

A humanized monoclonal antibody Fab fragment inhibiting the Von Willebrand Factor – platelet glycoprotein axis as an effective and safe antithrombotic

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EXECUTIVE SUMMARY

The K.U.Leuven Laboratory for Thrombosis Research has generated a humanized monoclonal antibody to the platelet glycoprotein (GP)Ib which may be useful in treating arterial thrombosis, acute ischemic stroke and the orphan disease thrombotic thrombocytopenic purpura. The GPIb antibody has also been shown to be highly instrumental to prevent xenotransplant rejection (porcine into human). The antiplatelet antibody is patented and has been shown to selectively inhibit VWF-dependent blood platelet adhesion to collagen at high shear stress.

The GPIb antibody has shown antithrombotic activity in animal models of arterial thrombosis and ischemic stroke. Preclinical studies show this antibody to have lower incidences of bleeding as compared to currently clinically available antiplatelet agents.

The K.U.Leuven Laboratory for Thrombosis seeks to establish a licensing arrangement or R&D collaboration to advance the humanized GPIb antibody to the clinic.

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ECONOMIC BURDEN AND MARKET

Cardiovascular disease (CVD) is a major cause of death and disability and of rising health care cost around the world. An estimated 16.7 million - or 29.2% of total global deaths - result from the various forms CVD (World Health Report, WHO).

According to the WHO, coronary artery disease (CAD) is responsible for 48% of deaths from CVD. Arterial thrombosis remains one of the most significant causes of CAD. Due to the rapidly aging population, the number of people that are presenting with risk factors for thrombotic events is increasing year by year. Although there are several marketed therapies, they are often associated with unwanted side effects. The vastness of the clinical problem clearly indicates that targeted therapies are needed.

According to the World Stroke Congress 2008, stroke accounts for 5.7 million deaths annually and the incidence is expected to increase by 30% over the next decade. In addition, at least 20 million people survive heart attacks and strokes every year, of which many require continuing costly clinical care. Stroke is already one of the top three causes of death and its incidence in the seven major markets is expected to increase in the next ten years because of an aging population. Long term disability caused by stroke is a major economic burden on healthcare systems, current treatment options are limited and general awareness of stroke is poor.

Antithrombotic drugs are one of the most rapidly growing sectors of the cardiovascular market. The antithrombotics market is expected to peak at just over \$20 billion in 2012, across the seven major markets. The antithrombotics market is dominated by antiplatelets, representing 60% of the total market share in 2007 (Business Insights). Future growth of this segment will be largely dependent on the generation of antiplatelets that combine efficacy with safety, allowing (1) to expand the patient potential and (2) to use antithrombotic drugs for treatment of indications where currently their use is very limited because of increased bleeding risks.

INTRODUCTION

Thrombosis is the formation of thrombus or clot inside a blood vessel resulting in obstruction of blood flow through the circulatory system. There are two forms of thrombosis, namely, a) arterial thrombosis, and b) venous thrombosis.

Arterial thrombosis occurs in arteries where blood is platelet-rich resulting in stroke and myocardial infarction. Venous thrombosis occurs in veins where blood is platelet-poor resulting in conditions such as pulmonary embolism and deep vein thrombosis. Arterial thrombosis is commonly treated by antiplatelet drugs while venous thrombosis is generally

treated by anticoagulants, either alone or in combination with other agents. Conditions where antiplatelet drugs are beneficial include coronary artery disease, cerebrovascular disease, peripheral arterial disease, congestive heart failure, atrial fibrillation etc.

Antiplatelet drugs have been shown to be associated with unwanted side effects like bleeding tendencies. The GPIb antibody developed by the K.U.Leuven Laboratory for Thrombosis has been shown to preventing VWF-dependent steps and thereby to specifically interfere with platelet adhesion and consequent platelet plug formation in the arterial circulation without affecting haemostasis in the slower blood flow vessels. The antibody thus has the potential to make the treatment of thrombosis more effective combined with an improved safety profile.

UNMET THERAPEUTIC NEED

Several antiplatelet drugs are currently used for the prevention and treatment of arterial thrombosis. All these drugs target either the activation or aggregation of platelet, being the late steps in thrombus formation. They do not prevent initial platelet activation and secretion of platelet activating and vasoactive components. Most of the currently used antiplatelet agents such as aspirin and clopidogrel inhibit a platelet amplification loop and by this only attenuate the platelet activation, indeed stronger stimuli can readily overcome the inhibition. GPIIb/IIIa inhibitors have a much more pronounced effect on thrombus formation. Unfortunately this is accompanied by a dramatically increased bleeding risk. Given the fact that an increasing number of people are presenting with risk factors for thrombotic events, there is a real unmet clinical need for developing novel and safer antithrombotic agents.

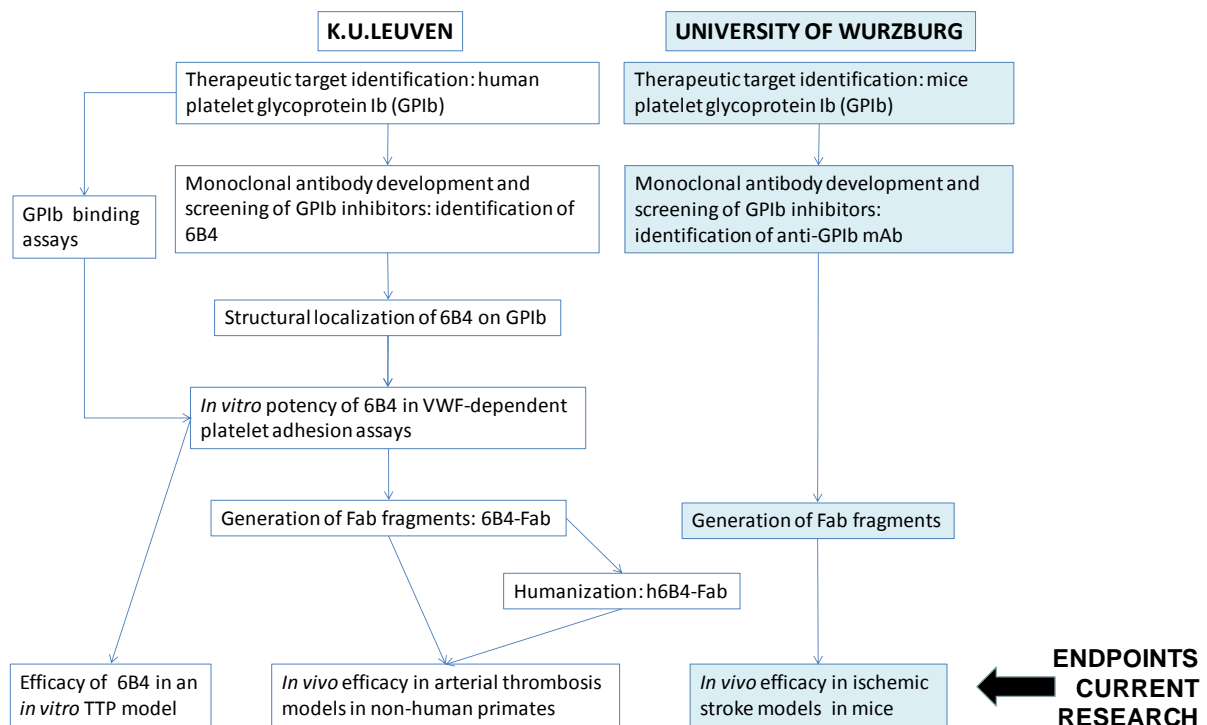
We believe our anti-GPIb antibody has high potential

- in the classical indication for antiplatelets as **an adjunct to PTCA or thrombolysis** for the prevention of cardiac ischemic complications.
- for treatment of **ischemic stroke**, the third leading cause of death and permanent disability in Industrialized Countries and for which treatment options are currently limited in view of the risk of intracerebral bleeding complications.
- for treatment of "**thrombotic thrombocytopenic purpura**" or TTP, a deadly disease due to uncontrolled production of ultralarge VWF-multimers that spontaneously bind to platelets (even in the absence of shear) and by this cause the formation of platelet aggregates that occlude small vessels with resulting organ failure.

OUR ANTITHROMBOTIC PROGRAM

The K.U.Leuven Laboratory for Thrombosis Research has developed and fully characterized monoclonal antibodies that by binding to either the von Willebrand Factor (VWF)-A3 domain or to the platelet glycoprotein (GP)Ib prevent VWF-dependent platelet adhesion to collagen¹⁻⁴ and by this have an antithrombotic effect⁵⁻⁸. VWF normally circulates in blood in an inactive globular form that does not interact with platelets. When however VWF is bound to collagen, exposed to the blood upon vessel damage as seen following rupture of an atherosclerotic plaque, in vessels with fast blood flow (high shear stress) such as in stenosed arteries, the shear that now is applied onto VWF results in an extended conformation of VWF that as a consequence presents its A1-domain, the binding site for GPIb. Our starting hypothesis therefore was that preventing VWF-dependent steps would interfere with platelet adhesion and consequent platelet plug formation specifically in the arterial circulation (MI, stroke) without affecting haemostasis in the slower blood flow vessels, which ultimately might result in an arterial antithrombotic effect with less risk for bleeding as compared to currently clinically available antiplatelet agents.

Key validation points supporting the hypothesis and current stage of development



- We have shown that GPIb Fab-fragments
 - abolish both ristocetin-, botrocetin- and shear-induced VWF-dependent platelet aggregation *in vitro* and that this inhibition was shear-dependent (more profound effect at higher shear)

- have a strong antithrombotic effect in medium and high shear conditions *in vivo* as shown in two ***baboon models of arterial thrombosis*** without side effects such as thrombocytopenia or increased bleeding time⁷⁻⁸, indeed showing the anticipated large therapeutic window.
- A fully recombinant and ***humanized version of GPIb -Fab-fragment*** was developed which maintains its inhibitory capacities *in vitro* and *ex vivo* and its safe antithrombotic effect after injection in two arterial thrombosis models in baboons^{3,5}.
- Identification of the paratope and epitope in GPIb antibody and GPIb, respectively, by using computer modeling and site-directed mutagenesis revealed all interactions between this antibody and its antigen at the amino acid level².
- In an ***ischemic stroke model in mice***, treatment with anti-GPIb reduced the ischemic lesions and improved the neurological scores without inducing bleeding complications as revealed by MRI. In contrast, blockade of platelet aggregation with anti-GPIIb/IIIa fragments had no positive effect on stroke size and functional outcome, but increased the incidence of intracerebral hemorrhage and mortality⁹. GPIb can bind different counterreceptors on epithelial cells, platelet and white blood cells such as VWF (being the main ligand), Mac-A or P-selectin. In recent work, we have shown that of these ligands VWF is critically involved in cerebral ischemia¹⁰. Targeted inhibition of the GPIb-VWF pathway is thus a very promising therapeutic option for safe treatment of ischemic stroke.
- The GPIb antibody prevents binding of blood platelets to ultralarge VWF multimers in an ***in vitro TTP model***. Currently, the antibody is evaluated in an *in vivo* TTP model in baboons.
- Finally, studies also revealed that platelet protein receptors including GPIb play an important role in amplification of complement activation during ***xenotransplant rejection***. Because of the increasing need for human donor organs and the limited numbers available, alternatives are being studied, a.o. transplantation of lungs from transgenic (“humanized”) pigs to humans. Although significant progress has been made recently, porcine lungs that are perfused with human blood nevertheless will rapidly undergo thrombosis, as porcine VWF secreted by the endothelial cells of the blood vessels in the porcine lung, spontaneously interacts with human GPIb causing platelet aggregates to form that occlude the vessels leading to cessation of lung function within 15 minutes. We have shown that the GPIb antibody inhibits porcine VWF binding to GPIb and thereby prolongs the patency of porcine lung perfused with human blood. At this moment, studies with the GPIb antibody on porcine lung/liver/heart transplanted in baboons are ongoing.

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ADVANTAGES OF OUR ANTIBODY

Most of the currently used antiplatelet agents such as aspirin and clopidogrel inhibit a platelet amplification loop and by this only attenuate the platelet activation, indeed stronger stimuli can readily overcome the inhibition. Likely also inhibitors of the collagen receptors GPVI (platelet-specific) and integrin $\alpha 2\beta 1$ (non platelet-specific) and thrombin PARs would belong to this group.

Other compounds aim to inhibit non-redundant pathways in which class the GPIIb/IIIa (integrin $\alpha IIb\beta 3$) inhibitors but also inhibitors of GPIb and of VWF are found. Indeed in order to be able to aggregate, platelets rely on the binding of fibrinogen to activated GPIIb/IIIa and inhibition of this pathway (by e.g. the mAb ReoPro[®] (abciximab)) has a much more pronounced effect on thrombus formation. Unfortunately this is accompanied by a dramatically increased bleeding risk. Furthermore because of still unresolved mechanisms, prophylactic treatment with orally available small molecule GPIIb/IIIa inhibitors is deleterious rather than beneficial, and hence application remains restricted to acute, short term treatment.

All these products act at relatively late steps in the platelet activation cascade and do not prevent initial platelet activation and secretion of platelet activating and vasoactive components. In contrast, the GPIb antibody acts at the very first steps, namely platelet adhesion, that prevents initial platelet activation and that by this is anticipated to provide a number of additional benefits:

1. GPIb/IX/V is platelet specific, only endothelial cells seem to be able to express GPIb under *in vitro* stress conditions.
2. GPIb/IX/V interaction with VWF is a prerequisite to form a thrombus under high shear conditions as occurs in arteries and in stenosed vessels.

3. The binding of GPIb/IX/V to VWF furthermore is the non-redundant first step in the formation of a platelet-rich thrombus, and hence a strong effect is anticipated, with possibly also an influence on other events such as restenosis, to some extent provoked by platelet-derived secretion products.

4. Since the GPIb-VWF interaction is especially important at high shear stress, the GPIb antibody is primarily active in the arterial vasculature and much less in the venous system. This results in a specific targeting of the effect to the high shear (stenosed) arterial side, with an anticipated lesser risk for bleeding problems and a much larger therapeutic window than currently clinically-used antithrombotics. Several compounds acting on the collagen-VWF-GPIb axis have been tested by us and others in a variety of animal models and in the mean time in phase I volunteer studies, and have nearly invariably shown a good antiplatelet and/or antithrombotic activity with minimal effects on the bleeding time or bleeding loss from standardised wounds.

5. Competitors in this field are Ablynx with a cameloid antibody and Archemix with a DNA aptamer both however directed against the VWF-A1 domain. However in mice KO-models, a more powerful antithrombotic effect was seen in GPIb-KO than in VWF-KO mice (Bergmeier et al., 2006), likely because GPIb in addition binds ligands other than VWF, but also involved in thrombosis.

INTELLECTUAL PROPERTY

The invention is protected by two patent families.

The first patent family concerns humanized antibody inhibition of the VWF-GPIb/V/IX interaction via the GPIb target for platelet adhesion formation disorders such as vascular thrombosis, cerebral thrombosis and metastasis. The patent family comprises **a granted patent** of Deckmyn et al. US7332162 B1, granted patent EP1200562B1 and the **pending divisional** US10/049,868.

A second patent family concerns anti GPIb antibodies for use in the treatment and prevention of stroke. It comprises the **US continuation in part** of Deckmyn et al. US61/124,293 which entered the international PCT phase in april 2009.