PROTEIN S ANTIBODIES, METHODS OF MAKING AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/020,505, filed on May 5, 2020 and to U.S. Provisional Patent Application No. 63/169,755 filed April 1, 2021. The contents of each of the preceding applications are incorporated by reference herein in their entirety.

BACKGROUND

[0002] Protein S (also known as ProS, ProS1) is a vitamin K-dependent plasma protein involved in the anti-coagulation cascade. The protein is multi-modular, comprising a γ-carboxy-glutamic acid domain (Gla domain), an epidermal growth factor-like domain (EGF domain), a thrombin-sensitive region (TSR), and a sex hormone binding globulin-like domain (SHBG-like domain). The protein is found in both a free form and as part of a complex with proteins such as C4 binding protein (C4BP) and tissue factor pathway inhibitor (TFPI). Among other functions, in its free form, Protein S is a cofactor in at least two pathways of the anti-coagulation cascade: (1) Protein S is a cofactor for plasma activated Protein C (APC), involved in the inactivation and degradation of coagulation factors Factor Va and Factor VIIIa; and (2) Protein S is also a cofactor for TFPI, also present in plasma, involved in the inactivation of coagulation factors Factor Xa and Factor VIIa.

[0003] Protein S is a potential therapeutic target for bleeding disorders, thus there is a need for agents that bind and modulate its activities within the coagulation pathway.

SUMMARY

[0004] Provided here are antibodies that bind Protein S, and methods of making and using such antibodies. In some ~~embodiment~~example~~s~~, the Protein S antibodies provided herein are useful for treating a bleeding disorder, or a condition characterized by reduced or impaired blood coagulation and/or clotting.

[0005] Accordingly, in one ~~aspect~~example, provided herein are antibodies that bind Protein S, wherein the antibodies are inhibitors of the cofactor activity of Protein S for activated Protein C (APC), inhibitors of the cofactor activity of Protein S for tissue factor pathway inhibitor (TFPI), or

inhibitors of the cofactor activity of Protein S for both APC and TFPI (dual inhibitor of cofactor activity), and wherein the antibody is human, humanized, or chimeric. In some preferred ~~embodiment~~examples, the antibodies provided herein specifically bind Protein S.

[0005a] In one aspect, the present disclosure refers to an antibody that binds Protein S, wherein the antibody is an inhibitor of the cofactor activity of Protein S for activated Protein C (APC), an inhibitor of the cofactor activity of Protein S for tissue factor pathway inhibitor (TFPI), an inhibitor of the cofactor activity of Protein S for both APC and TFPI, or is capable of promoting coagulation and/or modulating a component in the coagulation cascade and wherein the antibody is human, humanized, or chimeric.

[0006] In another ~~aspect~~example, provided herein are antibodies that bind Protein S, wherein the antibodies are capable of promoting coagulation and/or modulating a component in the coagulation cascade.

[0007] In another ~~aspect~~example, provided herein are exemplary Protein S antibodies comprising any one or more of the amino acid sequences of the complementarity determining region (CDR) sequences provided in Tables 1A, 1B, 1C, 2A, 2B, and 2C. In another ~~aspect~~example, the exemplary Protein S antibodies comprise any one of the CDR-L1 amino acid sequences of Table 1A; any one of the CDR-L2 amino acid sequences of Table 1B; any one of the CDR-L3 amino acid sequences of Table 1C; any one of the CDR-H1 amino acid sequences of Table 2A; any one of the CDR-H2 amino acid sequences of Table 2B; any one of the CDR-H3 amino acid sequences of Table 2B. In another ~~aspect~~example, provided herein are exemplary Protein S antibodies comprising the combinations of variable light chains and variable heavy chains presented in Table 4C. In another ~~aspect~~example, provided herein are the sequences of different antibodies as presented in Table 6. In another ~~aspect~~example, provided herein are nucleic acids encoding for any of the Protein S antibodies provided herein.

[0008] In another ~~aspect~~example, provided herein are pharmaceutical compositions comprising any one of the Protein S antibodies provided herein, and optionally a pharmaceutically acceptable excipient.

[0008a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody disclosed herein, and optionally a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to a nucleic acid encoding for the antibody disclosed herein. In one aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein.

[0009] Provided herein are methods of using the exemplary Protein S antibodies described herein. Accordingly, in one ~~aspect~~example, provided herein is an *in vitro* method of promoting the coagulation of blood, comprising contacting any one of the Protein S antibodies provided herein with a blood sample.

[0009a] In one aspect, the present disclosure refers to an *in vitro* method of promoting the coagulation of blood, comprising contacting the antibody disclosed herein with a blood sample.

[0010] In another ~~aspect~~example, provided herein is a method of promoting the coagulation of blood in a subject in need thereof, comprising administering to the subject any of the exemplary Protein S antibodies provided herein, or pharmaceutical compositions provided herein. In ~~exemplary embodiment~~examples, the route of administration is subcutaneous.

[0010a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for promoting the coagulation of blood in a subject in need thereof.

[0011] In another ~~aspect~~example, provided herein is a method of promoting the generation of thrombin in a subject in need thereof, comprising administering to the subject any of the Protein S antibodies provided herein, or pharmaceutical compositions provided herein.

[0011a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for promoting the generation of thrombin in a subject in need thereof.

[0012] In another ~~aspect~~example, provided herein is a method of treating a condition in a subject in need thereof, comprising administering to the subject any of the Protein S antibodies provided herein, or pharmaceutical compositions provided herein, wherein the condition is selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

[0012a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a condition in a subject in need thereof, wherein the condition is selected from the group consisting of: bleeding disorders, platelet disorders, trauma, and bleeding resulting from a surgery or a medical procedure.

[0013] In another ~~aspect~~example, provided herein is the use of any one of the Protein S antibodies or pharmaceutical compositions provided herein, for the treatment of a condition in a subject in need thereof. The condition may be selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

[0014] In another ~~aspect~~example, any one of the Protein S antibodies or pharmaceutical compositions provided herein, may be used for the manufacture of a medicament for the treatment of a condition in a subject in need thereof. The condition may be selected from the group consisting of: bleeding disorders, platelet disorders, trauma, bleeding resulting from a surgery or a medical procedure, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIG. 1** depicts a schematic diagram of Protein S showing the modular domains of Protein S.

[0016] **FIGS. 2A-2B** depict the results of screening assays performed to assess APC and TFPI cofactor activity, respectively, using control Protein S antibodies. In this and subsequent figures, ProS = Protein S; mAb = monoclonal antibody; pAb = polyclonal antibody; PRP = platelet rich plasma.

[0017] **FIGS. 3-4** depict peak thrombin levels generated by Protein S monoclonal antibodies, identified from hybridoma libraries, in the APC cofactor and TFPI cofactor screening assays, respectively.