Targeting GPVI

Platelets are blood cells that prevent the loss of extensive blood volumes however also contribute to arterial thrombosis. After a vessel is injured, platelets become activated by the exposed collagen and aggregate together, forming a platelet-fibrin thrombus. Whilst this is a crucial process in hemostasis, platelets also become activated due to rupture or erosion of an atherosclerotic plaque in atherothrombosis. Herein, occlusive platelet thrombi are formed, mediated by collagen that is present in the atherosclerotic plaque, leading to for example, myocardial infarction, stroke or transient ischemic attacks. These events are still leading causes of death world-wide, and patients are usually prescribed anti-platelet drugs to prevent a second thrombosis. Whilst the currently used drugs are effective in preventing the thrombosis, they are prone to causing unwanted bleeding events in some of the patients. Therefore, other treatment options are currently investigated. A promising approach is to prevent platelet-collagen interactions via inhibition of the collagen receptor, glycoprotein VI (GPVI).

In this thesis, we compared the various approaches used to interfere in the GPVI-dependent interaction of platelets with collagens. This was done by a whole blood flow chamber set up with collagen coatings, assessing the effects on thrombus formation. In Chapter 3, we investigated the effects of direct inhibition of GPVI on the receptor level with the antibody fragment 9O12, as well as by a recombinant GPVI fusion protein intended to mask GPVI motifs on the collagen fibers. These reagents are already being tested in clinical trials. In Chapters 3-7, we also studied a signaling blocker, PRT-060318, downstream of GPVI; novel anti-GPVI nanobodies (cameloid antibody fragments), and the antibody 6F1 inhibiting the α2β1 integrin, which is another collagen receptor on platelets. Chapters 3-4 in particular show strong effects on thrombus formation of the GPVI or signaling inhibition, which is a very promising finding.

The GPVI-directed nanobodies, are not only excellent tools to inhibit GPVI, but can also be used as imaging tools. In Chapters 4-5 we show how a fluorescently labeled non-inhibitory anti-GPVI nanobody can be utilized to investigate the clustering of GPVI on platelets. This process had never previously been shown in connection with thrombus formation. We also show that the GPVI clustering coincides with a higher degree of platelet activation and an increased thrombus size. These data can help the field to better understand the role of (clustered) GPVI, possibly also to find improved GPVI interventions.

Still additional work will need to be done, in the lab and in clinical trials, to define which approaches to inhibit platelet GPVI will prove to be most effective. In comparison to conventional antibodies, the use of small-sized nanobodies has advantages, but a modification will be needed to improve their half-life in the circulation, given that unmodified nanobodies are quickly excreted.

In Chapter 7 we investigated rare patients with the complex Noonan syndrome, which is sometimes associated with a bleeding phenotype. Platelet defects in these patients stem from a gain of function mutation in the *PTPN11* gene, which interferes in the GPVI signaling activity. We include additional data to the already existing work, using the Maastricht flow chamber to phenotype patient blood samples, in order to gain insight into the underlying mechanism of the platelet defect. In particular we showed that the mutation in these patients only partially affected the capability of their platelets to form thrombi. Based on extensive experiments, we concluded that platelets from patients showed a rescuing effect of the interaction between GPVI and integrin α2β1.

Chapters 3-6 furthermore illustrate the differences between various collagen preparations on the induction of platelet activation and thrombus

formation, as well as the effects on these processes of the tested inhibitors. It is concluded that the preparation of more physiological collagen types is needed for optimal point-of-care testing of blood samples. As well as that a move forward is needed towards more advanced vessel-on-a-chip models.

The studies presented in this thesis are of use to the scientific community as well as the general public, as the microfluidics set up bridges the gap between *in vitro* studies with isolated platelets or blood and *in vivo* animal models as well as clinical trials. Flow chambers provide a well-established micro-technique, where different platelet agonists and flow rates can be applied, thus making it possible to probe for overall effects and drawing additional conclusions about an outcome *in vivo*. This is of importance, as results from mouse studies in atherosclerosis, which process is fairly different between mouse and man, cannot always be translated into effects seen in human disease.

Taken together, with this thesis I come to two major conclusions: *i)* GPVI is indeed a promising target for a next generation of effective and safe anti-platelet drugs; *ii)* the flow chamber is a valuable tool to bridge *in vitro* and *in vivo* scientific studies, and to assist in the diagnosis of platelet-related dysfunctions.