

There is thus a need for a humanized antibody (i.e. an antibody without immunogenicity in human) with improved affinity, efficacy and half-life as compared to the antagonists of the prior art, as a means for efficiently and contentedly preventing and/or treating cardiovascular diseases in human. Moreover, these antagonists should preferably be easily purified.

In the art, anti-GPVI antibodies inducing a GPVI depletion phenotype were described (WO 2006/118350, WO 2011/073954, EP 2363416). In particular, WO 2006/118350 discloses an anti-GPVI antibody directed against all mammals. The epitope of GPVI bound by this antibody is described and corresponds to the loops 9 and 11 of the Ig-like C2-type domain 2 of GPVI, corresponding to amino acid residues 136-142 and 158-162, respectively.

However, GPVI depletion is undesirable since it cannot be controlled, and is irreversible (i.e. it lasts the lifetime of platelets, or even longer due to GPVI depletion on megakaryocytes).

In therapy, a rapid and safe antiplatelet effect is required. Therefore, antibodies not inducing a GPVI depletion phenotype, and preferably not inducing a decrease in platelet count *in vivo* (i.e. with a reversible effect) should be developed.

The Applicant developed an antibody anti-GPVI binding to a novel, undescribed, conformational epitope. Said antibody anti-GPVI has a strong affinity for human GPVI, and blocks GPVI interaction with its ligands (including collagen and fibrin), without decreasing the platelet count nor depleting GPVI *in vivo*. The present invention thus relates to an improved humanized neutralizing antibody specific to human GPVI.

SUMMARY

In one aspect of the present disclosure, there is provided an isolated humanized protein binding to human Glycoprotein VI (hGPVI), wherein said protein binds to a conformational epitope comprising:

- at least one amino acid residue from amino acid residues 114 to 142 of hGPVI (SEQ ID NO: 13) or from a sequence sharing at least 60% of identity over amino acid residues 114 to 142 of hGPVI (SEQ ID NO: 13); and
- at least one amino acid residue from amino acid residues 165 to 187 of hGPVI (SEQ ID NO: 13) or from a sequence sharing at least 60% of identity over amino acid residues 165 to 187 of hGPVI (SEQ ID NO: 13).

In one embodiment, the conformational epitope bound by the isolated humanized protein of the present disclosure comprises at least one amino acid residue from amino acid residues 121 to 135 of hGPVI (SEQ ID NO: 13) or from a sequence sharing at least 60% of identity over amino acid residues 121 to 135 of hGPVI (SEQ ID NO: 13); and at least one amino acid residue from amino acid residues 169 to 183 of hGPVI (SEQ ID NO: 13) or from a sequence sharing at least 60% of identity over amino acid residues 169 to 183 of hGPVI (SEQ ID NO: 13).

The present disclosure also relates to an isolated humanized protein binding to human Glycoprotein VI (hGPVI), wherein said protein has a K_D for binding to hGPVI less than 15 nM, wherein said K_D is measured by surface plasmon resonance using 960 to 1071 RU of soluble human GPVI and using PBS pH 7.4 as running buffer. In one embodiment, the isolated humanized protein of the present disclosure does not induce a GPVI depletion phenotype *in vivo*.

In a further aspect of the present disclosure, there is provided an isolated humanized protein binding to human Glycoprotein VI (hGPVI), wherein said protein has a K_D for binding to hGPVI less than 15 nM, wherein said K_D is measured by surface plasmon resonance using 960 to 1071 RU of soluble human GPVI and using PBS pH 7.4 as running buffer and wherein said isolated humanized protein does not induce a GPVI depletion phenotype *in vivo*.

In one embodiment, the protein of the present disclosure is an antibody molecule selected from the group consisting of a whole antibody, a humanized antibody, a single chain antibody, a Fv, a Fab; or an antibody fragment selected from the group consisting of a unibody, a domain antibody, and a nanobody; or a monomeric

antibody mimetic selected from the group consisting of an affibody, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPIn, an anticalin, an avimer, a fynomer, and a versabody.

In one embodiment, the antibody of the present disclosure is monovalent.

- 5 In one embodiment, the variable region of the heavy chain comprises at least one of the following CDRs:

VH-CDR1: GYTFTSYNMH (SEQ ID NO: 1);

In a further aspect of the present disclosure, there is provided a composition comprising the protein binding to human GPVI as defined hereinabove.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the protein binding to human GPVI as defined hereinabove and
5 at least one pharmaceutically acceptable excipient.

In one embodiment, the pharmaceutical composition of the present disclosure is for treating, or for use in treating, a GPVI-related condition.

In one embodiment, said GPVI-related condition is a cardiovascular disease selected from arterial and venous thrombosis, restenosis, acute coronary syndrome and ischemic
10 cerebrovascular accidents.

In a further aspect of the present disclosure, there is provided Use of the isolated humanized protein binding to human Glycoprotein VI (hGPVI) as defined hereinabove for the manufacture of a medicament for treating a GPVI-related condition.

The present disclosure further relates to the use of the antibody against human GPVI of
15 the present disclosure for detecting GPVI in a biological sample.

In another aspect of the present disclosure, there is provided an expression vector comprising at least one of SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12 or any sequence having a nucleic acid sequence that shares at least 60% of identity with said
20 SEQ ID NO: 10-12.

DETAILED DESCRIPTION

“Glycoprotein VI (GPVI)” is a platelet membrane glycoprotein that is involved in platelet-collagen interactions. GPVI is a transmembrane collagen receptor expressed on the surface of platelets. In one embodiment, the amino acid sequence of human GPVI is

SEQ ID NO: 13 (accession number: BAA89353.1) or any amino acid sequence presenting at least about 90% identity with SEQ ID NO: 13, preferably at least about 91, 92, 93, 94, 95, 96, 97, 98, 99% identity or more with SEQ ID NO: 13.

(SEQ ID NO: 13)

- 5 MSPSPTALFCLGLCLGRVPA (Signal peptide)

RSSQSLENSNGNTYLN (SEQ ID NO: 4), RVSNRFS (SEQ ID NO: 5) and LQLTHVPWT (SEQ ID NO: 6) or any CDR having an amino acid sequence that shares at least 60% of identity with said SEQ ID NO: 1-6.

- 5 **10.** The isolated humanized protein binding to human Glycoprotein VI (hGPVI) according to any one of claims **1** to **9**, wherein the amino acid sequence encoding the heavy chain variable region is SEQ ID NO: 7 and the amino acid sequence encoding the light variable region is SEQ ID NO: 8, or any sequence having an amino acid sequence that shares at least 60% of identity with said SEQ ID NO: 7 or 8.
- 10 **11.** The isolated humanized protein binding to human Glycoprotein VI (hGPVI) according to any one of claims **1** to **9**, wherein the amino acid sequence encoding the heavy chain variable region is SEQ ID NO: 7 and the amino acid sequence encoding the light variable region is SEQ ID NO: 9, or any sequence having an amino acid sequence that shares at least 60% of identity with said SEQ ID NO: 7 or 9.
- 15 **12.** A composition comprising the isolated humanized protein binding to human Glycoprotein VI (hGPVI) according to any one of claims **1** to **11**.
- 13.** A pharmaceutical composition comprising the isolated humanized protein binding to human Glycoprotein VI (hGPVI) according to any one of claims **1** to **11** and at least one pharmaceutically acceptable excipient.
- 20 **14.** Use of the isolated humanized protein binding to human Glycoprotein VI (hGPVI) according to any one of claims **1** to **11** for the manufacture of a medicament for treating a GPVI-related condition.
- 15.** The use according to claim **14**, wherein said GPVI-related condition is a cardiovascular disease selected from arterial and venous thrombosis, restenosis, acute coronary syndrome and cerebrovascular accidents due to atherosclerosis.
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