Comparative evaluation of the effects of tyrosine kinase inhibitors (TKIs) on endothelial function *in vivo* in mice

TKIs block the action of tyrosine kinases that regulate many cellular functions, including cell signalling, growth and division. These enzymes may be overactive in some types of cancer cells, promoting cell growth, which in turn may be effectively inhibited by the use of TKIs.

The introduction of TKIs radically changed the treatment of various cancers, including chronic myeloid leukaemia (CML). However, long-term use of TKIs is associated with unwanted vascular and procoagulant effects, which has a significant impact on the morbidity and mortality of patients due to adverse vascular events. The first introduced TKI was imatinib, however, due to the observed resistance to this drug, TKI of 2nd generation (nilotinib, dasatinib and bosutinib) and then of 3rd generation TKI (ponatinib) were introduced. While unwanted vascular effects have been observed many times with ponatinib and nilotinib treatment, imatinib treatment is considered to be free from significant adverse vascular and prothrombotic effects. These clinical observations suggest distinct effects of various TKIs on the cardiovascular system and vascular endothelium in particular.

As there are no studies that compare in experimental conditions *in vivo* toxicity profile of various TKIs in animals we plan to characterize the effects of TKIs, *in vivo* mice. In particular, we plan to assess whether TKIs, with good and limited safety profile as suggested in clinical studies in humans, would have a similar or different effect on the endothelial function in physiological and pathophysiological conditions in mice (healthy mice and mice with atherosclerosis, respectively). Accordingly, the aim of the study is to comprehensively compare the effects of tyrosine kinases inhibitors (TKIs) on endothelial function**.**

A state-of-the-art methodology based on magnetic resonance imaging (MRI) will be used to assess the effects of TKI on endothelial-dependent responses and changes of endothelial permeability in murine models *in vivo*. The assessment *in vivo* will be based on the detection of impaired vasodilatation induced by acetylcholine, which is related with impaired generation of endothelial nitric oxide (NO) or paradoxical vasoconstriction in response to acetylcholine in advanced stages of endothelial dysfunction. In addition, the endothelial function assessment will be complemented with the assessment of vasodilation resulting from increased blood flow after short-term occlusion (flow mediated-dilatation, FMD), which is the gold standard technique to assess endothelial function in humans. Finally, MR imaging will be also used to assess endothelial permeability with a contrast agent. The above MRI-based methods for the assessment of endothelial phenotype *in vivo* will be supplemented by classical methods of measurements of endothelial function using isolated vessels and biochemical measurements of NO production in *ex vivo* vessels.

We claim that this project will demonstrate whether TKIs (1st, 2nd, 3rd generation) with apparently distinct vascular safety profile in humans have a different effect on endothelium in healthy mice or in murine models of atherosclerosis. As such this project will help to better understand the mechanisms of vascular toxicity of TKIs and should open the novel methodological approach to assess vascular toxicity of compounds in preclinical studies based on the comprehensive assessment of endothelial function *in vivo* using unique MRI-based approach.