodimeric enzyme is composed of one α and one β subunit. In their C-termini, both subunits contain a catalytic domain. The catalytic domains of both subunits form the active site and a pseudo-symmetric site that is catalytically inactive. The N-terminal regions of both subunits form the heme-binding site homologous to the H-NOX (haem-nitric oxide/ oxygen) family of proteins and a predicted coiled-coil region between the heme-binding and catalytic regions. Earlier studies revealed that more than one molecule NO is required for activation of the enzyme; alternatively, one molecule NO in the presence of a GC stimulator/NO-sensitizer is sufficient for full activation. By using cryo-electron microscopy, the conformational changes underlying activation of the enzyme by NO binding to the heme were analysed. Binding of NO and an NO-GC stimulator leads to straightening of the coiled-coil domain and rearranges the catalytic domains into the active conformation. Interestingly, the cryo structures revealed that stimulators only fit into their binding pocket after the coiled-coil adopted the straightened conformation. Based on the cryo-structures, mutants were generated that are forced into either the inactive or the active/straightened conformation. In sum, the cryo electron microscopy structures and the mutants shed a new light on the molecular mechanism of NO-GC activation.

The next speaker, Jon O. Lundberg (Stockholm, Sweden), delivered a talk entitled "Is NO-ferroheme a signalling Entity?." Lundberg discussed the emerging concept of NO-ferroheme as a distinct and functional signaling molecule controlling vascular physiology (Kleschyov et al. 2023). Unlike free NO, NO-ferroheme exhibits unique stability and can activate the sGC–cGMP–PKG pathway independently of free NO intermediates. Experimental results demonstrated its ability to induce vascular relaxation and hypotension in animal models, particularly under conditions of NOS inhibition. He demonstrated that (sub-) nanomolar concentrations of NO-ferroheme dilates blood vessels. This vasodilatory response was blocked by the NO-GC inhibitor ODQ that oxidizes NO-GC's heme. Furthermore, the vasodilatory response was only marginally reduced by the NO scavengers Carboxy-PTIO and RBCs. In stark contrast, both scavengers almost completely blocked the dilatory effects of NO. Almost no NO-release was observed from NO-ferroheme, two orders of magnitude lower that from the NO donor DEA-NONOate. DEA-NONOate (1 μM) blocked mitochondrial respiration by interfering with the respiratory chain whereas NO-ferroheme (1 μM) did not. In rats, NO-ferroheme caused stronger BP reductions than equivalent NO concentrations. The resilience of NO-ferroheme to scavengers and its physiological implications underscore its pivotal role in vascular signaling, challenging traditional paradigms of NO bioactivity.

The third presentation was given by Mark Gladwin (Baltimore, MD, USA), and was entitled "Formation and signaling by an NO-ferroheme complex provides a novel pathway for intravascular and intracellular NO signaling." His lab proposed that NO-ferroheme complexes, formed through rapid reactions between NO and ferric heme in the presence of reduced thiols are capable of mediating stable and efficient NO signaling (DeMartino et al. 2023). They demonstrated that NO-ferroheme signaling may pass and survive hemoglobin/myoglobin-rich environments where traditional NO diffusion is constrained. Due to the lipophilic nature of heme, No-ferroheme can cross cell membranes and in plasma can bind to albumin. In the presence of oxyhemoglobin that readily scavenges NO, NO-ferroheme is rather stable. NO-ferroheme inhibited platelet aggregation and led to transient BP reductions in mice treated with the NOS inhibitor L-NAME. In sum, convincing data were presented that NO-ferroheme may mediate the effects of NO in the vasculature. This novel paradigm highlights the interplay between nitrite reduction, RBC endothelial NOS, and NO-ferroheme stability, offering new avenues for therapeutic exploration in vascular pathologies.

The session concluded with a presentation by Thorsten Kessler (Munich, Germany), entitled "cGMP in atherosclerosis: genetic and mechanistic insights." Thorsten Kessler pointed to the importance of NO/cGMP signaling in coronary artery disease. He reviewed the influence of common genetic variants associated with NO-cGMP pathways on coronary artery disease risk as described in published and unpublished work (Dang et al. 2020; Wobst et al. 2018). Although it is well known that reduced expression of NO-GC is a risk factor for coronary artery disease, the accompanying BP increases are not sufficient to explain increased atherosclerotic plaque formation. However, in mice lacking NO-GC selectively in platelets, increased atherosclerotic plaque formation can be observed. This can be attributed to increased leucocyte adhesion caused by reduced angiopoietin 1 release by NO-GC-deficient platelets. Similarly, also human platelets from risk allele carriers (reduced NO-GC content) displayed reduced angiopoietin 1 release. In platelets of WT mice, stimulation by the NO-GC stimulator BAY-747 increased angiopoietin 1 release and the supernatant of these platelets reduced neutrophil adhesion to endothelial cells. Lastly, Thorsten Kessler presented data on a genetic variant (rs7678555) linked to coronary artery disease in patients which is located in proximity of the PDE5A gene. Preliminary data obtained using knock down and overexpression in vascular SMCs suggest that the variant leads to enhanced expression of a long non-coding RNA that indirectly enhances PDE5A expression. In sum, besides genetic variants reducing activity of NO-GC also variants increasing activity of PDE5 represent monogenetic forms of coronary artery disease.

Emerging fields

The session entitled "Emerging fields" was chaired by Peter Sandner (Bayer AG, Wuppertal, Germany) and Robert Lukowski (University of Tübingen, Tübingen, Germany). This session focused on innovative findings in NO and cGMP signaling, with implications for cellular mechanisms and disease therapeutics.

The session was opened by Paola Corti (Baltimore, MD, USA), who presented on "Cytoglobin Regulates CO-dependent cilia motility and organ laterality during development." Her team demonstrated that Cygb2 deletion in zebrafish led to reversed heart orientation due to disrupted ciliary structure and reduced cGMP levels, linked to NO signaling defects. These defects were restored by exposure to NO donors or sGC stimulators, indicating cytoglobin's critical role in developmental NO signaling pathways. Similar disruptions were observed in cytoglobin-KO mice, underscoring its conserved function across species and highlighting its potential relevance in treating congenital ciliary disorders.

Svenja Stomberg (Braunschweig, Germany) introduced the "Discovery of the first isofrom-specific sGC activator." Runcaciguat selectively activates the GC-1 isoform while antagonizing GC-2, exposing previously unknown structural and functional distinctions between sGC isoforms. Their

findings support the hypothesis that precise modulation of GC-1 without affecting GC-2 may benefit patients with diseases involving dysregulated NO-sGC pathways.

Essam Metwally (Rahway, USA) presented a talk entitled "Structural insights into selective small molecule activation of PKG1α." His group identified allosteric activators that bind near the nucleotide binding domain of PKG1α, causing a structural shift that activates the enzyme without altering cGMP levels. Crystallographic data suggest these activators may provide an alternative route for regulating PKG1α, with potential therapeutic applications in vascular diseases, particularly in conditions like pulmonary arterial hypertension (PAH).

Leopold Jahn (Kassel, Germany) had a talk entitled "switchSENSE technology reveals distinct activation mechanisms for PKGI." His team demonstrated that cAMP induces a unique PKG I conformation distinct from that caused by cGMP. Using fluorescence proximity sensing and hydrodynamic diameter measurements, Jahn revealed how cAMP and cGMP shape PKG I's structural landscape. This research provides valuable insights into the specific structural states induced by each cyclic nucleotide, suggesting that distinct PKG I conformations could be selectively targeted in therapies for diseases involving cAMP/cGMP signaling interactions.

Crosstalk

The session entitled "Crosstalk" was chaired by Jon O. Lundberg (Stockholm, Sweden) and Michaela Kuhn (Wuerzburg, Germany). This session examined the interactions of cGMP signaling with other pathways via PDEs modulation and the role of cGMP in cellular crosstalk in cardiac and vascular cells, exploring novel therapeutic opportunities for heart disease.

John Pernow (Stockholm, Sweden) presented a talk entitled "Cardioprotective effects of erythrocytes mediated bysoluble guanylate cyclaseand release of cGMP." The findings of his team demonstrated that RBCs exposed to hypoxia release cGMP, activating cardiomyocyte PKG, enhancing cardiac function, and reducing infarct size in a bioassay Langendorff heart model. These protective effects were fully lost if RBCs from global sGC KO RBCs were used. Moreover, these effects were enhanced by nitrate administration both in mice as well as in humans. Pernow proposed that pharmacological enhancement of RBC-derived sGC could serve as a promising therapeutic strategy in ischemic heart disease.

Viacheslav O. Nikolaev (Hamburg, Germany) highlighted the "Regulation ofcardiac contractilityandhypertrophyby cGMPnanodomains." Focusing on the regulatory functions of PDE1 and PDE3, the team led by Nikolaev found that PDE1 inhibition boosts cGMP pools in NO-dependent signaling pathways, improving relaxation in cardiomyocytes. In contrast, ischemia-induced modifications in PDE3 suggest a maladaptive role in hypertrophy. These insights underscore the potential of PDE-targeted therapies to modulate localized cGMP signaling, offering new approaches for managing HF.

Michael Russwurm (Potsdam, Germany) explored "NO-induced cGMP in the heart:role of phosphodiesterases in physiology and pathophysiology." His team used a FRET-based cGMP indicator to show that NO-cGMP amplifies β-adrenergic cAMP responses in healthy myocytes by inhibiting PDE3. However, in hypertrophic hearts, increased PDE2 expression redirects cGMP to suppress cAMP responses, potentially exacerbating disease. These findings suggest that selective modulation of PDE activity may allow fine-tuning of cGMP and cAMP pathways in heart disease.

Virta Wagde (Würzburg, Germany) presented findings on "Role of phosphodiesterase 2 in ischemia-induced endothelial barrier dysfunction and cardiac inflammation." Her group found that TNFα-induced upregulation of PDE2 weakens the endothelial barrier by lowering cAMP and cGMP levels, increasing immune cell infiltration. Pharmacological inhibition of PDE2 mitigated TNFα-induced adhesion protein expression and preserved barrier integrity. Wagde's work identifies PDE2 as a promising target for reducing inflammation and vascular permeability in ischemic heart conditions.

Evening lecture

The evening lecture was delivered by Joseph Loscalzo from Harvard Medical School (Cambridge, MA, USA). Loscalzo is Hersey Professor of the Theory and Practice of Medicine and is a pioneering figure in the field of network medicine, has significantly advanced our understanding of complex diseases and opened new avenues for innovative treatments. His work in network medicine has been instrumental in shaping the field, providing valuable insights into the complex network of interactions that underlie human diseases and paving the way for the development of new and more effective treatments.

His research has not only advanced our understanding of complex diseases but has also opened new avenues for innovative treatments. In 2007, Loscalzo et al. published "Human disease classification in the postgenomic era" (Loscalzo et al. 2007). This paper established Network Medicine as a complex systems approach to understand human pathobiology. The authors propose a new classification system for human disease based on molecular and phenotypic features, rather than traditional symptom-based classifications. This approach has the potential to revolutionize the way we diagnose and treat diseases.

One of Loscalzo's most influential works is the 2011 publication, "Network medicine: a network-based approach to

human disease" (Barabasi et al. 2011). This paper provides a comprehensive overview of the principles that govern cellular networks and their implications for understanding disease. The authors argue that diseases are rarely the result of a single gene mutation, but rather the product of complex interactions within the cellular network. This perspective has shifted the way scientists approach the study of disease, leading to new insights and potential treatments.

The complex nature of polygenic diseases encompasses a broad range of underlying pathologies arising from heterogeneous molecular mechanisms and exhibiting a diversity of disease phenotypes. This complexity can be formalized using molecular interaction networks (e.g., the protein–protein interaction network) and analyzed using the basic tenets of graph theory. Among the features of these networks that can be discerned are included subnetworks that are specific to a particular disease (i.e., a disease module), unique mechanisms or pathways that govern the disease phenotype, and the identification of potential drug targets that exist within or near the disease module.

In his presentation entitled "Network approach to drug target identification and drug combinations: implications for cGMP-based therapeutics," Loscalzo discussed the computational approaches used to identify disease modules and drug targets, including approaches to drug repositioning or repurposing; experimental approaches to characterizing the dynamic interactions of drugs and their targets in real time in single cells; and efficient single-cell strategies for assessing combinations of drugs. These principles are illustrated with examples from specific diseases, and their implications for cell signaling pathways, including cGMP signaling, are considered. The strategies presented will ultimately contribute to and guide the ongoing evolution of precision medicine.

Loscalzo's work continues to inspire researchers around the world, and his contributions to the field of network medicine will undoubtedly continue to have a profound impact in the years to come.

Novel aspects of natriuretic peptide signaling

Natriuretic peptides (NP), discovered in the 1980s, represent a group of peptide hormones mainly comprising atrial (ANP), brain (BNP) and C-type (CNP) natriuretic peptides that are synthesized and secreted in and from the heart with three corresponding receptors, NPR-A, NPR-B, and NPR-C. Among their well-described physiological effects are the regulation of BP, fibrotic remodeling, fat metabolism, long-bone growth by impacting on cGMP and cAMP signaling (Chabardes et al. 1987; Goetze et al. 2020; Potter et al. 2009).

The first speaker of this session, Jean-Pierre Saint-Jeannette from the Department of Molecular Pathobiology (New York, NY, USA), during the talk "The functions of Npr3 during embryonic development" brought a new facet to the long-term established NP field and focused on their role in cell fate in the ectoderm of the developing *Xenopus* embryo. Using gene silencing approaches as well as pharmacological inhibitors and corresponding rescue experiments, they discovered a prominent role of cooperative action of Npr-1 with Npr-3 and the NP Nppc with strong influence on the cranio-facial skeleton and paired sensory organs development. Their important studies postulate a dual-function of Npr-3 during embryogenesis by spatio-temporari fine-tuning cGMP and cAMP levels in the segregation of these cell populations. Undoubtedly, these observations are of high translational relevance by aiding to explain and predict the impact of inherited mutations in the NPR-signaling system (Bartels et al. 2004).

The next speaker in the session, Sheila Collins from Vanderbilt University Medical Center (Nashville, TN, USA) discussed "Natriuretic peptide signaling – some old, some new – and roles in adipose biology." She approached the field from the metabolic angle and presented data on the role of NP signaling in adipocytes. It is well described that ANP and BNP exert their actions via NPR-A and CNP via NPR-B, while NPR-C represents the negative regulator that binds all three and leads to their degradation (Egom 2021; Rose & Giles 2008). In consequence the abundance of NPR-A and NPR-B versus the "clearance" receptor NPR-C dictates the signaling strength of generated cGMP. Collins and colleagues proposed here another mechanism on how NPR-C might additionally affect NPR-A/B signaling, namely via formation of receptor heterodimers that are subject of enhanced degradation with a decline in cGMP formation as an immediate consequence. Adipose tissue plays an unequivocal role in whole body metabolic homeostasis. In adipocytes, ANP and BNP exposure has strong lipolytic effects (Sengenes et al. 2000) as well as impacts browning and thermogenesis (Kimura et al. 2021). Collins and colleagues demonstrated that mRNA expression of two "negative" regulators of NPR-A/B signaling, NPR-C as well as phosphodiesterase 9 (PDE-9), are downregulated in adipocytes following beta-adrenergic stimulation of lipolysis/thermogenesis and may thus represent an interorgan crosstalk between ANP-released from the heart signaling to adipose tissue to match an increase in demand and protect from metabolic disease. Another important risk factor for the development of hypertension and heart hypertrophy besides metabolic derailment is increasing age, which is of utmost importance given the prevailing ageing and comorbid population (Prickett et al. 2024; Sangaralingham et al. 2011).

Thus, the third speaker of this session, Michaela Kuhn from the Institute of Physiology, University Wuerzburg (Germany), presented her work "Endothelial natriuretic peptide receptor-C: all clear?" that dissected the role of the NPR-C -the receptor lacking a GC domain- regarding

cardiovascular ageing in endothelial-specific KO mice. Importantly, neither age-dependent increase in BP nor cardiac fibrotic remodeling differed between KO and control mice, suggesting no direct involvement of endothelial NPR-C in these pathologies. However, importantly, age-dependent cardiac enlargement and hypertrophy were blunted and systolic and diastolic functions preserved in the absence of NPR-C in endothelial cells. This preservation in function was attributed to the elevation of cardiac cGMP levels that has been described before to protect from afterload-induced contractile dysfunction and hypertrophy (Blanton 2020; Mangmool et al. 2023; Petraina et al. 2022), thus positioning chronic NPR-C as a cardioprotective strategy to counteract a global disease.

Overall, this session intensified our current knowledge on NP signaling by adding novel aspects on its role in unperturbed embryonic development, a novel interorgan heart-adipose tissue signaling axis and the treatment of age-related comorbidities.

Natriuretic peptides, their receptors and beyond

This session intertwined with the previous session I as the focus was on NPs and their respective receptors. However, in this session, structural, mechanistic, and technological insights were in the limelight. NPs generated and released from the heart bind to the extracellular domain of their receptors NPR-A, B, C and in the case of NPR-A and B their binding activates the intracellular GC-domain that leads to increased generation of cGMP. However, the precise conformation and structural changes evoked in the receptor to NPs -bound vs. unbound state would in the future allow the precise generation of circumstance-specific pharmacological modulators.

The first speaker, Xin-Yuan Huang from the Department of Physiology and Biophysics, Cornell University (New York, NY, USA) presented "Unveiling the structure and activation mechanisms of full-length ANPR," his groundbreaking work combining cryo-electron microscopy with functional assays and molecular dynamics simulations to unveil the structure of the single-pass transmembrane full-length human NPR-A in the absence and presence of bound ANP. The application of ANP and BNP to patients with hypertension is severely limited due to the very short half-life of both, which is between approximately 2 min for ANP and 20 min for BNP and are consequently not applicable for oral administration. However, the generation of longer-lasting variants such as M-ANP definitively serve as promising additions to the anti-hypertensive regime (Liu et al. 2024).

In this context, the second speaker, Ana Calejo (Oslo, Norway), presented highly translatable data on the implementation of novel small molecule allosteric modulators of GC-A activity for the treatment of patients with cardiovascular diseases. In her talk, "Novel allosteric modulators of guanylyl cyclase A activity," data were presented on selective small molecular allosteric enhancers of NPR-A that increase the efficacy and potency of ANP and BNP in their ability to activate GC-A and cGMP production and on tolerability of the substances validated in an animal model.

The impressive impact of CNP on long-bone growth led to the groundbreaking discovery and clinical implementation of the drug vosoritide that is in clinical use for the treatment of the rare disease achondroplasia in children (Savarirayan et al. 2024; Simran et al. 2023). Treatment with vosoritide, a CNP analog, requires daily subcutaneous injections for several years and given that the patients are very young children, represents a substantial burden for patients and their relatives. Eric Schneider from ProLynx in San Francisco during his talk entitled "Development of a long acting c-natriuretic peptide suitable for QMo dosing" presented data on the development and validation of a long-acting CNP-analog conjugate that conveyed equal to greater efficacy when compared to vosoritide but requires less frequent—from 30 injections per month vosoritide to 1 injection using the novel analog—and hence more patient-friendly convenient dosing. This was achieved by employment of the half-life extension technology fusing linkers and involving the application in a polyethylene-glycol hydrogel microsphere that serves upon injection as a stationary depot with slow release. The novel analog was tested in mice and monkeys.

Arrhythmia is frequently occurring and manifests as an irregular heart rhythm in patients after myocardial ischemia or in HF (Kingma et al. 2023). Therapies often involve ablation therapy as drugs that affect action potential duration might lead to adverse side effects. Thus, identification of novel targets for an improved anti-arrhythmic therapy are invaluable. On the single cell level, a disbalance between overactivation of the beta-adrenergic signaling system due to high levels of circulating catecholamines leading to cellular cAMP overload in combination with diminished protective cGMP signaling has been described as one potential cause of the disease. Susanne Kämmerer from the Institute of Pharmacology and Toxicology, Technical University Dresden (Germany) proposed "The cGMP-induced PDE2 stimulation as a novel antiarrhythmic strategy" (Vettel et al. 2017). PDE-2 is a nodal point that converges the cAMP and cGMP signaling pathways by enhancing cAMP hydrolysis in response to cGMP-stimulation. Consequently, the data confirmed that exposure to NPs or stimulation of NO-GC conveyed anti-arrhythmic effects in murine models of cardiovascular disease.

In essence the talks presented in this innovative session provided proof-of-concept for the drugability of the NP-cGMP signaling system in the treatment of diseases as diverse as hypertension, achondroplasia and arrhythmia and

suggests that due to the ubiquitous presence of this prominent pathway in cells, tissues and organs, many more therapeutic options might arise during future research endeavors.

Technological advances & new applications

The Session entitled "Technological advances & new applications" was chaired by Dennis J. Stuehr (Cleveland, OH, USA) and Susanne Kämmerer (Dresden, Germany). This session aimed to broaden the scope of cyclic nucleotide research, covering non canonical roles in sensory transduction, optogenetics, pain and infectious disease.

During the first talk of the session Jian Yang (New York, NY, USA) presented his findings on "Conformational landscape of cGMP activation of the human cone photoreceptor CNG Channels." His work revealed that lipid interactions stabilize certain activation states, suggesting the membrane environment plays a critical role in tuning CNG channel responses. These findings enhance our understanding of CNG channelopathies and potential avenues for therapeutic modulation in visual disorders.

The following talk was by Shiqiang Gao (Wuerzburg, Germany), who presented "Development and applications of optogenetic tools for manipulation of cyclic nucleotides." His team introduced light-activated enzymes, such as Cyclop from *Blastocladiella emersonii*, to enable precise spatiotemporal control over cAMP and cGMP in targeted cells. These tools offer promising applications in cardiovascular, neurological, and metabolic research, providing a non-invasive method for controlling cyclic nucleotide pathways implicated in disease.

The pharmacologist Achim Schmidtko (Frankfurt, Germany) presented novel findings on PDE10A inhibition as a non-opioid approach to pain management. His study, entitled "Phosphodiesterase 10A inhibitors reverse acute and inflammatory pain in mice" showed that PDE10A inhibition elevates cGMP in pain-processing neurons, reducing acute and chronic inflammatory pain without developing tolerance. The experimental work by the team of Dr Schmidtko identifies PDE10A as a viable target for pain relief, addressing a critical need for effective and safe analgesic options.

Purnima Bhanot (New Jersey) reported on "PfPKG inhibitors for malaria chemo prevention." Her research demonstrated that P. falciparum PKG (PfPKG) is essential for multiple stages of the parasite life cycle. By selectively inhibiting PfPKG, they were able to block parasite invasion and egress in host cells, providing a basis for chemopreventive therapies that target parasite-specific pathways without host toxicity.

The session was closed by an interesting talk by the pharmacologist Harald Schmidt (Maastricht, Netherlands) entitled "Extending apo-sGC to haem-sensing proteins and a landscape of haem mimetic pharmacophores with broad applications." His team found that apo-sGC activators, developed for cardiovascular use, interact with a range of heme-responsive proteins beyond sGC, including REV-ERB receptors which are involved in metabolic regulation of circadian rhythm (Cho et al. 2012). These interactions suggest a broader pharmacological reach for heme-mimetic drugs, underscoring the need to account for off-target effects in their development.

Special session of the DPGT

The last session of the program was a Special Session led by the Chair of German Society for Experimental and Clinical Pharmacology and Toxicology (DGPT) Professor Roland Seifert (Hannover Medical School). Professor Seifert awarded the Naunyn–Schmiedeberg Medaille to Professor Franz Hoffman (Flockerzi & Biel 2024).

Outlook

The 11th anniversary meeting of the cGMP conference in 2024 was filled with novel insights into a non-canonical cGMP signaling and its role in cell–cell cross talk but also in novel potential therapeutic approaches of stimulators and activators of NO-GC. Specifically, the approval of two NO-GC stimulators for PH and HF, the success of a neprilysin inhibitor, and the presentation of clinical studies with NO-GC activators, the field seems to be expanding. There was also new data on novel compounds and treatment approaches of which some already started clinical testing. These preclinical and clinical data raise hope that in the future cGMP research may lead to new therapies for diseases like neurodegeneration or nephropathies. Therefore, the community is very much looking forward to the 12th cGMP conference which is currently planned to take place on June, 18th to 20st, 2026 in Kassel, Hesse Germany.

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