Mechanism-based diagnosis of cyclic GMPopathies and pharmacological interventions

Currently, causal disease mechanisms are not targeted by the prescribed therapies. Imprecise definitions of disease mechanisms prevents precision medicine. For example, reactive oxygen species (ROS) have been viewed solely as detrimental and as a consequence, it has been attempted to treat the appearance of ROS with broadly acting antioxidants. This concept has completely failed to achieve patient benefit. Thus, a new approach was proposed, which specifically targets the dysregulated ROS enzymatic sources, or repairing the affected enzymes, for instance, sGC (**Chapter 2**). sGC is a core enzyme of cGMP signalling, which is involved in physiology but also in pathophysiology of many diseases as a causal pathomechanism. Several compounds targeting the cGMP pathway have been developed in order to restore its dysregulation and some of them have been already authorised for clinical use. However, it is still not fully clear how to identify patients with cGMPopathies and treat only those within a heterogenous disease phenotype (**Chapter 3**).

First, the dichotomic view of sGC and apo-sGC pharmacology was critically reviewed. sGCs, which are already in the clinic, can stimulate not only sGC but also the damaged enzyme form, apo-sGC. sGCs also have synergistic effects in combination with sGCa on both in vitro and in vivo models of apo-sGC. Those synergistic effects were achieved with subthreshold doses of the compounds which indicates that such synergistic low dose drug combinations could be also used clinically (**Chapter 4**). Therefore, less side-effects would be expected, which for instance has been previously the reason for premature trial termination for cinaciguat, an sGCa. The development of sGCa, though, should be viewed carefully. These are not specific apo-sGC compounds, but rather heme-mimetics acting on a broad spectrum of heme-sensing proteins. Moreover, REV-ERB compounds also had effects on apo-sGC. These findings should be considered both in terms of binding promiscuity and off-target effects, but it can also mean that already available compounds can be repurposed and used as modulators for other disease relevant proteins as well.

A remaining knowledge gap was how to stratify the right patients for the right therapy e.g., the ones with cGMPopathy. Otherwise, the majority of previous clinical trials using cGMP interventions would most likely have been successful. A simple precise protocol was developed able to

detect cGMPopathies. It was validated in HFpEF where it showed a subgroup of 30% of the patients having higher levels of apo-sGC, and a subgroup of 25-33% of the patients with elevated NOX5 levels. Thus, these patients who appear to have dysfunctional ROCG signalling would most likely respond to a cGMP intervention. At the moment, the REPO-HFpEF clinical trial is about to start, in which, after ROCG-based biomarker stratification, HFpEF patients will receive an sGC stimulator (now knowing to stimulate both apo- and sGC), in combination with a NOS recoupling treatment. Thus, here the need for theranostic strategies is described, i.e. based on the causal disease mechanism applying the right pharmacological intervention for the right patient, stratified by mechanism-based biomarkers. This will lead to increased precision in medicine and enable the possibility to cure or even prevent diseases.