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MEETING ABSTRACTS

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ORAL PRESENTATIONS

Session 1 | Pre-Clinical Translation & Back-Translation

O1

Applying translational approaches for the nonclinical and clinical evaluation of the sGC stimulator CY6463 in CNS diseases

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Introduction: The NO-sGC-cGMP pathway plays a critical role in central nervous system (CNS) function and is impacted across a range of neurological and psychiatric diseases. NO is recognized as a key neurotransmitter that is produced on-demand within the CNS and can act through sGC and cGMP to govern a range of downstream effects. We have identified CY6463, a CNS-penetrant sGC stimulator, with demonstrated pharmacological effects in nonclinical and clinical studies. By acting as a selective positive allosteric modulator of sGC, CY6463 can amplify endogenous NO signaling while maintaining upstream spatial and temporal regulation. This enables the on-demand production of cGMP and propagation of downstream signals within the CNS.
Methods: A range of nonclinical studies were conducted to understand the in vitro and in vivo properties of CY6463 and supported advancement into clinical development. Phase 1 clinical studies included single-ascending dose, multiple-ascending dose and food interaction studies along with a translational pharmacology study in healthy elderly participants.
Results: This presentation will describe the nonclinical pharmacology of CY6463, along with clinical data from Phase 1 studies including the pharmacokinetic, safety, and pharmacodynamic results of our clinical translational pharmacology study in elderly participants. Furthermore, we will discuss our translational biomarker strategy that has been carried through into clinical studies in three separate patient populations and provide outlines of these clinical studies and updates on progress to date.
Conclusions: Applying a translational biomarker based approach to the development of CY6463 has enabled advancement of clinical studies in well-defined patient populations to help understand the potential opportunity for modulating sGC function in neuropsychiatric and neurodegenerative diseases.
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O2

sGC modulators as cognitive enhancers: neuronal and/or vascular?

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Introduction: Cognitive impairment is one of the main symptoms of Alzheimer's disease or Vascular dementia, which negatively impacts the quality of life of patients. Therefore, a pharmacological intervention that has memory enhancing effects would be beneficial to patients. Vascular dementia is characterized by impairments in cerebral blood flow, endothelial function and blood–brain barrier integrity. These processes are all physiologically regulated by the soluble guanylate cyclase (sGC)-cGMP signaling pathway in blood vessel cells. Additionally, neuronal cGMP signaling plays an important role in long-term potentiation underlying memory formation. Therefore, targeting the NO-sGC-cGMP pathway may be a therapeutic strategy for treating neuronal- and/or vascular-based dementias.
Methods: sGC stimulators acting on heme-bound sGC and one sGC activator acting on heme-free sGC were tested in the object location task (OLT) on acquisition memory processes, in healthy rodents and in deficit models. Vascular function and neuroplasticity were assessed.

Results: The non-brain penetrant sGC stimulators riociguat and vericiguat improved memory acquisition in the OLT in rodents. Riociguat attenuated memory deficits in a cerebral vasoconstriction model for memory impairment induced by sumatriptan. The effective doses of vericiguat had no effect on mean arterial blood pressure and cerebral blood volume. This suggests that non-brain penetrant sGC stimulators improve memory via an effect on the cerebral microvasculature. The brain penetrant sGC stimulator BAY-747 and activator runcaciguat both improved memory acquisition in the OLT in rats. Interestingly only BAY-747 reversed the NOS inhibitor L-NAME induced memory impairment. Both BAY-747 and runcaciguat increased mBDNF levels in the hippocampus. Both drugs also enhanced GluA1-containing AMPA receptors trafficking in a chemical LTP model for memory acquisition using mouse hippocampal slices. Yet, only for runcaciguat this involved phosphorylation of the receptor on S845.
Conclusions: Both sGC stimulators and activators have potential as cognitive enhancers. sGC stimulators have effects on microvasculature as well as neuroplasticity. Effects on neuroplasticity are also exerted by sGC activators, yet with different underlying mechanisms than sGC stimulators. Further elucidating these properties will help in determining which type of sGC modulator can optimally improve cognition in a vascular and/or neuronal type of dementia.
Funding: Funding was in part supported by a restricted grant from Bayer AG and Merck Sharp & Dohme Corp.

O3

Discovery and preclinical profiling of the oral sGC activator runcaciguat (BAY 1101042): a novel and effective treatment approach for chronic kidney disease?

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Peter Sandner is presenting on behalf of the entire research and development teams working on runcaciguat (BAY 1101042) in recent years, especially Michael G. Hahn [1], Thomas Lampe [1], Sherif El Sheikh 1], Niels Griebenow [1], Elisabeth Woltering [1], Karl-Heinz Schlemmer [1], Lisa Dietz [1], Michael Gerisch [1], Frank Wunder [1], Eva Maria Becker-Pelster [1], Thomas Mondritzki [1,2], Hanna Tinel [1], Andreas Knorr [1], Achim Kern [1], Dieter Lang [1],Tibor Schomber [1], Agnes Benardeau [2], Antje Kahnert [1], Laura Popp [1], Julia Vienenkoetter [1], Heidrun Ellinger-Ziegelbauer [1], Mira Pavkovic [1], Axel Kretschmer [1], Bettina Lawrenz [1], Elke Hartmann [1], Krystyna Siudak [1], Alexius Freyberger [1], Ina Hagelschuer [1], Jutta Meyer [1], Jan R. Kraehling [1], Ilka Mathar [1], Joachim Mittendorf [1], Hubert Truebel [2,4], Joerg Hueser [1], Volker Geiss [1], Frank Eitner [1,5], and Johannes-Peter Stasch [1,6];

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Introduction: The class of sGC activators has an unique mode of action by activating the oxidized and heme-free form of sGC which is not responsive to nitric oxide (NO). The sGC activators can restore cGMP signaling under oxidative stress conditions, which is present in a variety of diseases and which could potentially result in a broad therapeutic profile of the sGC activators. However, the first generation of sGC activators exhibits limitations and was discontinued. With the discovery of the novel, oral sGC activator runcaciguat (BAY 1101042), the second generation of sGC activators is available with improved solubility, permeability, metabolism, and drug-drug interactions parameters. The discovery and preclinical profiling of runcaciguat in vitro, ex vivo, and in vivo, in both mechanistic and disease models, will be presented.
Methods: Runcaciguat was broadly profiled in vitro, ex vivo, and in vivo. Runcaciguat was tested in mechanistic in vitro, and ex vivo assays and mechanistically studied in vivo models. Moreover, runcaciguat, was tested in different disease models, especially in models for CKD with different etiologies and comorbidities.
Results: Runcaciguat exhibits the profile of a potent and selective sGC activator, which independently of NO stimulates cGMP production in vitro, on the isolated sGC enzyme as in cellular tests. Ex vivo, runcaciguat can relax vascular tissues in different vascular beds. In vivo, runcaciguat leads to a dose-dependent decrease in blood pressure. In different rat CKD models, in renin transgenic (RenTG) and angiotensin-supplemented (ANG-SD) rats, but also in rats with diabetic and metabolic CKD, e.g. the Zucker diabetic fatty (ZDF) rat and ZSF-1 rat, runcaciguat significantly reduced proteinuria. In addition, biomarkers and histopathological markers of kidney damage were also significantly reduced. These kidney-protective effects were also significant at doses that did not or only moderately decrease systemic blood pressure.
Conclusions: In summary, these data demonstrate that runcaciguat (BAY 1101042) exhibits a typical profile of an oral sGC activator in vitro, ex vivo, and in vivo. Moreover, runcaciguat treatment leads to significant renal protection at doses that do not reduce blood pressure and is effective in hypertensive as well as diabetic and metabolic CKD models. Therefore, the sGC activator runcaciguat could represent an efficient treatment approach for CKD (Figure 1). Runcaciguat is currently developed in phase 2 in patients with chronic kidney disease (CONCORD trial, NCT04507061).

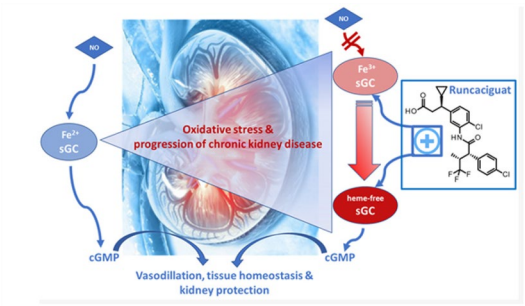

Figure 1. Targeting CKD with the sGC activator runcaciguat (BAY 1101042)

failure or neurological conditions and have reached preclinical studies. They may also have potential as drugs for BPH. Our aim was to investigate and localize PDE9 expression in the prostate as well as in the epididymis as an organ relevant to ejaculation.
Methods: Western blot and immunofluorescence (IF) used a monoclonal rabbit anti-PDE9 antibody. Comparison to PDE9 KO tissue confirmed the antibody could detect PDE9 in rodent and human tissue. The more detailed cellular localization of PDE9 in prostate and epididymis could be determined by probing membrane and cytosolic protein fractions using Western blot. For IF, prostate and epididymis sections of mouse, rat and men were available. RT-PCR was used to detect PDE9 transcripts in human prostatic SMCs and qPCR served to quantify transcripts in different mouse epididymis regions.
Results: PDE9 was found in SMCs in human and rodent prostate, as evidenced by co-localization with the SMC marker smooth muscle actin (SMA) in IF, it was also detected in vascular SMCs. PDE9 could be assigned to the membrane fraction in rat prostate by Western blot. The SMC localization of PDE9 was confirmed by RT-PCR in isolated human prostatic SMCs. Epididymis tissue revealed a staining pattern for PDE9 comparable to the prostate with a signal in the smooth muscle layer surrounding the epididymal duct and vascular SMCs. qPCR data showed a higher expression of PDE9 in the cauda epididymis.
Conclusions: Besides its expression in the epididymis, the SMC localization of PDE9 in the prostate identifies this PDE as a potential therapeutic target in the treatment of BPH. Its membrane localization in these organs supports the idea of interactions of PDE9 with distinct subcellular, e.g. natriuretic peptide-dependent, pools of cGMP.

P26

Brain-penetrant sGC stimulator BAY-747 and activator runcaciguat act as cognitive enhancers via differential neuroplastic mechanisms

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Introduction: Soluble guanylyl cyclase (sGC)-derived cGMP signaling plays a role in both neurons and the vasculature: neuronal cGMP signaling is involved in memory formation, while endothelial cGMP signaling plays a role in vascular health. Therefore, sGC may be a target to treat vascular cognitive impairment (VCI).
sGC can be targeted by stimulators acting on heme-bound sGC or by activators acting on oxidized/apo-sGC. However, first generations of sGC agonists do not cross the blood–brain barrier, potentially limiting the neuronal therapeutic effectiveness. Here, we compare novel brain-penetrant sGC stimulator BAY-747 and sGC activator runcaciguat for their potential as cognitive enhancers.
Methods: BAY-747 and runcaciguat were tested in vivo in the object location task (OLT) in rodents to assess their potential as cognitive enhancers. In addition, a NOS-inhibitor L-NAME-induced memory deficit model was used to elucidate the mechanistic differences. In vivo effects on neuroplasticity were also measured.
Results: Both BAY-747 and runcaciguat enhanced long-term memory in a natural forgetting model of the OLT. Interestingly, only BAY-747 attenuated the memory impairments induced by NOS-inhibitor L-NAME. This shows that sGC stimulators and activators may differ in efficacy depending on the underlying mechanism of memory impairment.
The neuroplastic effects underlying cognitive enhancement by BAY-747 and runcaciguat were assessed in vivo. While runcaciguat was more effective on the glutamatergic system, BAY-747 more strongly enhanced the neurotrophic system.
Conclusions: These data show that brain-penetrant sGC agonists act as cognitive enhancers via neuroplastic effects in vivo. Based on the underlying memory deficit mechanism, sGC stimulators and activators may differ in efficacy. Additionally, while sGC stimulator BAY-747 acts on the neurotrophic system, sGC activator runcaciguat more strongly enhances the glutamatergic system. This further indicates differential effects for sGC stimulators versus sGC activators. Interestingly, BAY-747 and runcaciguat are effective in a similar dose–response window, while BAY-747 shows a larger brain penetration rate (38–60% vs 10–13%). This suggests that pharmacokinetics and enzyme binding kinetics differ between BAY-747 and runcaciguat, which may influence the specific neuroplastic and cognition enhancing effects. Therefore, conclusions on superiority of one sGC agonist over the other cannot be drawn. Nevertheless, brain-penetrant sGC agonists show promise as cognitive enhancers with clear neuronal effects.
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P27

Inhibitory effects of C-type natriuretic peptide on PDGF-BB induced metabolic remodelling of lung pericytes from patients with pulmonary hypertension

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Swati Dabral and Michaela Kuhn contributed equally
Introduction: Pericytes are mural cells regulating the perfusion and barrier functions of the systemic and pulmonary microcirculation. A hyperproliferative phenotype and metabolic switch to higher glycolysis rates contribute to vascular pathologies such as pulmonary hypertension (PH) (1). Increased expression of growth factors such as platelet-derived growth factor-BB (PDGF-BB) has been implicated in the initiation and progression of these pathological alterations. We studied the effect of the endothelial hormone C-type natriuretic peptide (CNP), signalling through the cyclic GMP-producing guanylyl cyclase B (GC-B) receptor (2), on the PDGF-BB-induced hyperproliferative and metabolic switch of cultured human lung pericytes from control donors and patients with PH (1).
Methods: See below.
Results: CNP (0.01–100 nM) markedly and similarly increased intracellular cGMP levels in control and PH pericytes, demonstrating CNP/GC-B/cGMP signalling. These cGMP responses were not altered by PDGF-BB pretreatment (30 ng/ml, 30 min). Upon PDGF-BB stimulation, PH pericytes exhibited higher proliferation coupled with enhanced rate of glycolysis in comparison to control pericytes. Notably, these effects were significantly attenuated by CNP. Mass spectrometry-based metabolomic analyses revealed an upregulation of glycolytic intermediates in response to PDGF-BB, which was prevented by CNP. To dissect the underlying mechanisms, the expression levels of various components of the glycolytic pathway as well as of their well-known regulator hypoxia-inducible factor 1 alpha (HIF-1α) were analysed by immunoblotting. PDGF-BB markedly upregulated membrane glucose transporter 1 (GLUT1) and nuclear HIF-1α levels, and CNP prevented these alterations in both control and PH pericytes.
Conclusions: Taken together, our data show that CNP attenuates both the PDGF-BB-induced hyperproliferation and the increased glycolysis rate of human PH lung pericytes. Mechanistically, the protective actions of CNP are partly mediated by inhibition of HIF-1α-dependent GLUT1 induction. This results in reduction of cellular glucose uptake and concomitant glycolytic flux. To further characterize these findings, we are studying the downstream signalling pathways involved in CNP/GC-B mediated HIF-1α and GLUT1 regulation as well as glucose uptake of cultured lung pericytes. Understanding the effects and signalling