

ANTI-TMPRSS6 ANTIBODIES AND USES THEREOF

RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Patent Application Serial No. 63/187,150, filed May 11, 2021, the entire disclosure of which is hereby incorporated herein by reference.

SEQUENCE LISTING

[002] The content of the electronically submitted sequence listing in ASCII text file (Name: 728825_RGN9-002PC_ST25.txt; Size: 141.9 kilobytes; Date of Creation: May 10, 2022) is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[003] Disclosed herein are antibodies and antigen-binding fragments thereof that specifically bind to transmembrane serine protease 6 (TMPRSS6). Also disclosed herein are nucleic acids comprising nucleotide sequences that encode such antibodies, host cells comprising the same, and therapeutic methods of using the antibodies and antigen-binding fragments thereof.

BACKGROUND

[004] Transmembrane serine protease 6 (TMPRSS6; also known as Matriptase-2 or MTP-2) is a type II transmembrane serine protease comprised of a short N-terminal intracellular region followed by a single transmembrane domain, a single SEA domain, two CUB domains, three LDLRa repeat domains, and a C-terminal catalytic serine protease domain. TMPRSS6 is highly expressed in the liver and plays a role in iron homeostasis by negatively regulating hepcidin expression. High levels of hepcidin block iron export from cells by decreasing the levels of an iron efflux channel, ferroportin, from the cell surface. TMPRSS6 cleaves hemojuvelin (HJV), which results in decreased SMAD signaling and subsequent downregulation of the transcription of *HAMP*, the gene that encodes hepcidin.

[005] Attenuation of hepcidin expression levels can result in iron overload or hemochromatosis. Iron overload disorders are a group of disorders that lead to the storage of excess iron in the body. As the body cannot excrete excess iron, the excess iron is stored in certain organs, for example, the liver, heart, and pancreas. Excess iron that is stored in such organs can lead to organ damage and other associated diseases such as diabetes.

[006] Examples of iron overload disorders include hereditary hemochromatosis (HHC),

a genetic disorder in which too much iron from the diet is absorbed by the body, and secondary hemochromatosis, which may arise as a complication in patients with certain hematological disorders such as thalassemia (a type of hereditary anemia caused by decreased hemoglobin production) or myelodysplastic syndrome (a blood cancer which result in acquired anemia). In the case of beta-thalassemia and/or myelodysplastic syndrome, the administration of repeated blood transfusions for treating the anemia associated with disease can often result iron overload.

[007] Treatment options for iron overload disorders are limited and typically are required throughout a patient's lifetime. Treatment options include iron chelation therapy, regular removal iron-rich blood by phlebotomy or venesection, and dietary restrictions to reduce iron intake. There is a need in the art for additional and improved compositions and methods for the treatment of iron overload disorders.

BRIEF SUMMARY OF THE DISCLOSURE

[008] Disclosed herein are antibodies and antigen-binding fragments thereof that specifically bind the transmembrane serine protease 6 (TMPRSS6) protein (*i.e.*, anti-TMPRSS6 antibodies). In certain embodiments, the anti-TMPRSS6 antibodies are fully human antibodies that bind with high affinity to human TMPRSS6 and inhibit its protease activity.

[009] In one aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof binds to the catalytic domain of TMPRSS6 but does not bind to the catalytic triad of TMPRSS6.

[010] In certain embodiments, the antibody or antigen-binding fragment thereof further binds at a site comprised within the LDLRa domain 2 of TMPRSS6. In one embodiment, binding of the antibody or antigen-binding fragment thereof to TMPRSS6 does not directly occlude the catalytic triad of TMPRSS6. In other embodiments, binding of the antibody or antigen-binding fragment thereof to TMPRSS6 mediates allosteric regulation of TMPRSS6. In certain embodiments, binding of the antibody or antigen-binding fragment thereof to TMPRSS6 mediates allosteric inhibition of TMPRSS6.

[011] In another aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof is an allosteric inhibitor of TMPRSS6. In certain embodiments, the antibody or antigen-binding fragment maintains TMPRSS6 in an inactive conformation that is capable of binding to but not cleaving a TMPRSS6 substrate. In certain embodiments, the TMPRSS6 substrate is hemojuvelin (HJV).

[012] In another aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment binds within the catalytic domain of TMPRSS6 at a site that sterically occludes the catalytic triad of TMPRSS6. In certain embodiments, the antibody or antigen-binding fragment thereof competes with hemojuvelin (HHV) for binding to TMPRSS6.

[013] In another aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to human Transmembrane Protease Serine 6 (hTMPRSS6) protein (SEQ ID NO:126), wherein the antibody or antigen-binding fragment thereof interacts with one or more amino acids contained within the extracellular domain of hTMPRSS6, as determined by hydrogen/deuterium exchange.

[014] In certain embodiments, the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 125 to 133 of SEQ ID NO: 126; (b) amino acids 586-594 of SEQ ID NO: 126; (c) amino acids 626 to 650 of SEQ ID NO: 126; (d) amino acids 693 to 703 of SEQ ID NO: 126; (e) amino acids 704 to 724 of SEQ ID NO: 126; and/or (f) amino acids 780 to 805 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange. In other embodiments, the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 693 to 703 of SEQ ID NO: 126; and/or (b) amino acids 780 to 805 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange. In certain embodiments, the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 125 to 133 of SEQ ID NO: 126; (b) amino acids 586-594 of SEQ ID NO: 126; (c) amino acids 626 to 650 of SEQ ID NO: 126; and/or (d) amino acids 704 to 724 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange.

[015] In another aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof interacts with residues G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, and N791 of human TMPRSS6 (SEQ ID NO:126), as determined by cryoelectron microscopy structural modeling.

[016] In certain embodiments, the antibody or antigen-binding fragment thereof further interacts with residues V490, S501, T502, C503, I504, S505, and K508 of human TMPRSS6 (SEQ ID NO:126), as determined by cryoelectron microscopy structural modeling.

[017] In another aspect, the disclosure provides an isolated antibody or antigen-binding

fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof interacts with residues R597, R599, I601, D622, L710, R711, E712, G713, G714, P715, and I716 of human TMPRSS6, as determined by cryoelectron microscopy structural modeling.

[018] In another aspect, the disclosure provides an isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR); and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR), wherein the HCDR3 has an amino acid sequence selected from a HCDR3 sequence listed in Table 1.

[019] In certain embodiments, the LCDR3 sequence has an amino acid sequence selected from a LCDR3 sequence listed in Table 1. In certain embodiments, the antibody or antigen binding fragment comprises a HCDR1, HCDR2, and HCDR3 sequence listed in Table 1. In certain embodiments, the antibody or antigen binding fragment comprise a LCDR1, LCDR2, and LCDR3 sequence listed in Table 1.

[020] In certain embodiments, (a) the HCDR1 domain has an amino acid sequence selected from SEQ ID NOs: 4, 24, 34, 44, 64, 84, or 102; (b) the HCDR2 domain has an amino acid sequence selected from SEQ ID NOs: 6, 26, 36, 46, 66, 86, or 104; (c) the HCDR3 domain has an amino acid sequence selected from SEQ ID NOs: 8, 28, 38, 48, 68, 88, or 106; (d) the LCDR1 domain has an amino acid sequence selected from SEQ ID NOs: 12, 52, 72, 92, or 110; (e) the LCDR2 domain has an amino acid sequence selected from SEQ ID NOs: 14, 54, or 74; and (f) the LCDR3 domain has an amino acid sequence selected from SEQ ID NOs: 16, 56, 76, 94, or 112.

[021] In certain embodiments, the three heavy chain CDRs and three light chain CDRs comprise one of the following sets of CDRs: (a) SEQ ID NO: 4 (HCDR1), SEQ ID NO:6 (HCDR2), SEQ ID NO:8 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3); (b) SEQ ID NO: 24 (HCDR1), SEQ ID NO:26 (HCDR2), SEQ ID NO:28 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3); (c) SEQ ID NO: 34 (HCDR1), SEQ ID NO:36 (HCDR2), SEQ ID NO:38 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3); (d) SEQ ID NO: 44 (HCDR1), SEQ ID NO:46 (HCDR2), SEQ ID NO:48 (HCDR3), SEQ ID NO:52 (LCDR1), SEQ ID NO:54 (LCDR2), and SEQ ID NO:56 (LCDR3); (e) SEQ ID NO: 64 (HCDR1), SEQ ID NO:66 (HCDR2), SEQ ID NO:68 (HCDR3), SEQ ID NO:72 (LCDR1), SEQ

ID NO:74 (LCDR2), and SEQ ID NO:76 (LCDR3); (f) SEQ ID NO: 84 (HCDR1), SEQ ID NO:86 (HCDR2), SEQ ID NO:88 (HCDR3), SEQ ID NO:92 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:94 (LCDR3); or (g) SEQ ID NO: 102 (HCDR1), SEQ ID NO:104 (HCDR2), SEQ ID NO:106 (HCDR3), SEQ ID NO:110 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:112 (LCDR3).

[022] In certain embodiments, the three heavy chain CDRs and three light chain CDRs comprise the following set of CDRs: SEQ ID NO: 44 (HCDR1), SEQ ID NO:46 (HCDR2), SEQ ID NO:48 (HCDR3), SEQ ID NO:52 (LCDR1), SEQ ID NO:54 (LCDR2), and SEQ ID NO:56 (LCDR3). In other embodiments, the three heavy chain CDRs and three light chain CDRs comprise the following set of CDRs: SEQ ID NO: 102 (HCDR1), SEQ ID NO:104 (HCDR2), SEQ ID NO:106 (HCDR3), SEQ ID NO:110 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:112 (LCDR3).

[023] In another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that binds to TMPRSS6, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a HCVR and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a LCVR; wherein the HCVR comprises: (i) an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100; (ii) an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100; (iii) an amino acid sequence having at least 95% identity to an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100; or (iv) an amino acid sequence having no more than 12 amino acid substitutions with respect to a sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100.

[024] In certain embodiments, the LCVR comprises: (a) an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108; (b) an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108; (c) an amino acid sequence having at least 95% identity to an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108; or (d) an amino acid sequence having no more than 10 amino acid substitutions with respect to a sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108.

[025] In certain embodiments, the HCVR comprises an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100. In certain embodiments, the LCVR comprises an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108.

[026] In certain embodiments, the antibody or antigen-binding fragment thereof comprises HCVR/LCVR amino acid sequence pair selected from SEQ ID NOs: 2/10, 22/10, 32/10, 42/50, 62/70, 82/90, or 100/108. In certain embodiments, the antibody or antigen

binding-fragment comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 42/50. In other embodiments, the antibody or antigen binding-fragment comprises the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 100/108.

[027] In another aspect, the disclosure provides an antibody that binds to TMPRSS6, wherein the antibody comprises a heavy chain (HC) and a light chain (LC), wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 30, 40, 58, 78, 96 and 114.

[028] In certain embodiments, the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 60, 80, 98 and 116. In certain embodiments, the antibody comprises a HC/LC amino acid sequence pair selected from SEQ ID NOs: 18/20, 30/20, 40/20, 58/60, 78/80, 96/98 or 114/116. In certain embodiments, the antibody comprises the HC/LC amino acid sequence pair of 58/60. In other embodiments, the antibody comprises the HC/LC amino acid sequence pair of 114/116.

[029] In certain embodiments, the antibody or antigen binding fragments of the disclosure have one or more properties selected from: (a) is a fully human monoclonal antibody or antigen binding fragment thereof; (b) binds to human TMPRSS6 at 25°C and at 37°C with a dissociation constant (K_D) of less than about 21.2 nM, as measured in a surface plasmon resonance assay; (c) binds to monkey TMPRSS6 at 25°C and at 37°C with a K_D of less than about 25.7 nM, as measured in a surface plasmon resonance assay; (d) binds to mouse TMPRSS6 at 25°C and at 37°C with a K_D of less than about 703 nM, as measured in a surface plasmon resonance assay; (e) binds to cells expressing human TMPRSS6 with EC_{50} less than about 2.7 nM; (f) binds to cells expressing monkey TMPRSS6 with EC_{50} less than about 3.6 nM; (g) binds to cells expressing mouse TMPRSS6 with EC_{50} less than about 35 nM; (h) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6, with percent inhibition greater than about 90%; (i) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6 and exhibits an IC_{50} less than about 200 pM; (j) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6, with percent inhibition greater than about 42%; (k) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6 and exhibits an IC_{50} less than about 274 pM; (l) inhibits human TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 65%; (m) inhibits human TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 10 nM; (n) inhibits monkey TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 66%; (o) inhibits monkey TMPRSS6

at 25°C and at 37°C and exhibits an IC₅₀ less than about 50 nM; (p) inhibits mouse TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 58%; (q) inhibits mouse TMPRSS6 at 25°C and at 37°C and exhibits an IC₅₀ less than about 35 nM; (r) when administered to a subject in need thereof, decreases serum iron levels of the subject; (s) when administered to a subject in need thereof, increases serum hepcidin levels of the subject; (t) when administered to a subject in need thereof, increases mature red blood cell levels of the subject; (u) when administered to a subject in need thereof, increases mature red blood cell levels in the spleen and/or bone marrow of the subject; (v) when administered to a subject in need thereof, increases hemoglobin levels of the subject; or (w) when administered to a subject in need thereof, decreases transferrin saturation levels of the subject. In certain embodiments, the antibody is a fully human monoclonal antibody.

[030] In another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that competes for binding to TMPRSS6 with an antibody or antigen-binding fragment thereof disclosed herein.

[031] In another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that binds to the same epitope as an antibody or antigen-binding fragment thereof disclosed herein.

[032] In another aspect, the disclosure provides a pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof that binds to TMPRSS6 as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[033] In another aspect, the disclosure provides an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an antibody or antigen-binding fragment thereof as disclosed herein.

[034] In another aspect, the disclosure provides an isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an antibody or antigen-binding fragment thereof as disclosed herein.

[035] In another aspect, the disclosure provides a vector comprising a polynucleotide sequence disclosed herein.

[036] In another aspect, the disclosure provides a set of vectors comprising a first vector comprising a polynucleotide sequence that encodes a HCVR of an antibody or antigen-binding fragment thereof as disclosed herein and a second vector comprising a polynucleotide sequence that encodes a LCVR of an antibody or antigen-binding fragment thereof as disclosed herein.

[037] In another aspect, the disclosure provides a host cell comprising a vector or set of vectors as disclosed herein.

[038] In another aspect, the disclosure provides a method of producing an antibody or antigen-binding fragment thereof that binds specifically to TMPRSS6, comprising culturing a host cell of the disclosure under conditions permitting production of the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof so produced. In certain embodiment, the method further comprises formulating the antibody or antigen-binding fragment thereof as a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

[039] In another aspect, the disclosure provides a method of treating, preventing, or ameliorating at least one symptom or indication of a disease or disorder associated with iron overload, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof of the disclosure to a subject in need thereof.

[040] In certain embodiments, the disease or disorder is a congenital dyserythropoietic anemia, Diamond-Blackfan anemia, alpha thalassemia, beta thalassemia, a transfusion-dependent hemolytic anemia, myelodysplastic syndrome, sickle cell disease, polycythemia vera, hereditary hemochromatosis, or chronic liver disease. In certain embodiments, the disease or disorder is beta thalassemia. In certain embodiments, the disease or disorder is beta thalassemia major or beta thalassemia intermedia. In certain embodiments, the disease or disorder is myelodysplastic syndrome. In certain embodiments, the disease or disorder is myelodysplastic syndrome with ring sideroblasts. In certain embodiments, the disease or disorder is a transfusion-dependent hemolytic anemia. In certain embodiments, the disease or disorder is a transfusion-dependent hemolytic anemia due to pyruvate kinase deficiency, or a sideroblastic transfusion-dependent hemolytic anemia. In certain embodiments, the disease or disorder is a chronic liver disease. In certain embodiments, the disease or disorder is an alcohol related chronic liver disease, hepatitis C, or autoimmune hepatitis.

[041] In certain embodiments, the pharmaceutical composition is administered prophylactically or therapeutically to the subject in need thereof. In other embodiment, the pharmaceutical composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.

[042] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[043] FIG. 1 is a graphical depiction of the study plan for the mice forced running studies in Example 15.

[044] FIG. 2 shows the effect of REGN7999 treatment on running distance (Figure 2A) and lactate production over running distance (Figure 2B) in Hbb^{th3/+} mice. Control animals are wild-type (WT) C57BL/6 mice or Hbb^{th3/+} mice treated with isotype control antibody (REGN1945). Note: All figure data presented as Mean $\pm$ Standard Deviation (SD); P<0.05 indicate statistically significant differences under One-Way ANOVA and Tukey post-hoc multiple comparison test.

[045] FIG. 3 shows the running distance of wild-type (WT) C57BL/6 mice treated with either REGN7999 or isotype control antibody (REGN1945).

[046] FIG. 4 shows the levels of serum hepcidin (FIG. 4A), serum iron (FIG. 4B), and liver iron content (FIG. 4C) in Hbb^{th3/+} mice treated for 8 weeks with either REGN7999 or Acvr2b(L79D)-Fc. Control animals are wild-type (WT) C57BL/6 mice or Hbb^{th3/+} mice treated with isotype control antibody (REGN1945).

[047] FIG. 5 shows the mean body weight (g) of WT or Hbb^{th3/+} mice after 8 weeks of treatment with REGN7999, Acvr2b(L79D)-Fc, or isotype control antibody (REGN1945). WT mice treated with isotype control: circles, black solid line. Hbb^{th3/+} mice treated with isotype control: squares, gray solid line. Hbb^{th3/+} mice treated with REGN7999: triangles (up), black dotted line. Hbb^{th3/+} mice treated with Acvr2b(L79D)-Fc: triangles (down), gray dotted line.

[048] FIG. 6 shows the mean spleen weight (g) of WT or Hbb^{th3/+} mice after 8 weeks of treatment with REGN7999, Acvr2b(L79D)-Fc, or isotype control antibody (REGN1945).

[049] FIG. 7 shows the amount of splenic mature RBC (% CD44^{low}Ter119⁺ cells) from WT or Hbb^{th3/+} mice after 8 weeks of treatment with REGN7999, Acvr2b(L79D)-Fc, or isotype control antibody (REGN1945).

[050] FIG. 8 shows the amount of reticulocytes (% CD44^{high}Ter119⁺ cells) from WT or Hbb^{th3/+} mice after 8 weeks of treatment with REGN7999, Acvr2b(L79D)-Fc, or isotype control antibody (REGN1945).

DETAILED DESCRIPTION

[051] Before the present methods are described, it is to be understood that this disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is

for the purpose of describing particular embodiments only and is not intended to be limiting.

[052] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art in the related field. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the disclosed embodiments, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[053] The term “TMPRSS6”, also called “Matriptase-2” or “MTP-2” refers to transmembrane serine protease 6. TMPRSS6 is a type II transmembrane serine protease comprised of a short N-terminal intracellular region (residues 1-55 of human TMPRSS6 (SEQ ID NO: 126)) followed by a single transmembrane domain (residues 56-76 of SEQ ID NO: 126), a single SEA (sea urchin sperm protein, enteropeptidase, and agrin) domain (residues 84-209 of SEQ ID NO: 126), two CUB (Cls/Clr, urchin embryonic growth factor, and bone morphogenic protein (BMP)-1) domains (residues 213-336 of SEQ ID NO: 126 for CUB domain 1 and residues 335-452 of SEQ ID NO: 126 for CUB domain 2) three LDLRa (low-density-lipoprotein receptor, class A) repeat domains (residues 457-489 of SEQ ID NO: 126 for LDLRa domain 1, residues 492-526 of SEQ ID NO: 126 for LDLRa domain 2, and residues 530-567 of SEQ ID NO: 126 for LDLRa domain 3), and a C-terminal catalytic serine protease domain (residues 577-811 of SEQ ID NO: 126). The catalytic serine protease domain contains a catalytic triad of histidine (residue 617 of human TMPRSS6 (SEQ ID NO: 126)), aspartate (residue 668 of SEQ ID NO: 126), and serine residues (residue 762 of SEQ ID NO: 126) (Lee, *Acta Haematol.* (2009) 122(2-3): 87-96; Ramsay et al., *Front. Biosci.* (2008) 13:569-579). TMPRSS6 has been shown to play a role in iron homeostasis by acting as a negative regulator of hepcidin production through the cleavage of the BMP coreceptor hemojuvelin (HJV) (Dion et al., *Scientific Reports* (2018) 8:12562). The amino acid sequence of full-length human TMPRSS6 protein (isoform 2) is exemplified by the 811 amino acid sequence provided in UniProtKB/Swiss-Prot as accession number Q8IU80 and corresponds to NCBI accession number NP_705837.1 (TMPS6_HUMAN; SEQ ID NO: 126). Human TMPRSS6 protein is encoded by the nucleic acid sequence represented by NCBI Reference Sequence NM_153609.3 (SEQ ID NO: 127). The amino acid sequence of full-length cynomolgous monkey TMPRSS6 protein is exemplified by the amino acid sequence provided in UniProtKB/Swiss-Prot as accession number A0A2K5VAP0 and corresponds to NCBI accession number XP_005567441.1 (A0A2K5VAP0_MACFA; SEQ ID NO: 128). Cynomolgous monkey TMPRSS6 protein is encoded by the nucleic acid sequence

represented by NCBI Reference Sequence XM_005567384 (SEQ ID NO: 129). The amino acid sequence of full-length mouse Tmprss6 protein is exemplified by the amino acid sequence provided in UniProtKB/Swiss-Prot as accession number Q9DBI0 and corresponds to NCBI accession number NP_082178.2 (TMPS6_MOUSE; SEQ ID NO: 130). Mouse Tmprss6 protein is encoded by the nucleic acid sequence represented by NCBI Reference Sequence NM_027902.2 (SEQ ID NO: 131). The term "Tmprss6" includes recombinant Tmprss6 protein or a fragment thereof. The term also encompasses Tmprss6 protein or a fragment thereof coupled to, for example, histidine tag, mouse or human Fc, or a signal sequence such as ROR1 (for example, SEQ ID NOs: 117-119).

[054] The term "about", when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4.... 100.7, 100.8, 100.9).

[055] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "V_H") and a heavy chain constant region (comprised of domains C_{H1}, C_{H2} and C_{H3}). Each light chain is comprised of a light chain variable region ("LCVR or "V_L") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the disclosure, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[056] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995, FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* (2002) J Mol Biol 320:415-428).

[057] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[058] The fully human anti-TMPRSS6 monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present disclosure includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies disclosed herein may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to

the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic biological properties, reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present disclosure.

[059] Also disclosed herein are fully human anti-TMPRSS6 monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present disclosure includes anti-TMPRSS6 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[060] The term "human antibody", or "fully human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs disclosed herein may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", or "fully human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human FR sequences. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[061] The term "recombinant", as used herein, refers to antibodies or antigen-binding fragments thereof created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term refers to antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[062] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-8} M or less (*e.g.*, a smaller K_D denotes a tighter

binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to TMPRSS6. Moreover, multi-specific antibodies that bind to one domain in TMPRSS6 and one or more additional antigens or a bi-specific that binds to two different regions of TMPRSS6 are nonetheless considered antibodies that “specifically bind”, as used herein.

[063] The term “high affinity” antibody refers to those mAbs having a binding affinity to TMPRSS6, expressed as K_D , of at least 10^{-8} M; preferably 10^{-9} M; more preferably 10^{-10} M, even more preferably 10^{-11} M, as measured by surface plasmon resonance, *e.g.*, BIACORE™ or solution-affinity ELISA.

[064] By the term “slow off rate”, “Koff” or “kd” is meant an antibody that dissociates from TMPRSS6, with a rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, preferably $1 \times 10^{-4} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[065] The terms “antigen-binding portion” of an antibody, “antigen-binding fragment” of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms “antigen-binding fragment” of an antibody, or “antibody fragment”, as used herein, refers to one or more fragments of an antibody that retain the ability to bind to TMPRSS6 protein.

[066] In specific embodiments, antibody or antibody fragments of the disclosure may be conjugated to a moiety such as a ligand or a therapeutic moiety (“immunoconjugate”), a second anti-TMPRSS6 antibody, or any other therapeutic moiety useful for treating a TMPRSS6-associated disease or disorder.

[067] An “isolated antibody”, as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds TMPRSS6, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than TMPRSS6).

[068] The term “surface plasmon resonance”, as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[069] The term “ K_D ”, as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[070] The term “epitope” refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term “epitope” also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[071] In some embodiments, the antibodies and antigen-binding fragments disclosed herein comprise an epitope or part of an epitope comprising G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, and N791 of human TMPRSS6 (NP_705837.1; SEQ ID NO: 126). In some embodiments, the antibodies and antigen-binding fragments disclosed herein comprise an epitope or part of an epitope comprising V490, S501, T502, C503, I504, S505, and K508 of human TMPRSS6 (NP_705837.1; SEQ ID NO: 126). In some embodiments, the antibodies and antigen-binding fragments disclosed herein comprise an epitope comprising two parts of human TMPRSS6, the first part comprising G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, and N791 of human TMPRSS6, and the second part comprising V490, S501, T502, C503, I504, S505, and K508 of human TMPRSS6 (NP_705837.1; SEQ ID NO: 126).

[072] In other embodiments, the antibodies and antigen-binding fragments disclosed herein comprise an epitope or part of an epitope comprising R597, R599, I601, D622, L710, R711, E712, G713, G714, P715, and I716 of human TMPRSS6 (NP_705837.1; SEQ ID NO: 126).

[073] The term “cross-competes”, as used herein, means an antibody or antigen-binding fragment thereof binds to an antigen and inhibits or blocks the binding of another antibody or antigen-binding fragment thereof. The term also includes competition between two antibodies in both orientations, *i.e.*, a first antibody that binds and blocks binding of second antibody and vice-versa. In certain embodiments, the first antibody and second antibody may bind to the same epitope. Alternatively, the first and second antibodies may bind to different, but overlapping epitopes such that binding of one inhibits or blocks the binding of the second antibody, *e.g.*, *via*

steric hindrance. Cross-competition between antibodies may be measured by methods known in the art, for example, by a real-time, label-free bio-layer interferometry assay. Cross-competition between two antibodies may be expressed as the binding of the second antibody that is less than the background signal due to self-self binding (wherein first and second antibodies is the same antibody). Cross-competition between 2 antibodies may be expressed, for example, as % binding of the second antibody that is less than the baseline self-self background binding (wherein first and second antibodies is the same antibody).

[074] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST, or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[075] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) *Methods Mol. Biol.* 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine.

Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[076] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402, each of which is herein incorporated by reference.

[077] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[078] As used herein, the term "subject" refers to an animal, preferably a mammal, more preferably a human, in need of amelioration, prevention and/or treatment of a TMPRSS6-associated disease or disorder such as beta-thalassemia or myelodysplastic syndrome. The term includes human subjects who have or are at risk of having such a disease or disorder.

[079] As used herein, the terms "treat", "treating", or "treatment" refer to the reduction or amelioration of the severity of at least one symptom or indication of a TMPRSS6-associated disease or disorder due to the administration of a therapeutic agent such as an antibody or antigen binding fragment disclosed herein to a subject in need thereof. The terms include inhibition of

progression of disease or of worsening of a symptom/indication. The terms also include positive prognosis of disease, i.e., the subject may be free of disease or may have reduced disease upon administration of a therapeutic agent such as an antibody or antigen binding fragment disclosed herein. The therapeutic agent may be administered at a therapeutic dose to the subject.

[080] The terms “prevent”, “preventing” or “prevention” refer to inhibition of manifestation of a TMPRSS6-associated disease or disorder or any symptoms or indications of such a disease or disorder upon administration of an antibody or antigen binding fragment disclosed herein.

Antigen-Binding Fragments of Antibodies

[081] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to TMPRSS6 protein. An antibody fragment may include a Fab fragment, a F(ab')₂ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[082] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-

CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[083] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain $V_H - V_H$, $V_H - V_L$ or $V_L - V_L$ dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[084] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody disclosed herein include: (i) $V_H - C_H1$; (ii) $V_H - C_H2$; (iii) $V_H - C_H3$; (iv) $V_H - C_H1 - C_H2$; (v) $V_H - C_H1 - C_H2 - C_H3$; (vi) $V_H - C_H2 - C_H3$; (vii) $V_H - C_L$; (viii) $V_L - C_H1$; (ix) $V_L - C_H2$; (x) $V_L - C_H3$; (xi) $V_L - C_H1 - C_H2$; (xii) $V_L - C_H1 - C_H2 - C_H3$; (xiii) $V_L - C_H2 - C_H3$; and (xiv) $V_L - C_L$. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody disclosed herein may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[085] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an

antibody disclosed herein using routine techniques available in the art.

Preparation of Human Antibodies

[086] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present disclosure to make human antibodies that specifically bind to TMPRSS6.

[087] An immunogen comprising any one of the following can be used to generate antibodies to TMPRSS6 protein. In certain embodiments, the antibodies disclosed herein are obtained from mice immunized with a full length, native TMPRSS6 protein (See, for example, UniProtKB/Swiss-Prot accession number Q8IU80) or with DNA encoding the protein or fragment thereof. Alternatively, the protein or a fragment thereof may be produced using standard biochemical techniques and modified and used as immunogen.

[088] In some embodiments, the immunogen may be a recombinant TMPRSS6 protein or fragment thereof expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells (for example, SEQ ID NOs: 117-119)

[089] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to TMPRSS6 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[090] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable

domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[091] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody as disclosed herein, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[092] The anti-TMPRSS6 antibodies and antibody fragments disclosed herein encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind TMPRSS6 protein. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences disclosed herein encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment disclosed herein.

[093] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[094] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

[095] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant

change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[096] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[097] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[098] Bioequivalent variants of the disclosed antibodies may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-TMPRSS6 Antibodies Comprising Fc Variants

[099] Antibodies referred to herein typically have fully human variable regions, but may have human or mouse constant regions. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the Fc domain. In certain embodiments, the antibodies comprise a human IgG4 Fc. In one embodiment, the human IgG4 Fc comprises a serine to proline mutation in the hinge region (S108P) to promote dimer stabilization.

[0100] According to certain embodiments disclosed herein, anti-TMPRSS6 antibodies are

provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present disclosure includes anti-TMPRSS6 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, H/L/R/S/P/Q or K) and/or 434 (*e.g.*, A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.*, 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.*, M428L) and 434S (*e.g.*, N434S) modification; a 428L, 259I (*e.g.*, V259I), and 308F (*e.g.*, V308F) modification; a 433K (*e.g.*, H433K) and a 434 (*e.g.*, 434Y) modification; a 252, 254, and 256 (*e.g.*, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.*, T250Q and M428L); and a 307 and/or 308 modification (*e.g.*, 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0101] For example, the present disclosure includes anti-TMPRSS6 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present disclosure.

[0102] Also disclosed herein are anti-TMPRSS6 antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies disclosed herein may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies disclosed herein comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid

sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (See, e.g., U.S. Patent Application Publication 2014/0243504, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies and Antigen-Binding Fragments Thereof

[0103] In general, the disclosed antibodies or antigen-binding fragments thereof function by binding to TMPRSS6 protein and inhibiting its activity (e.g., an enzymatic activity). In some embodiments, the inhibited TMPRSS6 activity is proteolytic activity.

[0104] Disclosed herein are antibodies and antigen-binding fragments thereof that bind TMPRSS6 protein (*i.e.* anti-TMPRSS6 antibodies or antigen-binding fragments thereof). In certain embodiments, the anti-TMPRSS6 antibody or antigen-binding fragment thereof binds to the catalytic domain of TMPRSS6. In certain embodiments, the anti-TMPRSS6 antibody or antigen-binding fragment thereof binds to the catalytic domain of TMPRSS6 but does not bind to the catalytic triad of TMPRSS6. Also disclosed herein are antibodies and antigen-binding fragments thereof that bind at a site located within the LDLRa domain 2 of TMPRSS6 (e.g., residues 492-526 of human TMPRSS6 (SEQ ID NO: 126)). In some embodiments, the antibodies and antigen-binding fragments thereof may bind or interact with more than one site on TMPRSS6. For example, an antibody or antigen-binding fragment thereof that binds a site within the catalytic domain of TMPRSS6 may also bind at a site (e.g., secondary site) comprised within the LDLRa domain 2 of TMPRSS6.

[0105] Further disclosed herein are antibodies and antigen-binding fragments thereof that bind the catalytic domain of TMPRSS6 (e.g., residues 577-811 of human TMPRSS6 (SEQ ID NO: 126)), wherein the binding does not directly occlude the catalytic triad of TMPRSS6. Without being bound by theory, where binding of the anti-TMPRSS6 antibodies or antigen-binding fragments thereof does not directly occlude the catalytic triad of TMPRSS6, TMPRSS6 may retain its ability to bind a substrate, e.g., hemojuvelin (HJV). For example, in certain embodiments, the anti-TMPRSS6 antibody or antigen-binding fragment thereof binds to

TMPRSS6 and maintains TMPRSS6 in an inactive conformation that is capable of binding to but not cleaving a TMPRSS6 substrate. In certain embodiments, the anti-TMPRSS6 antibody or antigen-binding fragment thereof binds to TMPRSS6 and maintains TMPRSS6 in an inactive conformation that is capable of binding to but not cleaving hemojuvelin. Such anti-TMPRSS6 antibodies and antigen-binding fragments thereof may mediate allosteric regulation of TMPRSS6. In certain embodiments, the anti-TMPRSS6 antibody or antigen-binding fragment thereof mediates allosteric inhibition of TMPRSS6. Also disclosed herein are antibodies and antigen-binding fragments thereof that bind human TMPRSS6 protein and are allosteric inhibitors of TMPRSS6.

[0106] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to the catalytic domain of TMPRSS6, wherein the binding directly occludes the catalytic triad of TMPRSS6. Without being bound by theory, occlusion of the catalytic triad of TMPRSS6 may result in steric hindrance of substrate (*e.g.*, hemojuvelin) binding to TMPRSS6.

[0107] Disclosed herein are antibodies and antigen-binding fragments of antibodies that bind human TMPRSS6 protein (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 25 nM as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind human TMPRSS6 with a K_D of less than about 25 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 picomolar (pM), less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, or less than about 150 pM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments thereof bind human TMPRSS6 with a K_D in the range of about 128 pM to about 21.2 nM.

[0108] Also disclosed herein are antibodies and antigen-binding fragments of antibodies that bind cynomolgous monkey (*e.g.*, *Macaca fascicularis*) TMPRSS6 protein (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 30 nM as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind cynomolgous monkey TMPRSS6 with a K_D of less than about 30 nM, less than about 25 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 9 nM, less than about 8 nM, less than about 7 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less

than about 1 nM, less than about 900 picomolar (pM), less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 150 pM, less than about 100 pM, or less than about 75 pM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments thereof bind monkey TMPRSS6 with a K_D in the range of about 65 pM to about 25.7 nM.

[0109] Also disclosed herein are antibodies and antigen-binding fragments of antibodies that bind mouse TMPRSS6 protein (*e.g.*, at 25°C or at 37°C) with a K_D of less than about 750 nM as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind mouse TMPRSS6 with a K_D of less than about 750 nM, less than about 700 nM, less than about 650 nM, less than about 600 nM, less than about 550 nM, less than about 500 nM, less than about 450 nM, less than about 400 nM, less than about 350 nM, less than about 300 nM, less than about 250 nM, less than about 200 nM, less than about 150 nM, less than about 50 nM, less than about 1000 picomolar (pM), less than about 900 pM, less than about 800 pM, less than about 700 pM, less than about 600 pM, less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 150 pM, or less than about 100 pM, as measured by surface plasmon resonance, *e.g.*, using the assay format as defined in Example 3 herein, or a substantially similar assay. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof bind mouse TMPRSS6 with a K_D in the range of about 82.9 pM to about 703 nM.

[0110] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to cells expressing human TMPRSS6 at a EC_{50} of less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, less than about 900 pM, less than about 800 pM, or less than about 700 pM, as measured, *e.g.*, using an assay format as described in Example 5 herein, or a substantially similar assay. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof bind to cells expressing human TMPRSS6 at a EC_{50} in the range of about 670 pM to about 2.7 nM.

[0111] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to cells expressing cynomolgous monkey TMPRSS6 at a EC_{50} of less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, or less than about 1 nM, as measured, *e.g.*, using an assay format as described in Example 5 herein, or a substantially similar assay. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof bind to cells expressing cynomolgous monkey TMPRSS6 at a EC_{50} in the range of about 940 pM

to about 3.6 nM.

[0112] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to cells expressing mouse TMPRSS6 at a EC_{50} of less than about 40 nM, less than about 35 nM, less than about 30 nM, less than about 25 nM, less than about 20 nM, less than about 15 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM, or less than about 0.9 nM, as measured, *e.g.*, using an assay format as described in Example 5 herein, or a substantially similar assay. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof bind to cells expressing mouse TMPRSS6 at a EC_{50} in the range of about 840 pM to about 35 nM.

[0113] In one embodiment, an isolated recombinant antibody or antigen-binding fragment thereof is disclosed herein which binds specifically to TMPRSS6 protein, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (a) is a fully human monoclonal antibody; (b) binds to human TMPRSS6 at 25°C and at 37°C with a dissociation constant (K_D) of less than about 21.2 nM, as measured in a surface plasmon resonance assay; (c) binds to monkey TMPRSS6 at 25°C and at 37°C with a K_D of less than about 25.7 nM, as measured in a surface plasmon resonance assay; (d) binds to mouse TMPRSS6 at 25°C and at 37°C with a K_D of less than about 703 nM, as measured in a surface plasmon resonance assay; (e) binds to cells expressing human TMPRSS6 with EC_{50} less than about 2.7 nM; (f) binds to cells expressing monkey TMPRSS6 with EC_{50} less than about 3.6 nM; (g) binds to cells expressing mouse TMPRSS6 with EC_{50} less than about 35 nM; (h) comprises a HCDR1, HCDR2, and HCDR3 comprising an amino acid sequence selected from a HCDR1, HCDR2, and HCDR3 sequence listed in Table 1 and/or a LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence selected from a LCDR1, LCDR2, and LCDR3 sequence listed in Table 1; and/or (i) comprises a HCVR comprising an amino acid sequence selected from a HCVR sequence listed in Table 1 and a LCVR comprising an amino acid sequence selected from a LCVR sequence listed in Table 1.

[0114] Disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and decrease serum iron levels of a subject in need thereof, for example, as demonstrated in Examples 10, 11, and 14 herein.

[0115] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and increase serum hepcidin levels of a subject in need thereof, for example, as demonstrated in Examples 10, 11, and 14 herein.

[0116] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and increase mature red blood cell levels of a subject in need thereof. In

certain embodiments, the antibodies and antigen-binding fragments thereof that bind to TMPRSS6 increase mature red blood cell levels in the spleen and/or bone marrow of a subject in need thereof. Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and decrease the level of splenic reticulocytes of a subject in need thereof. Without being bound by theory, such antibodies and antigen-binding fragments thereof that bind to TMPRSS6 improve erythropoiesis of a subject in need thereof. In certain embodiments, the disclosed antibodies and antigen-binding fragments thereof bind to TMPRSS6 and improve erythropoiesis of a subject in need thereof, leading to a reduced need for secondary erythropoiesis in the spleen, for example, as demonstrated in Example 12 herein.

[0117] Also disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and increase hemoglobin levels of a subject in need thereof, for example, as demonstrated in Example 13 herein.

[0118] Disclosed herein are antibodies and antigen-binding fragments thereof that bind to TMPRSS6 and decrease transferrin saturation levels of a subject in need thereof, for example, as demonstrated in Example 14 herein.

[0119] Disclosed herein is an isolated recombinant antibody or antigen-binding fragment thereof which binds specifically to TMPRSS6 protein, wherein the antibody or antigen-binding fragment thereof exhibits one or more of the following characteristics when administered to a subject in need thereof: (a) decreases serum iron levels of the subject; (b) increases serum hepcidin levels of the subject; (c) increases mature red blood cell levels of the subject; (d) increases mature red blood cell levels in the spleen and/or bone marrow of the subject; (e) increases hemoglobin levels of the subject; or (f) decreases transferrin saturation levels of the subject.

[0120] Disclosed herein are anti-TMPRSS6 antibodies or antigen-binding fragments thereof that are useful for decreasing serum iron levels of a subject in need thereof. Also disclosed herein are antibodies or antigen-binding fragments thereof that are useful for increasing serum hepcidin levels of a subject in need thereof. Also disclosed herein are antibodies or antigen-binding fragments thereof that are useful for increasing mature red blood cell levels of a subject in need thereof. Also disclosed herein are antibodies or antigen-binding fragments thereof that are useful for increasing mature red blood cell levels in the spleen and/or bone marrow of a subject in need thereof. Also disclosed herein are antibodies or antigen-binding fragments thereof that are useful for increasing hemoglobin levels of a subject in need thereof. Also disclosed herein are antibodies or antigen-binding fragments thereof that are useful for decreasing transferrin saturation levels of a subject in need thereof.

[0121] Disclosed herein are anti-TMPRSS6 antibodies or antigen-binding fragments thereof that are useful for treating or preventing at least one symptom or indication that is associated with increased serum iron levels. In certain embodiments, the antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication that is associated with decreased serum hepcidin levels. In certain embodiments, the antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication that is associated with decreased mature red blood cell levels. In certain embodiments, the antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication that is associated with increased mature red blood cell levels in the spleen and/or bone marrow. In certain embodiments, the antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication that is associated with decreased hemoglobin levels. In certain embodiments, the antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication that is associated with increased transferrin saturation levels.

[0122] The antibodies and antigen-binding fragments thereof may possess one or more of the aforementioned characteristics, or any combinations thereof. Other characteristics of the disclosed antibodies and antigen-binding fragments thereof will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Inhibition of TMPRSS6

[0123] Disclosed herein are antibodies and antigen-binding fragments thereof that inhibit protease-dependent release of cell surface hemojuvelin (HJV) in the presence of human TMPRSS6, *e.g.*, antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6, with a percent inhibition greater than about 90% (*e.g.*, greater than about 89.2%, greater than about 89.4%, greater than about 89.6%, greater than about 89.8%, greater than about 90%, greater than about 90.2%, greater than about 90.4%, greater than about 90.6%, greater than about 90.8%), as measured, *e.g.*, using an assay format as described in Example 6 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit protease-dependent release of cell surface hemojuvelin (HJV) in the presence of human TMPRSS6, *e.g.*, antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6, exhibiting an IC_{50} value ranging from about 70 pM to about 200 pM (*e.g.*, about 69.3 pM, about 70 pM, about 80 pM, about 90 pM, about 100 pM, about 110 pM, about 120 pM, about 130 pM, about 140 pM, about 150 pM, about 160 pM, about 170 pM, about 180 pM, about

190 pM, about 202 pM), as measured, *e.g.*, using an assay format as described in Example 6 herein, or a substantially similar assay.

[0124] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit protease-dependent release of cell surface hemojuvelin (HJV) in the presence of mouse TMPRSS6, *e.g.*, antibodies and antigen-binding fragments thereof that inhibit mouse TMPRSS6, with a percent inhibition from about 42% to about 98.5% (*e.g.*, about 41.6%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%), as measured, *e.g.*, using an assay format as described in Example 6 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit protease-dependent release of cell surface hemojuvelin (HJV) in the presence of mouse TMPRSS6, *e.g.*, antibodies and antigen-binding fragments thereof that inhibit mouse TMPRSS6, exhibiting an IC₅₀ value ranging from about 82.6 pM to about 274 pM (*e.g.*, about 81.8 pM, about 90 pM, about 100 pM, about 110 pM, about 120 pM, about 130 pM, about 140 pM, about 150 pM, about 160 pM, about 170 pM, about 180 pM, about 190 pM, about 200 pM, about 210 pM, about 220 pM, about 230 pM, about 240 pM, about 250 pM, about 260 pM, about 276 pM), as measured, *e.g.*, using an assay format as described in Example 6 herein, or a substantially similar assay.

[0125] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6 with a percent inhibition from about 65% to about 98% (*e.g.*, about 64.5%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98.9%), as measured, *e.g.*, using a “CM bioassay” format as described in Example 7 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6 exhibiting an IC₅₀ value ranging from about 710 pM to greater than about 10 nM (*e.g.*, about 703 pM, about 750 pM, about 800 pM, about 850 pM, about 900 pM, about 950 pM, about 1 nM, about 5 nM, about 10.1 nM), as measured, *e.g.*, using a “CM bioassay” format as described in Example 7 herein, or a substantially similar assay.

[0126] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit monkey TMPRSS6 with a percent inhibition from about 66% to about 98% (*e.g.*, about 65.5%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 98.9%), as measured, *e.g.*, using a “CM bioassay” format as described in Example 7 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit monkey TMPRSS6 exhibiting an IC₅₀ value ranging from about 740 pM to greater than about 50 nM (*e.g.*, about 733 pM, about 750 pM, about 800 pM, about 850 pM, about 900 pM, about 950 pM, about 1 nM, about 10 nM, about 15 nM, about 20 nM, about 25

nM, about 30 nM, about 35 nM, about 40 nM, about 45 nM, about 50.5 nM), as measured, *e.g.*, using a “CM bioassay” format as described in Example 7 herein, or a substantially similar assay.

[0127] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6 (*e.g.*, at 25°C or at 37°C) with a percent inhibition from about 86% to about 90% (*e.g.*, about 85.2%, about 85%, about 91%), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit human TMPRSS6 (*e.g.*, at 25°C or at 37°C) exhibiting an IC₅₀ value ranging from about 360 pM to about 5.8 nM (*e.g.*, about 357 pM, about 400 pM, about 500 pM, about 600 pM, about 700 pM, about 800 pM, about 900 pM, about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay.

[0128] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit monkey TMPRSS6 (*e.g.*, at 25°C or at 37°C) with a percent inhibition from about 81% to about 94% (*e.g.*, about 80.2%, about 85%, about 90%, about 94.9%), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit monkey TMPRSS6 (*e.g.*, at 25°C or at 37°C) exhibiting an IC₅₀ value ranging from about 660 pM to about 30 nM (*e.g.*, about 654 pM, about 700 pM, about 750 pM, about 800 pM, about 850 pM, about 900 pM, about 950 pM, about 1 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30.3 nM), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay.

[0129] Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit mouse TMPRSS6 (*e.g.*, at 25°C or at 37°C) with a percent inhibition from about 58% to about 103% (*e.g.*, about 57.5%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 104%), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay. Also disclosed herein are antibodies and antigen-binding fragments thereof that inhibit mouse TMPRSS6 (*e.g.*, at 25°C or at 37°C) exhibiting an IC₅₀ value ranging from about 1.5 nM to about 35 nM (*e.g.*, about 2 nM, about 10 nM, about 15 nM, about 20 nM, about 25 nM, about 30 nM, about 35.3 nM), as measured, *e.g.*, using a “HEK293 bioassay” format as described in Example 7 herein, or a substantially similar assay.

[0130] In one embodiment, an isolated recombinant antibody or antigen-binding fragment thereof is disclosed herein which binds specifically to TMPRSS6 protein, wherein the antibody

or fragment thereof exhibits one or more of the following characteristics: (a) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6, with percent inhibition greater than about 90%; (b) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6 and exhibits an IC_{50} less than about 200 pM; (c) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6, with percent inhibition greater than about 42%; (d) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6 and exhibits an IC_{50} less than about 274 pM; (e) inhibits human TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 65%; (f) inhibits human TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 10 nM; (g) inhibits monkey TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 66%; (h) inhibits monkey TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 50 nM; (i) inhibits mouse TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 58%; (j) inhibits mouse TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 35 nM; (k) is a fully human monoclonal antibody or antigen-binding fragment thereof; (l) comprises a HCDR1, HCDR2, and HCDR3 comprising an amino acid sequence selected from a HCDR1, HCDR2, and HCDR3 sequence listed in Table 1 and/or a LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence selected from a LCDR1, LCDR2, and LCDR3 sequence listed in Table 1; and/or (m) comprises a HCVR comprising an amino acid sequence selected from a HCVR sequence listed in Table 1 and a LCVR comprising an amino acid sequence selected from a LCVR sequence listed in Table 1.

[0131] The antibodies and antigen-binding fragments thereof may possess one or more of the aforementioned characteristics, or any combinations thereof. Other characteristics of the disclosed antibodies and antigen-binding fragments thereof will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[0132] Disclosed herein are anti-TMPRSS6 antibodies which interact with one or more amino acids found within one or more regions of the TMPRSS6 protein molecule. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned domains of the TMPRSS6 protein molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino

acid sequences) located within either or both of the aforementioned domains of the protein molecule. In one embodiment, the epitope is a conformational epitope.

[0133] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in *Antibodies*, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) *Methods Mol. Biol.* 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) *Prot. Sci.* 9: 487-496).

[0134] Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. For example, see Example 8 of the instant disclosure. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. See, e.g., Ehrling (1999) *Analytical Biochemistry* 267: 252-259; Engen and Smith (2001) *Anal. Chem.* 73: 256A-265A.

[0135] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies disclosed

herein into groups of antibodies binding different epitopes.

[0136] The present disclosure includes anti-TMPRSS6 antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antibodies listed in Table 1. Likewise, Also disclosed herein are anti-TMPRSS6 antibodies that compete for binding to TMPRSS6 protein or a fragment thereof with any of the specific exemplary antibodies listed in Table 1. For example, the present disclosure includes anti-TMPRSS6 antibodies that cross-compete for binding to TMPRSS6 protein with one or more antibodies listed in Table 1.

[0137] One can determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-TMPRSS6 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-TMPRSS6 antibody disclosed herein, the reference antibody is allowed to bind to a TMPRSS6 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the TMPRSS6 protein molecule is assessed. If the test antibody is able to bind to TMPRSS6 following saturation binding with the reference anti-TMPRSS6 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-TMPRSS6 antibody. On the other hand, if the test antibody is not able to bind to the TMPRSS6 protein following saturation binding with the reference anti-TMPRSS6 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-TMPRSS6 antibody disclosed herein.

[0138] To determine if an antibody competes for binding with a reference anti-TMPRSS6 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a TMPRSS6 protein under saturating conditions followed by assessment of binding of the test antibody to the TMPRSS6 molecule. In a second orientation, the test antibody is allowed to bind to a TMPRSS6 molecule under saturating conditions followed by assessment of binding of the reference antibody to the TMPRSS6 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the TMPRSS6 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to TMPRSS6. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0139] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, *Cancer Res.* 1990

50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0140] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0141] The disclosure encompasses a human anti-TMPRSS6 monoclonal antibody conjugated to a therapeutic moiety (“immunoconjugate”), to treat a TMPRSS6-associated disease or disorder (*e.g.*, an iron overload disorder). As used herein, the term “immunoconjugate” refers to an antibody which is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a peptide or protein or a therapeutic agent. The antibody may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to TMPRSS6 protein. The type of therapeutic moiety that may be conjugated to the anti-TMPRSS6 antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081.

Therapeutic Administration and Formulations

[0142] The disclosure provides therapeutic compositions comprising the anti-TMPRSS6 antibodies or antigen-binding fragments thereof disclosed herein. Therapeutic compositions in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes,

oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0143] Various delivery systems are known and can be used to administer the pharmaceutical composition disclosed herein, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.* (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0144] The use of nanoparticles to deliver the antibodies disclosed herein is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., *et al.* 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles may be developed and conjugated to antibodies contained in pharmaceutical compositions to target cells. Nanoparticles for drug delivery have also been described in, for example, US 8257740, or US 8246995, each incorporated herein in its entirety.

[0145] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0146] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known.

[0147] The disclosed pharmaceutical compositions can be delivered subcutaneously or

intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition as disclosed herein. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0148] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

Therapeutic Uses of the Antibodies

[0149] The disclosed anti-TMPRSS6 antibodies and antigen-binding fragments thereof are useful for the treatment, and/or prevention of a disease or disorder or condition associated with TMPRSS6 and/or for ameliorating at least one symptom associated with such disease, disorder or condition. In certain embodiments, the disclosed anti-TMPRSS6 antibody or antigen-binding fragment thereof may be administered at a therapeutic dose to a subject with a disease or disorder or condition associated with TMPRSS6.

[0150] In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of a TMPRSS6-associated disease or disorder. In certain embodiments, the TMPRSS6-associated disease or disorder is an iron overload disorder. In certain embodiments, the antibodies or antigen-binding fragments thereof of the present disclosure are useful for treating or preventing at least one symptom of a congenital dyserythropoietic anemia, Diamond-Blackfan anemia, alpha thalassemia, beta thalassemia, a transfusion-dependent hemolytic anemia, myelodysplastic syndrome, sickle cell disease, polycythemia vera, hereditary hemochromatosis, primary hemochromatosis, secondary hemochromatosis, severe juvenile hemochromatosis, or chronic liver disease.

[0151] In certain embodiments, the disclosed antibodies or antigen-binding fragments

thereof are useful for treating or preventing at least one symptom, complication, or indication of beta thalassemia. In certain embodiments, the beta thalassemia is beta thalassemia major or beta thalassemia intermedia. Symptoms of beta thalassemia include fatigue, weakness, pale or yellowish skin, facial bone deformities, slow growth, abdominal swelling, dark urine, and other complications (*e.g.*, complications due to therapy). Examples of complications of moderate to severe beta thalassemia include iron overload, which can be a result of either the disease or from frequent blood transfusions, and is associated with damage to organs such as the heart, the liver, and the endocrine system (*e.g.*, hormone-producing glands that regulate processes throughout the body), and increased risk of infection. Complications of severe beta thalassemia can also include bone deformities, enlarged spleen, slowed growth rates, and heart problems.

[0152] In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom, complication, or indication of hemochromatosis. Symptoms of hemochromatosis include, for example, joint pain, abdominal pain, weakness, fatigue, diabetes, loss of sex drive, impotence, heart failure, liver failure, bronze or gray skin color, and memory fog. Complications due to hemochromatosis include, for example, liver problems, pancreas problems, heart problems, reproductive problems, and skin color changes.

[0153] In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom, complication, or indication associated with iron overload. Iron overload can be the result of a genetic disease or disorder, or the result of receiving frequent blood transfusions, consuming high levels of supplemental iron, and/or receiving injections comprising iron. Genetic disorders that result in iron overload include hereditary hemochromatosis, various anemias including sickle-cell disease and sideroblastic anemia, African iron overload (*e.g.*, where affected individuals drink a beverage that contains a high amount of iron), enzyme deficiencies (*e.g.*, deficiencies in pyruvate kinase, deficiencies in glucose-6-phosphate dehydrogenase), and protein transport disorders such as aceruloplasminemia and atransferrinemia.

[0154] Symptoms of iron overload include, for example, fatigue (*e.g.*, chronic fatigue), joint pain, abdominal pain, liver disease (*e.g.*, cirrhosis, liver cancer), diabetes mellitus, issues with the heart (*e.g.*, irregular heart rhythm, heart attack, heart failure), skin color changes (*e.g.*, bronze, ashen-gray green), loss of period, loss of interest in sex, impotence, infertility, hypogonadism, issues with bones (*e.g.*, osteoarthritis, osteoporosis), hair loss, enlarged liver or spleen, hypothyroidism, hypopituitarism, depression, adrenal function problems, early onset neurodegenerative disease (*e.g.*, Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy,

multiple sclerosis), and elevated markers such as blood sugar, liver enzymes, serum iron, serum transferrin, and serum ferritin.

[0155] In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication associated with receiving blood transfusions, *e.g.*, receiving frequent and/or regular blood transfusions. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication associated with a physical trauma that results in blood loss. In certain embodiments, the physical trauma that results in blood loss requires treatment with a blood transfusion. As such, a symptom or indication associated with a physical trauma that results in blood loss can be any symptom or indication (*e.g.*, iron overload) that is associated with treatment of the physical trauma with a blood transfusion. For example, a physical trauma can be a traumatic accident (*e.g.*, leading to a loss of limb, blunt force trauma, a gash) resulting in blood loss requiring a blood transfusion. In certain embodiments, the required blood transfusion(s) is a large volume blood transfusion.

[0156] In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of myelodysplastic syndrome. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of myelodysplastic syndrome with ring sideroblasts. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof of the present disclosure are useful for treating or preventing at least one symptom or indication of a transfusion-dependent hemolytic anemia. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of a transfusion-dependent hemolytic anemia due to pyruvate kinase deficiency (*i.e.*, hemolytic anemia), or a sideroblastic transfusion-dependent hemolytic anemia (*i.e.*, sideroblastic anemia). In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of sickle-cell anemia. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of dyserythropoietic anemia. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of erythropoietic porphyria. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication of a chronic liver disease. In certain embodiments, the disclosed antibodies or antigen-binding fragments thereof are useful for treating or preventing at least one symptom or indication

of an alcohol related chronic liver disease, hepatitis C, or autoimmune hepatitis.

[0157] Also disclosed is the use of the disclosed antibodies or antigen-binding fragments thereof prophylactically in subjects at risk for suffering from a Tmprss6-associated disease or disorder.

[0158] In some embodiments, the disclosed antibodies or antigen-binding fragments thereof are used for the preparation of a pharmaceutical composition or medicament for treating subjects suffering from a disease, disorder, or condition disclosed herein. In another embodiment, the disclosed antibodies or antigen-binding fragments thereof are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating or ameliorating a disease, disorder or condition disclosed herein.

Combination Therapies

[0159] Combination therapies may include one or more of the disclosed antibodies or antigen-binding fragments thereof and any additional therapeutic agent that may be advantageously combined with the disclosed antibodies or antigen-binding fragments thereof. The disclosed antibodies or antigen-binding fragments may be combined synergistically with one or more drugs or therapy used to treat a Tmprss6-associated disease or disorder. In some embodiments, the disclosed antibodies or antigen-binding fragments may be combined with a second therapeutic agent to ameliorate one or more symptoms of said disease or condition.

[0160] Depending upon the disease, disorder or condition, the antibodies or antigen-binding fragments disclosed herein may be used in combination with one or more additional therapeutic agents. For example, wherein the disease, disorder or condition is associated with iron overload, the antibodies or antigen-binding fragments disclosed herein may be used in combination with one or more iron reduction therapies and/or therapies that are currently employed for treating the iron overload.

[0161] In certain embodiments, iron chelation therapy may be employed for treating the iron overload. Iron chelation therapy is the pharmacological removal of iron with an iron-chelating agent. Examples of iron-chelating agents include, deferoxamine, deferasirox, and deferiprone. (see, *e.g.*, Mobarra et al., *Int. J. Hematol. Oncol. Stem Cell Res.* (2016) 10(4): 239-247). Other examples of iron-chelating agents are known to those of skill in the art, *e.g.*, those reviewed in Hatcher et al., *Future Med. Chem.* (2009) 1(9):1643-70.

[0162] Other therapies that may be employed for treating the iron overload include the use of agents such as, without limitation, vitamin E, wheat germ oil, tocophersolan, indicaxanthin, folic acid, agents that increase fetal hemoglobin levels (*e.g.*, hydroxyurea, histone

deacetylase inhibitors and/or DNA methyl transferase inhibitors (*e.g.*, butyrate derivatives, azacitidine, decitabine (5-aza 2-deoxycytidine) and trichostatin-A), hemin, pomalidomide, thalidomide, cytokines such as stem cell factor (SCF) and transforming growth factor beta (TGF beta)), agents to manage ulcers, agents to manage infection (*e.g.*, antibiotics and antivirals), agents to treat thrombosis, or agents to treat anemia (*e.g.*, luspatercept).

[0163] Iron overload can also be treated with stem cell or bone marrow transplantation, blood transfusions, or phlebotomy.

[0164] As used herein, the term “in combination with” means that additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-TMPRSS6 antibodies or antigen-binding fragments disclosed herein. The term “in combination with” also includes sequential or concomitant administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments and a second therapeutic agent.

[0165] The additional therapeutically active component(s) may be administered to a subject prior to administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments thereof. For example, a first component may be deemed to be administered “prior to” a second component if the first component is administered 1 week before, 72 hours before, 60 hours before, 48 hours before, 36 hours before, 24 hours before, 12 hours before, 6 hours before, 5 hours before, 4 hours before, 3 hours before, 2 hours before, 1 hour before, 30 minutes before, or less than 30 minutes before administration of the second component.

[0166] In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments thereof. For example, a first component may be deemed to be administered “after” a second component if the first component is administered 30 minutes after, 1 hour after, 2 hours after, 3 hours after, 4 hours after, 5 hours after, 6 hours after, 12 hours after, 24 hours after, 36 hours after, 48 hours after, 60 hours after, 72 hours after or more after administration of the second component.

[0167] In other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments thereof. “Concurrent” administration, for purposes of the present disclosure, includes, *e.g.*, administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments thereof and an additional therapeutically active component to a subject in a single dosage form, or in separate dosage forms administered

to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route (*e.g.*, both the anti-TMPRSS6 antibody and the additional therapeutically active component may be administered intravenously, etc.); alternatively, each dosage form may be administered via a different route (*e.g.*, the disclosed antibody or antigen-binding fragment may be administered intravenously, and the additional therapeutically active component may be administered orally). Administering the components in a single dosage form, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of one or more of the disclosed anti-TMPRSS6 antibodies or antigen-binding fragments thereof "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of the disclosed anti-TMPRSS6 antibody or antigen-binding fragment thereof "in combination with" an additional therapeutically active component.

[0168] Also disclosed herein are pharmaceutical compositions in which an anti-TMPRSS6 antibody or antigen-binding fragment disclosed herein is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Diagnostic Uses of the Antibodies

[0169] The disclosed antibodies and antigen-binding fragments thereof may be used to detect and/or measure TMPRSS6 in a sample, *e.g.*, for diagnostic purposes. Disclosed herein are uses of the disclosed antibodies and antigen-binding fragments thereof in assays to detect a TMPRSS6-associated disease or disorder. Exemplary diagnostic assays for TMPRSS6 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-TMPRSS6 antibody or antigen-binding fragment disclosed herein that is labeled with a detectable label or reporter molecule. The disclosed antibodies and antigen-binding fragments thereof may also be used as a capture ligand to selectively isolate TMPRSS6 from samples from a subject. Alternatively, an unlabeled anti-TMPRSS6 antibody or fragment can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I ; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β -galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure TMPRSS6 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell

sorting (FACS).

[0170] Samples that can be used in TMPRSS6 diagnostic assays include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either TMPRSS6 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of TMPRSS6 protein in a particular sample obtained from a healthy subject (*e.g.*, a patient not afflicted with a disease associated with TMPRSS6) may be measured to initially establish a baseline, or standard, level of TMPRSS6. This baseline level of TMPRSS6 can then be compared against the levels of TMPRSS6 measured in samples obtained from individuals suspected of having a TMPRSS6-associated condition, or symptoms associated with such condition.

[0171] The anti-TMPRSS6 antibodies or antigen-binding fragments thereof may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

EXAMPLES

[0172] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions disclosed herein, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, room temperature is about 25°C, and pressure is at or near atmospheric.

Example 1: Generation of Human Antibodies to Transmembrane Serine Protease 6 (TMPRSS6)

[0173] Human antibodies to TMPRSS6 protein were generated in a VELOCIMMUNE[®] mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions. The mice were immunized and boosted with human TMPRSS6 DNA by hydrodynamic DNA delivery.

[0174] Anti-TMPRSS6 antibodies were isolated directly from antigen-positive mouse B cells without fusion to myeloma cells, as described in U.S. Patent No. 7,582,298, herein

specifically incorporated by reference in its entirety. Using this method, several fully human anti-TMPRSS6 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained.

[0175] Exemplary antibodies generated as disclosed above were designated as mAb37746, mAb37763, mAb37777, mAb37699, mAb41422, mAb41465, and mAb41450, having HCVR, HCDR1, HCDR2, HCDR3, LCVR, LCDR1, LCDR2, and LCDR3 amino acid and nucleic acid sequences as identified in Table 1 and Table 2.

[0176] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Heavy and Light Chain Variable Region Amino Acid and Nucleotide Sequences

[0177] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable region sequences, CDR sequences, and heavy and light chain sequences of selected exemplary anti-TMPRSS6 antibodies.

]

Table 1: Amino Acid Sequence Identifiers

Antibody ID	SEQ ID NOs:							
	HCVR	H-CDR1	H-CDR2	H-CDR3	LCVR	L-CDR1	L-CDR2	L-CDR3
mAb37746	2	4	6	8	10	12	14	16
mAb37763	22	24	26	28	10	12	14	16
mAb37777	32	34	36	38	10	12	14	16
mAb37699	42	44	46	48	50	52	54	56
mAb41422	62	64	66	68	70	72	74	76
mAb41465	82	84	86	88	90	92	14	94
mAb41450	100	102	104	106	108	110	14	112

[0178] The corresponding nucleic acid sequence identifiers of the selected exemplary anti-TMPRSS6 antibodies are set forth in Table 2.

Table 2: Nucleic Acid Sequence Identifiers

Antibody ID	SEQ ID NOs:							
	HCVR	H-CDR1	H-CDR2	H-CDR3	LCVR	L-CDR1	L-CDR2	L-CDR3
mAb37746	1	3	5	7	9	11	13	15
mAb37763	21	23	25	27	9	11	13	15
mAb37777	31	33	35	37	9	11	13	15
mAb37699	41	43	45	47	49	51	53	55
mAb41422	61	63	65	67	69	71	73	75
mAb41465	81	83	85	87	89	91	13	93
mAb41450	99	101	103	105	107	109	13	111

[0179] The antibodies may have a human or mouse Fc isotype. As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (e.g., an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG1 or a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Table 1 – will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the Fc domain.

[0180] Unless indicated otherwise, all antibodies used in the following Examples comprise a human IgG4 Fc comprising a serine to proline mutation in the hinge region (S108P). Exemplary antibodies of mAb37746, mAb37763, mAb37777, mAb37699, mAb41422, mAb41465, and mAb41450 comprising a human IgG4 Fc comprising a serine to proline mutation in the hinge region (S108P) were designated as REGN7969, REGN7971, REGN7973, REGN8023, REGN7974, REGN7977, and REGN7999, respectively. Table 3 sets forth the amino acid sequence identifiers of full-length heavy chain and light chain sequences of these antibodies.

Table 3

Antibody ID	Antibody Name	SEQ ID NOs:			
		HC		LC	
		DNA	PEP	DNA	PEP
mAb37746	REGN7969	17	18	19	20
mAb37763	REGN7971	29	30	19	20
mAb37777	REGN7973	39	40	19	20
mAb37699	REGN8023	57	58	59	60

mAb414 22	REGN79 74	77	78	79	80
mAb414 65	REGN79 77	95	96	97	98
mAb414 50	REGN79 99	113	114	115	116

Example 3: Antibody binding to TMPRSS6 as determined by Surface Plasmon Resonance

Experimental Procedure

[0181] The equilibrium dissociation constant (K_D) for TMPRSS6 binding to different TMPRSS6 monoclonal antibodies (mAbs) was determined using a real-time surface plasmon resonance biosensor using a Biacore 4000 instrument. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25°C and 37°C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with a human Fc specific mouse mAb to capture different TMPRSS6 mAbs. Several TMPRSS6 reagent proteins were used for the experiment including human TMPRSS6 extracellular domain with a S762A mutation expressed with a C-terminal myc-myc-hexahistidine tag (referred to as hTMPRSS6_S762A-MMH; REGN5330; SEQ ID NO: 117), *Macaca fascicularis* TMPRSS6 extracellular domain with a S751A mutation expressed with a C-terminal myc-myc-hexahistidine tag (referred to as mfTMPRSS6_S751A-MMH; REGN5977; SEQ ID NO: 119), and mouse TMPRSS6 extracellular domain with a S762A mutation expressed with a C-terminal myc-myc-hexahistidine tag (referred to as mTMPRSS6_S762A-MMH; REGN6848; SEQ ID NO: 118). Different concentrations (100nM to 3.7nM, 3-fold serial dilution) of the TMPRSS6 reagent proteins prepared in HBS-EP running buffer were injected over the TMPRSS6 mAb captured surface for 150 seconds at a flow rate of 30 μ L/min and their dissociation in HBS-ET running buffer was monitored for 10 minutes. At the end of each cycle, the TMPRSS6 mAb captured surface was regenerated using a 12 second injection of 20mM phosphoric acid.

[0182] The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0 (BioLogic Software) curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life ($t_{1/2}$) were calculated from the kinetic rates as:

$$K_D(M) = \frac{k_d}{k_a}, \quad \text{and} \quad t_{1/2}(min) = \frac{\ln(2)}{60 * k_d}$$

Results

[0183] Binding kinetics parameters of the different selected TMPRSS6 mAbs to each TMPRSS6 reagent protein are shown in Tables 4 through 9.

Table 4: Binding kinetics parameters of different TMPRSS6 mAbs binding to hTMPRSS6_S762A-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7969	142 ± 0.6	89	4.04E+05	1.82E-04	4.50E-10	64
REGN7971	164 ± 5	103	4.55E+05	1.47E-04	3.22E-10	79
REGN7973	152 ± 2.8	134	2.52E+05	1.57E-04	6.26E-10	73
REGN7974	156 ± 3	191	2.59E+05	6.80E-04	2.63E-09	17
REGN7977	138 ± 0.4	159	2.18E+05	3.68E-04	1.69E-09	31
REGN7999	131 ± 0.8	176	2.67E+05	6.49E-05	2.43E-10	178
REGN8023	160 ± 1.1	205	6.71E+05	8.60E-05	1.28E-10	134

[0184] As shown in Table 4, the selected TMPRSS6 mAbs bound to hTMPRSS6_S762A-MMH at 25°C with K_D values ranging from about 128pM to about 2.63nM.

Table 5: Binding kinetics parameters of different TMPRSS6 mAbs binding to hTMPRSS6_S762A-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7969	145 ± 3.6	110	2.22E+05	9.75E-04	4.40E-09	12
REGN7971	187 ± 4.6	169	2.61E+05	6.37E-04	2.44E-09	18
REGN7973	176 ± 2.7	164	5.95E+04	1.26E-03	2.12E-08	9
REGN7974	181 ± 3.2	206	1.11E+05	2.35E-03	2.12E-08	4.9
REGN7977	131 ± 1.2	134	1.13E+05	1.24E-03	1.10E-08	9
REGN7999	106 ± 1.5	129	1.54E+05	3.54E-04	2.30E-09	33
REGN8023	147 ± 1.8	205	4.18E+05	9.35E-04	2.24E-09	12

[0185] As shown in Table 5, the selected TMPRSS6 mAbs bound to hTMPRSS6_S762A-MMH at 37°C with K_D values ranging from about 2.24nM to about 21.2nM.

Table 6: Binding kinetics parameters of different TMPRSS6 mAbs binding to mfTMPRSS6_S751A-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7969	142 ± 0.6	92	4.19E+05	1.82E-04	4.35E-10	63
REGN7971	168 ± 2	109	4.87E+05	1.38E-04	2.83E-10	84
REGN7973	157 ± 1.4	132	2.17E+05	5.14E-04	2.37E-09	22
REGN7974	160 ± 1.6	188	2.68E+05	1.92E-03	7.18E-09	6
REGN7977	137 ± 0.5	155	2.25E+05	6.97E-04	3.09E-09	17
REGN7999	130 ± 0.5	185	6.60E+05	7.23E-05	1.10E-10	160
REGN8023	161 ± 3.3	203	8.91E+05	5.79E-05	6.50E-11	199

[0186] As shown in Table 6, the selected TMPRSS6 mAbs bound to mfTMPRSS6_S751A-MMH at 25°C with K_D values ranging from about 65pM to about 7.18nM.

Table 7: Binding kinetics parameters of different TMPRSS6 mAbs binding to mfTMPRSS6_S751A-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7969	138 ± 1.3	105	2.83E+05	9.24E-04	3.27E-09	12
REGN7971	178 ± 1.8	160	3.63E+05	6.10E-04	1.68E-09	19
REGN7973	168 ± 1.2	145	1.32E+05	3.40E-03	2.57E-08	3.4
REGN7974	173 ± 2	183	2.51E+05	4.05E-03	1.61E-08	2.9
REGN7977	128 ± 0.9	133	2.07E+05	2.47E-03	1.19E-08	4.7
REGN7999	104 ± 1	143	4.08E+05	3.06E-04	7.50E-10	38
REGN8023	144 ± 1.6	206	6.32E+05	6.34E-04	1.00E-09	18

[0187] As shown in Table 7, the selected TMPRSS6 mAbs bound to mfTMPRSS6_S751A-MMH at 37°C with K_D values ranging from about 750pM to about 25.7nM.

Table 8: Binding kinetics parameters of different TMPRSS6 mAbs binding to mTMPRSS6_S762A-MMH at 25°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
REGN7969	140 ± 0.4	4	NB*	NB*	NB*	NB*
REGN7971	169 ± 1.2	5	IC [#]	IC [#]	IC [#]	IC [#]
REGN7973	158 ± 0.9	7	IC [#]	IC [#]	IC [#]	IC [#]
REGN7974	160 ± 0.5	3	NB*	NB*	NB*	NB*

REGN7977	136 ± 0.9	20	2.88E+05	6.70E-02	2.33E-07	0.17
REGN7999	128 ± 0.4	178	4.16E+05	3.45E-05	8.29E-11	334
REGN8023	157 ± 1.5	209	5.99E+05	1.10E-03	1.84E-09	10

- NB* indicates that no binding was observed under the particular experimental conditions of this Example.
- IC[#] indicates that the observed binding data were inconclusive and binding kinetic parameters could not be determined under the particular experimental conditions of this Example.

[0188] As shown in Table 8, three out of seven selected TMPRSS6 mAbs bound to mTMPRSS6_S762A-MMH at 25°C with K_D values ranging from about 82.9pM to about 233nM.

Table 9: Binding kinetics parameters of different TMPRSS6 mAbs binding to mTMPRSS6_S762A-MMH at 37°C

mAb Captured	mAb Capture Level (RU)	100nM Ag Bound (RU)	k _a (1/Ms)	k _d (1/s)	K _D (M)	t _{1/2} (min)
REGN7969	128 ± 0.8	2	NB*	NB*	NB*	NB*
REGN7971	170 ± 2.2	15	IC [#]	IC [#]	IC [#]	IC [#]
REGN7973	162 ± 4.1	12	IC [#]	IC [#]	IC [#]	IC [#]
REGN7974	164 ± 0.9	3	NB*	NB*	NB*	NB*
REGN7977	123 ± 0.7	9	3.23E+05	2.27E-01	7.03E-07	0.05
REGN7999	99 ± 0.8	130	5.05E+05	1.60E-04	3.17E-10	72
REGN8023	138 ± 1.4	189	2.69E+05	6.66E-03	2.47E-08	1.7

- NB* indicates that no binding was observed under the particular experimental conditions of this Example.
- IC[#] indicates that the observed binding data were inconclusive and binding kinetic parameters could not be determined under the particular experimental conditions of this Example.

[0189] As shown in Table 9, three out of seven selected TMPRSS6 mAbs bound to mTMPRSS6_S762A-MMH at 37°C with K_D values ranging from about 317pM to about 703nM.

Example 4: Cross-competition between selected anti-TMPRSS6 monoclonal antibodies

Experimental Procedure

[0190] Binding competition between TMPRSS6 monoclonal antibodies (mAbs) was determined using a real time, label-free bio-layer interferometry (BLI) assay on the Octet HTX biosensor platform (Pall ForteBio Corp.). The entire experiment was performed at 25°C in 10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% v/v Surfactant Tween-20, 1mg/mL BSA, 0.02% NaN₃, pH7.4 (HBS-EBT) buffer with the plate shaking at a speed of 1000rpm. To assess whether 2 mAbs are able to compete with one another for binding to their respective epitopes on TMPRSS6,

a recombinant protein comprised of the extracellular domain of human TMPRSS6 expressed with a S762A mutation and a C-terminal myc-myc-hexahistidine tag (referred to as hTMPRSS6_S762A-MMH; REGN5330SEQ ID NO: 117) was first captured onto anti-penta-His antibody (HIS1K) coated Octet biosensor tips by submerging the biosensor tips for 1 minute in wells containing 10 µg/mL solution of hTMPRSS6_S762A-MMH. The hTMPRSS6_S762A-MMH captured biosensor tips were then saturated with the first TMPRSS6 mAb (referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 4 minutes. The biosensor tips were then subsequently dipped into wells containing 50µg/mL solution of the second TMPRSS6 mAb (referred to as mAb-2) for 3 minutes. The biosensor tips were washed in HBS-EBT buffer in between every step of the experiment. The real-time binding response was monitored during the entire course of the experiment and the binding response at the end of every step was recorded.

Results

[0191] The response of mAb-2 binding to hTMPRSS6_S762A-MMH pre-complexed with mAb-1 was compared and the competitive/non-competitive behavior of different selected TMPRSS6 mAbs was determined as shown in Table 10.

Table 10: Cross-competition between anti-TMPRSS6 monoclonal antibodies

mAb-1	mAb-2 shown to compete with mAb-1
REGN7974	REGN7974
	REGN7973
	REGN7977
	REGN7999
REGN7973	REGN7974
	REGN7973
	REGN7977
	REGN7999
REGN7977	REGN7974
	REGN7973
	REGN7977
	REGN7999
REGN7999	REGN7974
	REGN7973
	REGN7977
	REGN7999
REGN7969	REGN7969
	REGN7971

	REGN8023
REGN7971	REGN7969
	REGN7971
	REGN8023
	REGN7969
REGN8023	REGN7971
	REGN8023

Example 5: Antibody binding to cells expressing TMPRSS6

Experimental Procedure

[0192] In order to assess cell binding by the disclosed anti-TMPRSS6 antibodies, HEK293 cells were engineered to stably overexpress full length TMPRSS6 from human (NCBI Reference Sequence NM_001289001), cynomolgous monkey/*Macaca fascicularis* (NCBI Reference Sequence XM_005567384), and mouse (NCBI Reference Sequence NM_027902.2), and these cell lines are referred to as HEK293/hTMPRSS6, HEK293/mfTMPRSS6, and HEK293/mTMPRSS6, respectively. In addition, HEK293 cells were engineered to stably overexpress a catalytically inactive full length human TMPRSS6, where the Serine at position 753 of NCBI Reference Sequence NM_001289001 was changed to an Alanine. This cell line is referred to as HEK293/hTMPRSS6 (S762A). All of the cell lines were unsorted, except for the mouse TMPRSS6 cell line (HEK293/mTMPRSS6), which was sorted for high expression of mouse TMPRSS6.

[0193] To assess the binding of anti-TMPRSS6 antibodies of the present disclosure to the receptor expressed on the cell surface, antibodies (including an IgG subclass control) were serially diluted in assay buffer (PBS without Ca^{++} and Mg^{++} containing 2% FBS) from 375nM to 22.9pM (plus a sample containing assay buffer alone without test molecule) and incubated with 0.5×10^6 cells/well at 4°C for 30 minutes in assay buffer. After incubation with primary antibodies, the cells were stained with 4.0µg/mL of Alexa Fluor®-647 conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., anti-human # 109-607-003) at 4°C for 30 minutes. Cells were fixed using BD CytoFix™ (Becton Dickinson, # 554655) and analyzed on either the IQue® or IQue®Plus Flow Cytometers (Intellicyt®). Unstained and secondary antibody alone controls were also tested for all cell lines. The results were analyzed using ForeCyt® (IntelliCyt®) software to determine the geometric means of fluorescence for viable cells and binding ratio was calculated by normalizing the geometric mean value of the test condition by the geometric mean value of the corresponding unstained cells.

Results

[0194] Table 11 shows binding of selected anti-TMPRSS6 antibodies to cells engineered to express hTMPRSS6, hTMPRSS6 (S762A), mfTMPRSS6, and mTMPRSS6.

Table 11: Binding of anti-TMPRSS6 antibodies to HEK293/TMPRSS6 cells

	MFI – Normalized to Unstained Control									
TMPRSS6 expressed in HEK293 cells	No TMPRSS6		hTMPRSS6		hTMPRSS6 (S762A)		mfTMPRSS6		mTMPRSS6	
mAb	Max. Fold Binding	EC ₅₀ (M)	Max. Fold Binding	EC ₅₀ (M)	Max. Fold Binding	EC ₅₀ (M)	Max. Fold Binding	EC ₅₀ (M)	Max. Fold Binding	EC ₅₀ (M)
REGN7969	3	No Binding	52	6.7E-10	170	2.0E-09	50	1.2E-09	1	No Binding
REGN7971	3	No Binding	66	9.7E-10	209	1.7E-09	75	1.5E-09	1	No Binding
REGN7973	4	No Binding	58	2.7E-09	145	2.0E-09	91	3.3E-09	42	3.5E-08
REGN7974	3	No Binding	54	2.4E-09	234	2.3E-09	50	3.6E-09	1	No Binding
REGN7977	5	3.1E-08	69	1.9E-09	156	1.6E-09	78	2.4E-09	125	9.1E-09
REGN7999	4	No Binding	50	2.2E-09	420	2.2E-09	79	2.0E-09	421	4.5E-09
REGN8023	3	No Binding	47	7.0E-10	300	2.0E-09	52	9.4E-10	296	8.4E-10
hIgG Subclass Control	2	No Binding	1	No Binding	3	No Binding	1	No Binding	1	No Binding
Anti-Human 2" alone	2	No Binding	1	No Binding	2	No Binding	1	No Binding	1	No Binding

[0195] As shown in Table 11, the selected anti-TMPRSS6 antibodies showed binding to human, cynomolgous monkey, and human S762A TMPRSS6 expressed on HEK293 cells. The range of maximum fold binding ratios and EC₅₀ values were about 47 to 69-fold and about 670pM to 2.7nM for hTMPRSS6 expressing cells, about 145 to 420-fold and about 1.6 to 2.3nM for hTMPRSS6 (S762A) expressing cells, and about 50 to 91-fold and about 940pM to 3.6nM for mfTMPRSS6 expressing cells. For the four mAbs that bound to mTMPRSS6 expressing cells, the range of maximum fold binding ratios and EC₅₀ values were about 42 to 421-fold and about 840pM

to 35nM. The selected anti-TMPRSS6 antibodies demonstrated binding to HEK293 parental cells, with binding ratios of about 3 to 5-fold. The IgG subclass control antibody (hIgG Subclass Control) and secondary antibody alone (Anti-Human 2nd alone) samples demonstrated binding ratios ranging from about 1 to 3-fold.

Example 6: Detection of human Hemojuvelin (HJV)

Experimental Procedure

[0196] In order to further characterize the ability of anti-TMPRSS6 monoclonal antibodies (mAbs) to inhibit TMPRSS6, a cell-based bioassay with two different readouts was utilized. TMPRSS6 acts as a negative regulator of hepcidin expression by cleaving cell surface hemojuvelin (HJV). See, e.g., Rausa et al. *J. Cell. Mol. Med.* (2015) 19:879-888; and Lee P., *Acta Haematol.* (2009) 122:87-96.

[0197] For the assay, flow cytometry was used to measure cell surface human hemojuvelin (hHJV), and an ELISA assay was performed to quantify the level of soluble hHJV in HEK293 cells expressing full length human HJV and either human or mouse TMPRSS6. The co-expression bioassay cell lines were engineered using hHJV-expressing HEK293 cells as a parental cell line (hHJV; amino acid 1-426, NCBI Reference Sequence NP_998818.1). The parental cell line with stable expression of human HJV was selected with 500 µg/mL of G418 in Dulbecco's modified Eagle Media (DMEM) containing 10% (v/v) fetal bovine serum, 100 units of penicillin, 100 µg of streptomycin and 292 µg/ml of L-glutamine. These hHJV expressing HEK293 cells (HEK293.hHJV) were then enriched for high hHJV cell surface expression by FACS (Anti-hemojuvelin antibody; Abcam ab54431, Donkey anti-mouse IgG AF647; Invitrogen A-31571). To express TMPRSS6, HEK293.hHJV were transduced with lentiviral supernatants for either full length human TMPRSS6 (hTMPRSS6; amino acids 1-811, NCBI Reference Sequence NM_153609.3) or full-length mouse TMPRSS6 (mTMPRSS6; amino acids 1-811, NCBI Reference Sequence NM_027902.2) with a C-terminal triple myc tag. The resulting cell lines were referred to as HEK293.hHJV/3xMyc.hTMPRSS6 and HEK293.hHJV/3xMyc.mTMPRSS6, respectively). The lentivirus transduced cells were additionally selected with 500 µg/mL G418 and 100 µg/mL hygromycin B for 2 weeks, and sorted for high expressing TMPRSS6 populations using anti-Myc-AF647 antibody.

[0198] For the ELISA, assay cell lines were plated in flat bottom 96-well plates with 1 x 10⁴ cells/well. The antibodies of the present disclosure as well as controls were serially diluted (from 0nM to 200nM), then were added to each well containing a final volume of 100 µL, and incubated overnight at 37°C. The following day, supernatants were harvested, and an ELISA was

performed using Human RGM-C/Hemojuvelin DuoSet ELISA kit (R&D Systems, DY3720-05) according to the manufacturer's protocol. Optical density was obtained using SpectraMax i3 (Molecular Devices) and results analyzed with Prism software (GraphPad) to obtain IC₅₀ values. The inhibition percentage was calculated with the following equation:

$$\text{Inhibition, [\%]} = \frac{\text{Soluble HJV}_{\text{Baseline}} - \text{Soluble HJV}_{\text{Antibody}}}{\text{Soluble HJV}_{\text{Baseline}} - \text{Soluble HJV}_{\text{Protease Inhibitor}}} \times 100$$

[0199] In this equation, “*Soluble HJV_{Baseline}*” indicates the amount of soluble HJV from the supernatant of HJV-TMPRSS6 over-expression cells in the absence of antibody or protease inhibitor treatment. “*Soluble HJV_{Antibody}*” indicates the amount of soluble HJV from the maximum concentration of antibody treated assay samples, and “*Soluble HJV_{Protease Inhibitor}*” indicates the amount of soluble HJV from the serine protease inhibitor, Aprotinin (Sigma, Cat: 10236624001), treated sample.

[0200] For the FACS analysis, HEK293.hHJV/3xMyc.hTMPRSS6 cells or HEK293.hHJV/3xMyc.mTMPRSS6 cells were treated with 50 nM of anti-TMPRSS6 antibodies overnight. The following day, cells were harvested for surface staining. To stain cell surface hHJV in assay cell lines, cells were blocked with FcR blocker (Miltenyi Biotec, 130-059-901) and stained with an anti-hemojuvelin antibody (Abcam ab54431, 1:400, on ice, 1 hour) followed by incubation with a donkey anti-mouse IgG AF647 antibody (Invitrogen A-31571, 1:2000, in ice, 40 minutes). The stained cells were measured using an Accuri C6 flow cytometer (BD).

[0201] In the ELISA assay, a serine protease inhibitor, Aprotinin (2 µg/mL), and protease inhibitor cocktail (Sigma, Cat: P-1860, 1:200) were included as controls to block protease activity in HEK293.hHJV/3xMyc.TMPRSS6 cells. For FACS, Aprotinin (2 µg/mL), was included as a control to block protease activity in HEK293.hHJV/3xMyc.TMPRSS6 cells.

Results

[0202] Table 12 shows inhibition of protease-dependent release of HJV from the cell surface by anti-TMPRSS6 antibodies.

Table 12: Blocking Efficacy of TMPRSS6 Antibodies in HEK293.hHJV/3xMyc.TMPRSS6 Over-expression Cell Lines as measured by ELISA

Assay Cell Lines	HEK293.hHJV/3xMyc.hTMPRSS6		HEK293.hHJV/3xMyc.mTMPRSS6	
<i>mAb</i>	Inhibition (%)	IC ₅₀ [M]	Inhibition (%)	IC ₅₀ [M]
REGN7969	> 99	7.12E-11	< 1	N/A
REGN7971	89.9	7.74E-11	10.2	N/A
REGN7973	> 99	1.99E-10	9.4	N/A
REGN7974	> 99	1.54E-10	11.8	N/A
REGN7977	> 99	1.27E-10	46.4	N/A
REGN7999	> 99	1.02E-10	98.5	8.26E-11
REGN8023	> 99	1.49E-10	42.0	2.74E-10
Isotype Control	15.7	N/A	5.2	N/A
Untreated	0.0	N/A	< 1	N/A
Aprotinin	> 99	N/A	> 99	N/A
Protease inhibitor cocktail	>99	N/A	>99	N/A

N/A – Not applicable.

[0203] As shown in Table 12, all selected TMPRSS6 antibodies demonstrated >90% inhibition of protease-dependent release of HJV from the cell surface in human TMPRSS6 expressing cells and IC₅₀ values ranging from about 70 to about 200 pM. Two antibodies, REGN7999 and REGN8023, showed inhibitory effects in mouse TMPRSS6 expressing cells, demonstrating about 98.5% and about 42% inhibition of mouse TMPRSS6 protease activity and IC₅₀ values of about 82.6 pM and about 274 pM, respectively.

[0204] Table 13 shows the inhibition of cell surface HJV expression by anti-TMPRSS6 antibodies.

Table 13: Hemojuvelin Cell Surface Expression Level in HEK293.hHJV/3xMyc.TMPRSS6 Over-expression Cell Lines as measured using flow cytometry

Assay Cell Lines	HEK293.hHJV/3xMyc.hTMPRSS6		HEK293.hHJV/3xMyc.mTMPRSS6	
<i>mAb</i>	Geometric Mean Fluorescence Intensity	Median Fluorescence Intensity	Geometric Mean Fluorescence Intensity	Median Fluorescence Intensity
REGN7969	47932	67517	8394	7813
REGN7971	61850	84416	8228	7472
REGN7973	46109	63527	8190	7441
REGN7974	40885	56698	6956	6224

REGN7977	49022	63785	9128	7030
REGN7999	30702	40146	23698	33576
REGN8023	55465	77830	18957	19405
Isotype Control	11684	12981	7750	7233
Untreated	13342	15024	9605	8897
Aprotinin	42301	58097	26671	38081

[0205] As shown in Table 13, all selected TMPRSS6 antibodies prevented cleavage of HJV from the surface of HEK293.hHJV/3xMyc.hTMPRSS6 cells. REGN7999 and REGN8023 also prevented cleavage of HJV from the surface of HEK293.hHJV/3xMyc.mTMPRSS6 cells.

Example 7: Inhibition of TMPRSS6 enzymatic activity

Experimental Procedure

[0206] In order to assess anti-TMPRSS6 antibody inhibition of TMPRSS6 enzymatic activity, two bioassays were established. In both bioassays, anti-TMPRSS6 antibodies or an IgG subclass control were serially diluted in assay buffer (PBS + Ca⁺⁺ and Mg⁺⁺) from 500nM to 122.1pM (plus a sample containing assay buffer alone without test molecule).

[0207] The first bioassay ("CM bioassay") was run with the extracellular domain of TMPRSS6 produced as conditioned media (CM). CM was made by engineering CHO cells to produce and secrete the extracellular domain of Human TMPRSS6 (Amino Acids 68-802 of NCBI Reference Sequence NM_001289001) or cynomolgous monkey TMPRSS6 (Amino Acids 66-800, V673M, of NCBI Reference Sequence XM_005567384), both fused to an N-terminal 8 x Histidine. For the CM bioassay, antibodies were incubated with either 0.2% hTMPRSS6 CM or 0.75% mfTMPRSS6 CM at 25°C for 30 minutes, with all percent CM being expressed as %v/v.

[0208] The second bioassay ("HEK293 bioassay") was run with the HEK293/hTMPRSS6, HEK293/mfTMPRSS6, and HEK293/mTMPRSS6 cell lines, which stably express TMPRSS6 from human, cynomolgous monkey, and mouse, respectively. Details on the preparation of these cell lines can be found in Example 5. For the HEK293 bioassay, cells were plated at 10,000 cells/well (for 30 minute pre-incubation) and 20,000 cells/well (for overnight pre-incubation) in a 96-well plate in DMEM High Glucose + 10% FBS + Pen/Strep/L-Glutamine (DMEM Complete) and incubated at 37°C in 5% CO₂. For the HEK293 cell bioassay with 30 minute pre-incubation condition, on the day after cell plating, media was removed from cells, all cells were washed once in assay buffer, antibodies were added to cells, and incubated at 25°C for 30 minutes. For the overnight pre-incubation condition, on the day after cell plating, antibodies were added to cells still in DMEM Complete without washing and incubated at 37°C in 5% CO₂ overnight. The following

day media (including antibodies) was removed from cells, and all cells were washed once in assay buffer.

[0209] At the conclusion of antibody/TMPRSS6 incubations and plate washing for all assays, 150uM of a fluorescent TMPRSS6 peptide substrate (Boc-Gln-Ala-Arg-AMC, Enzo BML-P237) dissolved in assay buffer was added to the antibody treated cells or CM/antibody mixture, and incubated for 1h at 37°C in 5% CO₂. After the 1h incubation, fluorescence was measured on an Envision plate reader (Perkin Elmer) with an excitation wavelength of 380nm and an emission wavelength of 460nm. The results were analyzed using nonlinear regression (4-parameter logistics) with Prism software (GraphPad) to obtain IC₅₀ values. The percentage of inhibition was calculated with the RFU (relative fluorescence units) values by using the following equation:

$$\% \text{ Inhibition} = 100 \times \frac{RFU_{\text{Baseline}} - RFU_{\text{Inhibition}}}{RFU_{\text{Baseline}} - RFU_{\text{Background}}}$$

[0210] In this equation “RFU_{Baseline}” is the fluorescence value from either a certain concentration of TMPRSS6 CM or a certain number of HEK293/TMPRSS6 cells without antibodies, “RFU_{Inhibition}” is the fluorescence value with maximum concentration of a particular antibody with the aforementioned concentration of TMPRSS6 CM or a certain number of HEK293/TMPRSS6 cells, and “RFU_{Background}” is the fluorescence value without any TMPRSS6 CM or HEK293/TMPRSS6 cells.

Results

[0211] Seven anti-TMPRSS6 antibodies were selected and tested for their ability to inhibit the enzymatic activity of TMPRSS6 CM and HEK293/TMPRSS6 cells. Tables 14 through 16 show the results of these experiments.

Table 14: Anti-TMPRSS6 antibody inhibition of TMPRSS6 CM

Constant TMPRSS6 CM concentration	0.2% hTMPRSS6 CM		0.75% mfTMPRSS6 CM	
mAb	%inhibition	IC ₅₀ [M]	%inhibition	IC ₅₀ [M]
REGN7969	71	>1.0E-08	66	>5.0E-08
REGN7971	65	>1.0E-08	68	>5.0E-08

REGN7973	93	2.6E-08	81	>5.0E-08
REGN7974	85	4.0E-07	69	>5.0E-08
REGN7977	94	2.3E-08	89	5.0E-08
REGN7999	98	3.0E-09	98	1.7E-09
REGN8023	98	7.1E-10	98	7.4E-10
hIgG Subclass control	No Inhibition	No Inhibition	No Inhibition	No Inhibition

[0212] As shown in Table 14, all of the selected antibodies showed inhibition of about 0.2% hTMPRSS6 CM ranging from about 65% to about 98%, with IC₅₀ values for ranging from about 710pM to greater than about 10nM. All of the antibodies disclosed herein also showed inhibition of about 0.75% mfTMPRSS6 CM ranging from about 66% to about 98%, with IC₅₀ values ranging from about 740pM to greater than about 50nM.

Table 15: Anti-TMPRSS6 antibody inhibition of HEK293/TMPRSS6 cells with 30 minute pre-incubation at 25°C

10,000 cells/well with 30 minute pre-incubation with antibodies at 25C						
HEK293/TMPRSS6 species	hTMPRSS6		mfTMPRSS6		mTMPRSS6	
mAb	% inhibition	IC ₅₀ [M]	% inhibition	IC ₅₀ [M]	% inhibition	IC ₅₀ [M]
REGN7969	87	1.9E-09	87	8.8E-09	No inhibition	No inhibition
REGN7971	86	9.7E-10	87	3.1E-09	No inhibition	No inhibition
REGN7973	89	5.8E-09	85	2.5E-08	No inhibition	No inhibition
REGN7974	86	5.1E-09	81	3.0E-08	No inhibition	No inhibition
REGN7977	86	3.6E-09	87	8.1E-09	58	3.5E-08
REGN7999	86	3.0E-09	90	6.5E-09	88	3.6E-09
REGN8023	90	3.6E-10	94	6.6E-10	82	1.5E-09
hIgG Subclass control	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition

[0213] As shown in Table 15, when antibodies were pre-incubated for 30 minutes with 10,000 HEK293/TMPRSS6 cells, all of the selected antibodies showed inhibition of HEK293/hTMPRSS6 cells ranging from about 86% to about 90%, with IC₅₀ values for ranging

from about 360pM to about 5.8nM. All of the selected antibodies also showed inhibition of HEK293/mfTMPRSS6 cells ranging from about 81% to about 94%, with IC₅₀ values ranging from about 660pM to about 30nM. Three of the selected antibodies (REGN7977, REGN7999, REGN8023) showed inhibition of HEK293/mTMPRSS6 cells ranging from about 58% to about 88%, with IC₅₀ values for the inhibiting antibodies ranging from about 1.5nM to about 35nM.

Table 16: Anti-TMPRSS6 antibody inhibition of HEK293/TMPRSS6 cells with overnight pre-incubation at 37°C

20,000 cells/well with overnight pre-incubation with antibodies at 37C						
HEK293/TMPRSS6 species	hTMPRSS6		mfTMPRSS6		mTMPRSS6	
mAb	% inhibition	IC ₅₀ [M]	% inhibition	IC ₅₀ [M]	% inhibition	IC ₅₀ [M]
REGN7969	88	1.2E-09	89	1.3E-08	No inhibition	No inhibition
REGN7971	90	1.4E-09	89	6.0E-09	No inhibition	No inhibition
REGN7973	88	2.2E-09	42	> 1.0E-08	No inhibition	No inhibition
REGN7974	86	2.6E-09	31	> 1.0E-08	No inhibition	No inhibition
REGN7977	87	1.7E-09	81	> 1.0E-08	97	1.4E-08
REGN7999	89	2.1E-09	91	2.1E-08	103	2.1E-09
REGN8023	90	1.1E-09	91	2.8E-09	98	1.2E-09
hIgG Subclass control	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition	No Inhibition

[0214] As shown in Table 16, when antibodies were pre-incubated overnight with 20,000 HEK293/TMPRSS6 cells, all of the selected antibodies showed inhibition of HEK293/hTMPRSS6 cells ranging from about 86% to about 90%, with IC₅₀ values ranging from about 1.1nM to about 2.6nM. All of the selected antibodies also showed inhibition of HEK293/mfTMPRSS6 cells ranging from about 31% to about 91%, with IC₅₀ values ranging from about 2.8nM to greater than about 10nM. Three of the selected antibodies (REGN7977, REGN7999, REGN8023) showed inhibition of HEK293/mTMPRSS6 cells ranging from about 97% to about 103%, with IC₅₀ values for the inhibiting antibodies ranging from about 1.2nM to about 14nM. An IgG subclass control antibody did not demonstrate any measurable inhibition of TMPRSS6 in any of the formats tested.

Example 8: Epitope mapping on human TMPRSS6 by HDX-MS

Experimental Procedure

[0215] Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) was performed to determine the amino acid residues of human TMPRSS6 that interact with a subset of TMPRSS6 monoclonal antibodies of the present disclosure, REGN7999 and REGN8023. A general description of the HDX-MS method is set forth in *e.g.*, Ehring, *Analytical Biochemistry* (1999) 267(2):252-259; and Engen and Smith, *Anal. Chem.* (2001) 73:256A-265A.

[0216] The HDX-MS experiments were performed on a customized platform, consisting of a custom HDX automation system for deuterium labeling and quenching, a Waters Acquity Binary Solvent Manager for sample digestion and loading, another Waters Acquity Binary Solvent Manager for analytical gradient, and a Thermo Q Exactive HF mass spectrometer for peptide identification and mass measurement.

[0217] The D₂O labeling solution was prepared as PBS buffer in D₂O at pD 7.0 (10 mM phosphate buffer, 140 mM NaCl, and 3 mM KCl, equivalent to pH 7.4 at 25°C). For deuterium labeling, 10 µL of recombinant human TMPRSS6 extracellular domain expressed with a S762A mutation and a C-terminal myc-myc-Hexahistidine tag (referred to as hTMPRSS6_S762A-MMH; REGN5330; SEQ ID NO: 117) premixed with either REGN7999 or REGN8023 were incubated at 20°C with 90 µL of D₂O labeling solution for various time-points in duplicates (non-deuterated control as 0 min; deuterium-labeling for 5 min or 10 min). The deuteration reaction was quenched by adding 90 µL of quench buffer (0.5 M TCEP-HCl, 4 M urea, and 0.5% formic acid) to each sample for a 90-second incubation at 20°C. The quenched samples were then injected into the LC system for online pepsin/protease XIII digestion. The digested peptides were trapped by a C18 column (2.1 mm x 5 mm, Waters) and separated by another C18 column (2.1 mm x 50 mm, Waters) at -5°C with a 20-minute gradient from 0%-90% B (mobile phase A: 0.5% formic acid and 4.5% acetonitrile in water, mobile phase B: 0.5% formic acid in acetonitrile). The eluted peptides were analyzed by a Thermo Q Exactive HF mass spectrometry in LC-MS/MS or LC-MS mode.

[0218] The LC-MS/MS data from the undeuterated hTMPRSS6_S762A-MMH samples were searched against a database including amino acid sequences of human TMPRSS6 protein, pepsin, protease XIII, and their reversed sequences using the Byonic search engine (Protein Metrics). The search parameters were set as default using non-specific enzymatic digestion and human glycosylation as common variable modification. The list of identified peptides was then imported into HDExaminer software 3.1 (Sierra Analytics) to calculate the deuterium uptake (D_{uptake}) and percentage of deuterium uptake (%D) for all deuterated samples. %D was calculated using the following formula:

$$\text{Percentage of deuterium uptake (\%D)} = \frac{D_{\text{uptake for peptide at each time point}} \times 100\%}{\text{Maximum } D_{\text{uptake of the peptide}}}$$

Results

[0219] A total of 294 peptides from human TMPRSS6 were identified from both hTMPRSS6_S762A-MMH alone and hTMPRSS6_S762A-MMH in complex with REGN7999 samples, representing about 79.2% sequence coverage of human TMPRSS6. Any peptide that exhibited a decrease of 5% or more in percentage of deuterium uptake was defined as significantly protected ($\Delta\%D < -5\%$).

[0220] Table 17 shows the human TMPRSS6 peptides with significant protection upon formation of hTMPRSS6_S762A-MMH-REGN7999 complex as compared to hTMPRSS6_S762A-MMH alone. Peptides corresponding to amino acids 693-703 SHFEPGLHCW (SEQ ID NO: 120) and 780-805 LVSWGLGCGRPNYFGVYTRITGVISW (SEQ ID NO: 121) on human TMPRSS6 were significantly protected by REGN7999.

Table 17: Human TMPRSS6 peptides with significant protection by REGN7999

	5 min			10 min			
Human TMPRSS6	hTMPRSS6-REGN7999	hTMPRSS6		hTMPRSS6-REGN7999	hTMPRSS6		
Residues*	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	$\Delta\% D$
693-698	0.42	0.61	-0.19	0.37	0.62	-0.25	-8.2
693-703	0.85	1.08	-0.23	0.53	1.15	-0.62	-5.9
695-703	0.39	1.18	-0.79	0.4	1.16	-0.76	-14.4
780-798	5.41	6.14	-0.73	5.3	6.18	-0.88	-5.6
781-783	0.32	0.40	-0.08	0.32	0.39	-0.07	-8.3
781-796	4.89	5.61	-0.72	4.91	5.59	-0.68	-6.0
781-798	5.30	6.04	-0.74	5.35	6.00	-0.65	-5.1
784-796	3.18	3.66	-0.48	3.14	3.59	-0.45	-5.2
792-798	0.43	0.79	-0.36	0.43	0.77	-0.34	-7.7
793-798	0.45	0.63	-0.18	0.44	0.66	-0.22	-5.5

797-805	2.15	2.56	-0.41	2.12	2.53	-0.41	-6.5
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*Sequence numbering corresponds to NCBI accession number NP_705837.1

[0221] A total of 302 peptides from human TMPRSS6 were identified from both hTMPRSS6 S762A-MMH alone and hTMPRSS6 S762A-MMH in complex with REGN8023 samples, representing about 79.6% sequence coverage of human TMPRSS6. Any peptide that exhibited a decrease of 5% or more in percentage of deuterium uptake was defined as significantly protected ($\Delta\%D < -5\%$).

[0222] Table 18 shows the human TMPRSS6 peptides with significant protection upon formation of hTMPRSS6 S762A-MMH -REGN8023 complex as compared to hTMPRSS6 S762A-MMH alone. Peptides corresponding to amino acids 125-133 LITSTRLGT (SEQ ID NO: 122), 586-594 GEWPWQASL (SEQ ID NO: 123), 626-650 STVLWTVFLGKQVWQNSRWPGEVSFK (SEQ ID NO: 124), and 704-724 ITGWGALREGGPISNALQKVD (SEQ ID NO: 125) on human TMPRSS6 were significantly protected by REGN8023.

Table 18: Human TMPRSS6 peptides with significant protection by REGN8023

Human TMPRSS 6	5 min			10 min			$\Delta\%D$
	hTMPRSS6 -REGN8023	hTMPRSS 6		hTMPRSS6 -REGN8023	hTMPRSS 6		
Residues*	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	
125-131	1.73	2.02	-0.29	1.73	1.98	-0.25	-6.0
125-133	2.59	3.03	-0.44	2.61	2.99	-0.38	-6.5
126-133	2.51	2.92	-0.41	2.56	2.90	-0.34	-6.9
586-589	0.07	0.15	-0.08	0.08	0.14	-0.06	-7.7
586-594	0.60	0.93	-0.33	0.60	0.93	-0.33	-6.2
626-630	1.51	1.66	-0.15	1.53	1.65	-0.12	-5.2
628-630	0.03	0.18	-0.15	0.03	0.16	-0.13	-15.4
628-631	0.04	0.15	-0.11	0.06	0.14	-0.08	-5.2
628-633	0.08	0.28	-0.20	0.10	0.29	-0.19	-5.4

634-639	1.93	2.45	-	0.52	1.92	2.45	-	0.53	-14.6
634-642	3.46	4.04	-	0.58	3.47	4.00	-	0.53	-8.9
634-644	3.84	4.44	-	0.60	3.87	4.41	-	0.54	-7.9
634-646	4.81	5.38	-	0.57	4.84	5.35	-	0.51	-6.0
634-649	5.99	6.63	-	0.64	6.02	6.59	-	0.57	-5.2
634-650	6.15	6.94	-	0.79	6.24	6.92	-	0.68	-5.9
704-710	1.50	1.83	-	0.33	1.51	1.84	-	0.33	-7.2
704-711	2.32	2.69	-	0.37	2.34	2.68	-	0.34	-6.6
704-713	3.22	3.61	-	0.39	3.20	3.57	-	0.37	-5.3
704-718	3.88	5.37	-	1.49	3.94	5.31	-	1.37	-13.2
704-719	4.56	5.99	-	1.43	4.50	6.02	-	1.52	-12.6
704-720	4.89	6.87	-	1.98	4.79	6.91	-	2.12	-16.3
711-720	2.28	3.72	-	1.44	2.32	3.67	-	1.35	-22.2
711-724	3.52	5.68	-	2.16	3.64	5.60	-	1.96	-20.8
714-724	2.86	4.70	-	1.84	2.88	4.62	-	1.74	-22.1

*Sequence numbering corresponds to NCBI accession number NP_705837.1

Example 9: Epitope mapping on human TMPRSS6 by CryoEM

Experimental Procedure

[0223] Cryoelectron Microscopy (CryoEM) was used to further characterize the epitopes relevant to selected TMPRSS6 antibodies.

[0224] For the preparation of Fab fragments, REGN7999 and REGN8023 IgGs were cleaved into F(ab')₂ and Fc fragments using Fabricator enzyme (Genovis) following standard protocols from the manufacturer. F(ab')₂ was reduced into Fab using 2-mercaptoethylamine (2-MEA, ThermoFisher) followed by Fc fragment removal using CaptureSelect IgG-Fc (ms) affinity resin (ThermoFisher). Fab fragments were further purified by injection into a size exclusion chromatography (SEC) column (Superdex 200 Increase 15/300 GL, GE healthcare) connected to an AKTA Avant 25 chromatography system (GE healthcare). Running buffer contained 25 mM

Tris-HCl pH 7.5, 150 mM NaCl. Peak fractions were pooled and concentrated in a 30 kDa cutoff centrifugal filter (Millipore Sigma) for subsequent use in complex preparation.

[0225] The human TMPRSS6 protein used in this experiment encompasses the extracellular domain residues G77-T811 of human TMPRSS6 fused to a C-terminal Myc-Myc-hexahistidine tag and contains a mutation in the catalytic domain (S762A) that renders the protein catalytically inactive (referred to as human TMPRSS6_S762A-MMH). For complex formation, 200 µg of purified human TMPRSS6_S762A-MMH was mixed with 250 µg each of REGN7999 Fab and REGN8023 Fab and then incubated on ice for approximately 30 minutes. The mixture was injected into an SEC column (Superdex 200 Increase 15/300 GL, GE) connected to an AKTA Avant 25 chromatography system (GE healthcare). SEC running buffer contained 25 mM Tris-HCl pH 7.5, 150 mM NaCl. Fractions from a single monodisperse peak corresponding to the human TMPRSS6 S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex were pooled and concentrated in a 30 kDa cutoff centrifugal filter (Millipore Sigma) to a concentration of 3.5 mg/mL measured using a Nanodrop instrument (ThermoFisher).

[0226] CryoEM sample preparation and data collection were performed as follows. Freshly purified human TMPRSS6_S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex was diluted two-fold to 1.75 mg/mL in buffer containing 25 mM Tris-HCl pH 7.5, 150 mM NaCl for cryoEM grid preparation. n-Dodecyl-β-D-maltopyranoside (Anatrace) was added to a final concentration of ~60 µM immediately before pipetting 3.5 µL of the mixture onto a UltrAufoil R1.2/1.3, 300 mesh grid (Quantifoil). Excess liquid was blotted away using filter paper and the grid was plunge frozen into liquid ethane cooled by liquid nitrogen using a Vitrobot Mark IV (ThermoFisher) operated at 4° C and 100% humidity. The grid was then inserted into a Titan Krios G3i microscope (ThermoFisher) equipped with a K3 camera (Gatan). 3,580 movies were collected in counted mode at a nominal magnification of 81,000x (0.85 Å pixel size). Each movie contained 46 dose fractions over a 2 second exposure, and the total acquired dose per Å² was ~40 electrons.

[0227] CryoEM data processing and map generation was performed as follows. CryoEM data were processed initially using Cryosparc v2.14.2. Movies were motion corrected by Patch motion correction and CTF parameters were estimated by Patch CTF estimation. Particles were initially picked using Blob picker to generate 2D class averages for template picking. 927,835 particles were picked using Template picker. After 2D classification, Ab initio reconstruction, and Heterogenous refinement, 94,036 particles corresponding to the REGN5330 -- REGN7999 Fab -- REGN8023 Fab complex were identified. These particles were refined to 3.4 Å resolution reconstruction using non-uniform refinement. The resulting map was used as an initial reference model for 3D classification in Relion. The map used for model building was calculated using a

Relion version 3.1 single particle processing pipeline. Movies were motion-corrected using MotionCor2 and CTF parameters were estimated using gctf. 3,411 aligned micrographs were selected for further processing on the basis of having a CTF resolution estimation of better than 4 Å. 1,445,534 particles were picked from the micrographs using 2D template-based autopicking and extracted as 3x binned particle images (2.55 Å per pixel). Three rounds of 2D classification were conducted to remove false positives and broken or incomplete complexes, resulting in 1,034,494 particles. These particles were subjected to 3D classification requesting 8 classes, using an initial reference model calculated in CryoSparc. 270,021 particles from the two best classes corresponding to human TMPRSS6 S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex were re-extracted as unbinned particle images and subjected to 3D refinement, which produced a 3.4 Å resolution map in which the Fabs and catalytic domains were well resolved, but the remaining N-terminal domains of the protein were poorly resolved. To better resolve the N-terminal domains, focused classification was carried out without alignment, applying a soft mask that excluded the Fabs and catalytic domain. A single class containing 146,035 particles was identified as having improved density for the N-terminal domains. Refinement of these particles resulted in a 3.6 Å resolution map. Two rounds of CtfRefine and Bayesian polishing were followed by three additional focused 3D classification runs without alignment in which soft masks surrounding the CUB and SEA domains were applied, resulting in a final set of 105,142 particles. These particles were then refined into a 3.3 Å resolution (FSC=0.143) map with improved features in N-terminal domains. For model building, the map was filtered to its local resolution values calculated in Relion and sharpened to a B factor of -76 Å².

[0228] Model building and refinement was performed as follows. Manual model building was carried out using Coot version 0.8.9 and real space refinements were done in Phenix version 1.17. A homology model of the human TMPRSS6 catalytic domain S762A mutant was generated from a published crystal structure of the human Matriptase-1 catalytic domain (PDB 4IS5) using the Sculptor program in Phenix. This model was docked into the cryoEM map of human TMPRSS6 S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex using Phenix AutoDock and manually adjusted. The LDLRa domains were built into the density manually using a published crystal structure of an LDLR domain (PDB 1AJJ) as a guide. Manual building of CUB domains 1 and 2 were guided by a published crystal structure of Cubilin (PDB 3KQ4). Correct sequence register was corroborated by density for side chains of bulky residues and N-linked glycosylations, as well as the positions of disulfide bridges. The N-terminal SEA domain was not built due to poor fragmented density. The current model encompasses human TMPRSS6 residues 212-811, excepting residues 573-581 and 662-664, which are located in disordered linkers. Homology

models for Fab fragments of REGN7999 and REGN8023 were made from a previously determined REGN Fab structure. Unambiguous docking of Fab models into their respective densities was aided by clearly interpretable side chain densities corresponding to their distinct CDR sequences. After docking, the models were adjusted manually followed by real space refinement of the entire human TMPRSS6 S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex.

Results

[0229] *CryoEM structure of human TMPRSS6 S762A-MMH -- REGN7999 Fab -- REGN8023 Fab complex:* To better understand how anti-TMPRSS6 antibodies bind to and inhibit TMPRSS6, a protein complex consisting of a catalytically inactive human TMPRSS6 ectodomain (human TMPRSS6 S762A-MMH) bound to the Fab fragments of anti-TMPRSS6 antibodies REGN7999 and REGN8023 was isolated by SEC and its three-dimensional structure was determined by single particle CryoEM. At an overall resolution of 3.3 Å, the CryoEM reconstruction was sufficient for building an atomic model for ~80% of the human TMPRSS6 ectodomain, encompassing its two CUB domains, three LDLRa domains, and catalytic serine protease domain. The reconstruction also allowed unambiguous docking of REGN7999 and REGN8023 Fab fragments and manual re-fitting of their variable regions. The local resolution at the paratope-epitope interfaces of both Fabs ranges from 3.1-3.5 Å, allowing for accurate determination of residue-level interactions between the antibodies and TMPRSS6. The structure of the tertiary complex also confirms that REGN7999 and REGN8023 bind at non-overlapping epitopes.

[0230] *REGN7999 epitope and structural mechanism of inhibition:* Residues contributing to the REGN7999 epitope on TMPRSS6 are summarized in Table 18. These residues are defined as TMPRSS6 residues with non-hydrogen atoms that are within 4 Å of non-hydrogen atoms of REGN7999 and can involve hydrogen bonds, charge-charge interactions, or hydrophobic/van der Waals interactions. The REGN7999 epitope can be split into two patches on TMPRSS6. The first patch resides on the catalytic domain and consists of TMPRSS6 residues G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, and N791. Contacts at this patch are mediated by CDRs H3, L2, L3 and a single residue from framework region (FR) L3. The second patch resides on LDLRa repeat domain 2 and consists of residues V490, S501, T502, C503, I504, S505, and K508. The paratope for the second patch is comprised of a continuous stretch of light chain residues spanning portions of CDR-L2 and FR-L3. The CUB domains and SEA domain are far removed from the REGN7999 binding interface and do not contribute to its epitope on TMPRSS6. Data from hydrogen deuterium exchange (HDX) mass spectrometry experiments on

REGN7999 (Example 8) generally agrees with the catalytic domain patch observed in the CryoEM structure, but did not highlight residues from LDLRa domain 2 as contributing to the epitope.

[0231] Although its epitope includes a patch on the catalytic domain, REGN7999 does not contact the catalytic site triad (H617, D668 and S672) where cleavage occurs, nor is it oriented such that it sterically occludes substrate access to the active site. The location of a substrate bound to TMPRSS6 has not been experimentally determined, but this location can be inferred from a homologous structure of matriptase serine protease domain in complex with peptide inhibitor, reported in Yuan et al, *BMC Struct Biol.* (2011) 11:30. These structural observations suggest that binding of soluble hemojuvelin to the TMPRSS6 ectodomain is not perturbed by the presence of REGN7999. Without being bound to any theory, it is possible that REGN7999 may inhibit TMPRSS6 by allosterically ‘trapping’ TMPRSS6 in an inactive conformation that is capable of binding but not cleaving substrate.

[0232] *REGN8023 epitope and structural mechanism of inhibition:* Residues contributing to the REGN8023 epitope on TMPRSS6 are summarized in Table 19. These residues are defined as TMPRSS6 residues with non-hydrogen atoms that are within 4 Å of non-hydrogen atoms of REGN8023 and can involve hydrogen bonds, charge-charge interactions, or hydrophobic/van der Waals interactions. The REGN8023 epitope resides exclusively on the catalytic domain and consist of TMPRSS6 residues R597, R599, I601, D622, L710, R711, E712, G713, G714, P715, and I716. The epitope observed in this structure generally agrees with data from HDX mass spectrometry experiments. The REGN8023 paratope is largely comprised within the heavy chain and includes CDRs H1, H2, H3, L1, L3 as well as FR-H3.

[0233] REGN8023 binds the TMPRSS6 catalytic domain such that the heavy chain does not directly contact the catalytic triad but appears to be in a position that would occlude substrate access to the active site, based on the model described above. Without being bound by any theory, it is possible that REGN8023 inhibits TMPRSS6 through steric hindrance of substrate binding to the active site. This is also suggested by binding data demonstrating that REGN8023 competes with soluble hemojuvelin for binding to TMPRSS6 ectodomain protein.

Table 19: Anti-TMPRSS6 antibody epitopes. Epitope residues are defined as amino acids with non-hydrogen atoms within 4 Å of non-hydrogen atoms of antibody

Antibody	TMPRSS6 epitope residues*	
	LDLRa domain 2	Catalytic domain
REGN7999	V490, S501, T502, C503, I504, S505, K508	G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, N791

REGN8023	N/A	R597, R599, I601, D622, L710, R711, E712, G713, G714, P715, I716
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*Sequence numbering corresponds to NCBI accession number NP_705837.1

Example 10: Comparison of REGN7999 and REGN8023 in female WT mice

Experimental Procedure

[0234] Two in vivo experiments were performed to analyze the effects of selected TMPRSS6 antibodies on serum hepcidin and serum iron. For both studies, female wild-type mice with humanized HJV were utilized (Study 1: ages 11-13 weeks and Study 2: ages 6-9 weeks). In both studies, mice were pre-bled at Day 7. In the first study (Study 1), two selected TMPRSS6 antibodies, REGN7999 and REGN8023, as well as an isotype control antibody, were subcutaneously administered to mice at 10 mg/kg on days 0, 2 and 7 of the study. Mice were then taken down at day 9 and terminal bleeds were collected for analysis. In the second study (Study 2), the same two TMPRSS6 antibodies, REGN7999 and REGN8023, as well as an isotype control antibody, were subcutaneously administered to mice at 25 mg/kg on days 0, 2 and 7 of the study. Mice were then taken down at day 14 and terminal bleeds were collected for analysis.

[0235] From both studies, serum iron was measured from terminal bleeds using a Siemens ADVIA Chemistry XPT instrument. Serum hepcidin was measured from terminal bleeds using a Hepcidin-murine-compete ELISA kit (Intrinsic Lifesciences, Cat: HMC-001, Lot: 5142019) as per manufacturer's instructions.

Results

[0236] Serum iron (expressed as ng/mL) and serum hepcidin (expressed as µg/dL) measurements from Study 1 are shown in Table 20 and from Study 2 are shown in Table 21. □

Table 20: Serum Iron and hepcidin levels in humanized HJV mice in response to TMPRSS6 antibody treatment in Study 1 (10 mg/kg)

Measurement	Isotype Control Ab	REGN7999	REGN8023
Serum Hepcidin (ng/mL) Mean±SD	390.2±175.4	652.0±175.4*	590±160.9
Serum Iron (µg/dL) Mean±SD	148.8±37.78	79.63±18.15*	112±51.41

[0237] As shown in Table 20, in Study 1, REGN7999 treatment increased serum hepcidin and reduced serum iron significantly in healthy REGN7999-treated mice as compared with isotype control treated mice. REGN8023 treatment also increased serum hepcidin and reduced serum iron in healthy REGN8023-treated mice, although this was not statistically significant as compared to

isotype control treated mice. In Table 20, all values are mean $\pm$ SD, n=6-8 per group. One Way ANOVA; *p<0.05 vs. Isotype control Ab.

Table 21: Serum Iron and hepcidin levels in humanized HJV mice in response to TMPRSS6 antibody treatment in Study 2 (25 mg/kg)

Measurement	Isotype Control Ab	REGN7999	REGN8023
Serum Hepcidin (ng/mL) Mean $\pm$ SD	220.4 $\pm$ 110.9	609.4 $\pm$ 289.6*	363.4 $\pm$ 108.1
Serum Iron (μ g/dL) Mean $\pm$ SD	156.8 $\pm$ 25.02	58.00 $\pm$ 11.14*	86.00 $\pm$ 17.77*

[0238] As shown in Table 21, in Study 2, REGN7999 treatment increased serum hepcidin and reduced serum iron significantly in healthy REGN7999-treated mice as compared with isotype control treated mice. REGN8023 treatment also increased serum hepcidin and reduced serum iron in healthy REGN8023-treated mice, although only the serum iron levels reached statistical significance as compared to isotype control treated mice. In Table 21, all values are mean $\pm$ SD, n=4-5 per group. One Way ANOVA; *p<0.05 vs. Isotype control Ab.

[0239] The data from both studies suggests that the selected anti-TMPRSS6 antibodies are able to block TMPRSS6 function resulting in the observed effects on hepcidin and serum iron levels.

Example 11: Effect of anti-TMPRSS6 on liver iron in a mouse model of hemochromatosis

[0240] To further investigate the efficacy of anti-TMPRSS6 antibody REGN7999 in a relevant disease model, a type 1 hereditary hemochromatosis study was performed. Hereditary hemochromatosis is an iron overload disease caused by a mutation in HFE protein, which leads to inappropriately low hepcidin levels (Finberg et al. *Blood* (2011) 117(17):4590-4599).

Experimental Procedure

[0241] Male mice homozygous for a deletion of the mouse HFE gene on a 100% C57BL/6 background (referred to as HFE^{-/-}), were used as a mouse model of type 1 hereditary hemochromatosis. HFE^{-/-} mice (n=5-7) were subcutaneously injected weekly with 10 mg/kg of isotype control or REGN7999, except during week 1 when 2 injections were administered. A wild type mouse control group was included and administered isotype control. After 8 weeks, mice were euthanized using CO₂ asphyxiation. A terminal bleed was taken and serum hepcidin was measured using the Hepcidin Murine-Compete ELISA kit (Intrinsic Lifesciences, Cat: HMC-001, Lot: 02032020) as per the manufacturer's protocol. After the mice were sacrificed, their livers were flash frozen. Later these livers were processed to measure liver iron content, based on the method described by Torrance and Bothwell, *S. Afr. J. Med. Sci.* (1968) 33(1):9-11.

Results

[0242] Both serum hepcidin (reported in ng/mL) and liver iron content (reported in µg/g dry weight) values are shown in Table 22.

Table 22: REGN7999 Effects on Serum Hepcidin and Liver Iron Content in a Mouse Model of Hereditary Hemochromatosis

	HFE ^{+/+} (wild type) mice Isotype Control treatment	HFE ^{-/-} mice Isotype Control treatment	HFE ^{-/-} mice REGN7999 treatment
Serum hepcidin (ng/mL)	364.8±96.89	339.3±150.7	1631±231.4* **
Liver iron content (mg/g dry weight)	338.4±60.03	771.1±68.37*	618.7±51.17* **

[0243] As shown in Table 22, after 8 weeks of treatment with REGN7999, HFE^{-/-} mice had significantly increased serum hepcidin (about 1631 ng/mL) as compared to those treated with isotype control (about 339.3 ng/mL). Also shown in Table 22, after 8 weeks of treatment with REGN7999, HFE^{-/-} mice had a significantly lower liver iron content (about 618.7 µg/g dry weight) as compared to those treated with isotype control (about 771.1 µg/g dry weight). In Table 22, all values are mean ± SD, n=5-7 per group. One Way ANOVA; *p<0.05 vs. HFE^{+/+} isotype control; **p<0.05 vs. HFE^{-/-} isotype control.

Example 12: Effect of anti-TMPRSS6 in a mouse model of beta thalassemia

[0244] In order to evaluate the efficacy of two anti-TMPRSS6 antibodies disclosed herein, studies were performed in a murine model of beta thalassemia intermedia, an iron overload disorder associated with ineffective erythropoiesis and iron dysregulation.

[0245] Mice were generated with a heterozygous deletion of $\beta 1$ and $\beta 2$ genes, which recapitulate beta thalassemia intermedia in humans (Ginzburg, *Blood* (2011) 118(16):4321-4330), and homozygous expression of human hemojuvelin (HJV) in place of mouse HJV (referred to as Hbb^{th3/+} x HJV^{HumIn}) in a C57BL/6/129S6 background. Hbb^{th3/+} x HJV^{HumIn} mice (n=10-19) were subcutaneously injected weekly with 10 mg/kg of either REGN7999, REGN8023, or isotype control (except in week 1 when 2 injections were administered). A wild type (WT) mouse control group was included and administered isotype control in a similar manner. After 8 weeks, mice were euthanized using CO₂ asphyxiation, and whole blood was collected via cardiac puncture and placed in serological blood collection tubes either with or without EDTA. Serum hepcidin was measured using the commercially available Hepcidin Murine-Compete ELISA kit (Intrinsic

Lifesciences, Cat: HMC-001, Lot: 08232019) according to the manufacturers' instructions. Serum iron and a full hematology panel, including reticulocytes and mature red blood cells (RBC), was measured using a Siemens ADVIA Chemistry XPT instrument and the Oxford Science GENESIS Hematology System.

[0246] Additionally, after the mice were sacrificed, their livers and spleens were flash frozen until further processing. Livers samples were later processed to measure liver iron content, based on the method described by Torrance and Bothwell, *S. Afr. J. Med. Sci.* (1968) 33(1):9-11. Spleens were minced and filtered through a 70 mm strainer into a tube containing 10 mL autoMACS® buffer (Miltenyi Biotec, Cat: 130-091-221). To harvest bone marrow from femurs, hip-ends were excised from mice and placed in a tube containing 100 mL of autoMACS® buffer supplemented with bovine serum albumin (BSA) (Miltenyi Biotec, Cat: 130-091-376). Tubes were quickly spun (10 s, 400 x g) to release bone marrow into solution.

[0247] Bone marrow and spleen cells were spun at 400 x g for 10 minutes at 4°C followed by incubation with anti-mouse CD45 magnetic beads (Miltenyi Biotec, Cat: 130-052-301) for 20 minutes on ice. This was followed by magnetic separation on multi-24 columns (Miltenyi Biotec, Cat: 130-095-691). The flow-through, enriched in both mature and precursor RBCs, was collected and incubated with anti-mouse CD16/CD32 Fc block (BD Biosciences, clone 2.4G2, Cat: 553142) for 10 minutes. This was followed by a 30-minute incubation with the following commercial antibodies (all rat anti-mouse): FITC anti-Ter119, Clone TER-119, Cat:557915; APC anti-CD44, Clone IM7, Cat: 561862; APC-Cy7 anti-CD45, Clone 30-F11, Cat: 557659; APC-Cy7 anti-CD11b, Clone M1/70, Cat: 557657; and APC-Cy7 anti-Ly6G, Clone 1A8, Cat: 560600; all from BD Biosciences.

[0248] AADvanced (Thermofisher, S10349) was added for 30 minutes to exclude dead cells from the analysis. All staining procedures were performed on ice and compensation controls were included using OneComp eBeads™ (Invitrogen, Cat: 01-1111-42). A minus-One control panel was prepared for correct gating of target populations. Samples were measured on a CytoFLEX LX Flow Cytometer (Beckman-Coulter) using fluorescence minus-one-controls to gate target populations.

[0249] As shown in Table 23, after 8 weeks treatment with REGN7999 or REGN8023, Hbb^{th3/+} x HJV^{HumIn} mice had significantly increased serum hepcidin levels (about 1510 ng/mL and about 1454 ng/mL, respectively) compared to both WT and Hbb^{th3/+} x HJV^{HumIn} mice treated with isotype control (about 225 ng/mL and about 511 ng/mL, respectively).

[0250] Additionally, after 8 weeks of treatment with REGN7999 or REGN8023, Hbb^{th3/+} x HJV^{HumIn} had a significant reduction (about 50 µg/dL) in serum iron compared to isotype control

(about 128 $\mu\text{g/dL}$, Table 23). In isotype control treatment groups, $\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice accumulated more liver iron than WT mice. This increase was more pronounced in female mice (about 466 $\mu\text{g/g}$ to about 985 $\mu\text{g/g}$ dry weight) compared to male mice (about 215 $\mu\text{g/g}$ to about 364 $\mu\text{g/g}$ dry weight) (Table 23). The reduction of liver iron content in response to REGN7999 and REGN8023 also had greater effects in female mice after 8 weeks of treatment. In Table 23, all values are mean $\pm$ SD, n=10-19 per group. One Way ANOVA; * $p<0.01$ vs. WT isotype control; ** $p<0.05$ vs. $\text{Hbb}^{\text{th3/+}}$ isotype control.

Table 23: Effect of REGN7999 and REGN8023 on Serum Hepcidin, Serum Iron, and Liver Iron Content in a Mouse Model of beta-thalassemia

Treatment Group	Serum Hepcidin (ng/mL)	Serum Iron (mg/dL)	Liver Iron (mg/g dry weight) Female Cohort	Liver Iron (mg/g dry weight) Male Cohort
Wild type mice Isotype Control Treatment	225 $\pm$ 107	167 $\pm$ 27	466 $\pm$ 64	215 $\pm$ 49
$\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice Isotype Control Treatment	511 $\pm$ 232	128 $\pm$ 31*	985 $\pm$ 190*	364 $\pm$ 123*
$\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice REGN7999 Treatment	1510 $\pm$ 911* **	51 $\pm$ 23* **	718 $\pm$ 177**	293 $\pm$ 119
$\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice REGN8023 Treatment	1454 $\pm$ 624* **	56 $\pm$ 34 * **	732 $\pm$ 182**	264 $\pm$ 79

[0251] REGN7999 or REGN8023 administration in $\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice led to an increase in red blood cells (about 10.51 M/ μL and about 10.18 M/ μL , respectively) compared to those mice treated with isotype control (about 8.72 M/ μL ; Table 24). Eight weeks of treatment with REGN7999 or REGN8023 in $\text{Hbb}^{\text{th3/+}}$ x $\text{HJV}^{\text{HumIn}}$ mice led to a reduction in splenic reticulocytes (from about 38% in isotype control to about 18%; Ter-119⁺/CD44^{high}) and increased levels of mature RBCs (Ter-119⁺/CD44^{low}) in both spleen and bone marrow, indicating improved erythropoiesis and less need of secondary erythropoiesis in the spleen (Table 25). Also observed

was a more significant reduction in spleen weight in $Hbb^{th3/+}$ x HJV^{HumIn} mice treated with anti-TMPRSS6 REGN7999 (about 0.142 g) or REGN8023 (about 0.130 g) compared to isotype control (about 0.301 g) (Table 24). In Table 24, all values are mean $\pm$ SD, n=10-19 per group. One Way ANOVA; *p<0.05 vs. WT isotype control; **p<0.05 vs. $Hbb^{th3/+}$ isotype control. In Table 25, all values are mean $\pm$ SD, n=10-19 per group. One Way ANOVA; *p<0.001 vs. WT isotype control; **p<0.0001 vs. $Hbb^{th3/+}$ isotype control.

Table 24: Effect of REGN7999 and REGN8023 on Red Blood Cells and Spleen Weight in a Mouse Model of Beta-thalassemia

Treatment Group	Number of Animals (n)	Red Blood Cells (M/mL)	Spleen Weight (g)
Wild type mice Isotype Control Treatment	16-19	10.05 $\pm$ 0.87	0.076 $\pm$ 0.014
$Hbb^{th3/+}$ x HJV^{HumIn} mice Isotype Control Treatment	15-16	8.72 $\pm$ 0.27*	0.301 $\pm$ 0.112*
$Hbb^{th3/+}$ x HJV^{HumIn} mice REGN7999 Treatment	11-14	10.51 $\pm$ 0.87**	0.142 $\pm$ 0.053* **
$Hbb^{th3/+}$ x HJV^{HumIn} mice REGN8023 Treatment	10-13	10.18 $\pm$ 0.47**	0.130 $\pm$ 0.046**

Table 25: Effect of REGN7999 and REGN8023 on Bone Marrow and Splenic Reticulocytes and Red Blood Cells in a Mouse Model of Beta-thalassemia

Treatment Group	Bone Marrow Reticulocytes (% CD44 ^{high} Ter119 ⁺)	Bone Marrow Mature Red Blood Cells (% CD44 ^{low} Ter119 ⁺)	Spleen Reticulocytes (% CD44 ^{high} Ter119 ⁺)	Spleen Mature Red Blood Cells (% CD44 ^{low} Ter119 ⁺)
Wild type mice Isotype Control Treatment	45.6 $\pm$ 5.4	42.0 $\pm$ 5.4	17.0 $\pm$ 5.4	80.5 $\pm$ 5.4
$Hbb^{th3/+}$ x HJV^{HumIn} mice Isotype Control Treatment	48.1 $\pm$ 10.5	25.8 $\pm$ 11.6*	38.0 $\pm$ 8.2*	51.0 $\pm$ 13.5*
$Hbb^{th3/+}$ x HJV^{HumIn} mice REGN7999 Treatment	40.4 $\pm$ 6.8	46.2 $\pm$ 7.8**	18.1 $\pm$ 3.2**	80.1 $\pm$ 4.3**
$Hbb^{th3/+}$ x HJV^{HumIn} mice REGN8023 Treatment	41.5 $\pm$ 9.4	45.7 $\pm$ 10.1**	18.5 $\pm$ 5.6**	78.2 $\pm$ 7.9**

Example 13: Effect of REGN7999 in a mouse model of beta-thalassemia

[0252] In order to further evaluate the efficacy of REGN7999 disclosed herein, studies were performed in a murine model of beta-thalassemia intermedia, as described in Example 12.

[0253] Mice were generated with a heterozygous deletion of $\beta 1$ and $\beta 2$ genes, which recapitulate beta thalassemia intermedia in humans (Ginzburg, *Blood* (2011) 118(16):4321-4330), and homozygous expression of human hemojuvelin (HJV) in place of mouse HJV (referred to as $Hbb^{th3/+} \times HJV^{HumIn}$) in a C57BL/6/129S6 background. $Hbb^{th3/+} \times HJV^{HumIn}$ mice (n=7-9; see, Example 12 for details on the generation of these mice) were subcutaneously injected weekly with 5 mg/kg of REGN7999 or isotype control (except 2 injections were administered in week 1). A wild type (WT) mouse control group was included and administered isotype control in a similar manner. After 8 weeks, mice were processed and samples were collected as described in Example 12. Serum hepcidin, serum iron and a full hematology panel, including reticulocytes and mature red blood cells were measured as described in Example 12.

[0254] Additionally, after the mice were sacrificed, their livers were flash frozen and later processed as described in Example 12. Both serum Hepcidin (reported in ng/mL) and liver iron content (reported in $\mu\text{g/g}$ dry weight) values are shown in Table 26.

Table 26: Effect of REGN7999 on Serum Hepcidin, Serum Iron, and Liver Iron Content in a Mouse Model of Beta-thalassemia

	Wild type mice Isotype Control Treatment	$Hbb^{th3/+} \times HJV^{HumIn}$ mice Isotype Control Treatment	$Hbb^{th3/+} \times HJV^{HumIn}$ mice REGN7999 Treatment
Serum Hepcidin (ng/mL)	376.8 $\pm$ 217.6	713.1 $\pm$ 435.8	1781 $\pm$ 1053* **
Serum Iron (mg/dL)	138.6 $\pm$ 60.92	102.3 $\pm$ 40.69	66.71 $\pm$ 25.53*
Liver Iron Content ($\mu\text{g/g}$ dry weight)	372.1 $\pm$ 83.96	547.2 $\pm$ 162.7*	513.9 $\pm$ 95.83

[0255] As illustrated in Table 26, after 8 weeks of treatment with 5 mg/kg REGN7999, $Hbb^{th3/+} \times HJV^{HumIn}$ mice had significantly increased (about 1781 ng/mL) serum hepcidin levels compared to both WT and $Hbb^{th3/+} \times HJV^{HumIn}$ mice treated with isotype control (about 376.8 ng/mL and about 713.1 ng/mL, respectively). Also shown in Table 26, treatment with REGN7999 led to a significant reduction (about 66 $\mu\text{g/dL}$) in serum iron compared to $Hbb^{th3/+} \times HJV^{HumIn}$ mice

treated with isotype control (about 138 $\mu\text{g/dL}$). In isotype treated control study groups, an increase in liver iron content from about 372 $\mu\text{g/g}$ dry weight in wild type mice to about 547 $\mu\text{g/g}$ dry weight in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice was observed. While treatment with REGN7999 did not significantly reduce liver iron content compared to isotype control in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice (about 513.9 and about 547.2 $\mu\text{g/g}$ dry weight, respectively), a significant reduction was seen compared to wild type mice treated with isotype control (Table 26). In Table 26, all values are mean $\pm$ SD, n=7-9 per group; One Way ANOVA * $p < 0.05$ vs. WT isotype control; ** $p < 0.05$ vs. $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ isotype control.

Table 27: Effect of REGN7999 on Hematocrit, Red Blood Cells, and Hemoglobin in a Mouse Model of Beta-thalassemia

	Wild type mice Isotype Control Treatment	$\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice Isotype Control Treatment	$\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice REGN7999 Treatment
Hematocrit (%)	36.92 $\pm$ 1.924	28.31 $\pm$ 1.969*	30.57 $\pm$ 3.216**
Red Blood Cells (M/mL)	9.52 $\pm$ 0.81	8.93 $\pm$ 0.68	11.2 $\pm$ 0.51 * **
Hemoglobin (g/dL)	13.02 $\pm$ 0.73	9.22 $\pm$ 0.54*	10.1 $\pm$ 0.65* **

[0256] As shown in Table 27, hematocrit, red blood cell count, and hemoglobin levels were lower in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice treated with isotype control as compared to wild type mice, as expected. Hematocrit was reduced from about 37% in wild type mice to about 28% in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice treated with 5 mg/kg isotype control. Treatment with REGN7999 did not show any effect compared to isotype control on hematocrit in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ (Table 27). Red blood cells, (reported in M/ μL) went from about 9.52 in wild type mice to about 8.93 in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice treated with isotype control. After 8 weeks of treatment with REGN7999 in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice significantly increased red blood cells to about 11.2 M μL (Table 27). In wild type isotype control treated mice, hemoglobin levels were about 13 g/dL, and, as shown in Table 27, treatment with isotype control in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice significantly reduced hemoglobin to about 9.22 g/dL. Treatment with REGN7999 increased hemoglobin levels to about 10.1 g/dL after 8 weeks of treatment in $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ mice. Without being bound by theory, these data support the idea that restricting iron by modulating the hepcidin pathway has a beneficial effect on red blood cells. In Table 27, all values are mean $\pm$ SD, n=6-9 per group; One Way ANOVA * $p < 0.05$ vs. WT isotype control; ** $p < 0.05$ vs. $\text{Hbb}^{\text{th3/+}} \times \text{HJV}^{\text{HumIn}}$ isotype control.

Example 14: Pharmacokinetics (PK) and pharmacodynamics (PD) in cynomolgus monkey

[0257] The objective of this study was to determine the pharmacokinetic (PK) characteristics and pharmacokinetic/pharmacodynamic (PK/PD) relationship of two anti-TMPRSS6 human monoclonal antibodies (mAbs). For this study, REGN7999 and REGN8023 were administered as a single intravenous (IV) infusion to female cynomolgus monkeys.

[0258] As summarized in Table 28, 21 female cynomolgus monkeys were assigned to one of 6 groups (n=3 or 4 animals per group; 5 mg/kg or 15 mg/kg dose groups). Animals received a single IV infusion of 5 mg/kg REGN7999, 5 mg/kg REGN8023, 15 mg/kg REGN7999, 15 mg/kg REGN8023, or 15 mg/kg isotype control antibody (Table 28). Blood samples for the determination of total drug concentrations were collected from all animals at pre-dose and at various times throughout the 56 day in-life period. Concentrations of total human mAb in serum were determined using a qualified anti-human Fc enzyme-linked immunosorbent assay (ELISA). The PK parameters were estimated using noncompartmental analysis (NCA). Blood samples for pharmacodynamics were collected at various time points throughout the course of the study and analyzed using standard clinical chemistry (Beckman Coulter AU680 analyzer) and hematology (Avida 120 analyzer) analyses. Serum hepcidin was measured using the commercially available Hepcidin Murine-Compete ELISA kit (Intrinsic Lifesciences, Cat: HMC-001, Lot: 08232019) according to the manufacturers' instructions.

Table 28: Summary of Doses and Dose Groups

Group No.	Test or Control Article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Animals
1	Isotype control antibody	15	7.5	3
2	REGN7999	5	2.5	3
3	REGN7999	15	7.5	4
4	REGN8023	5	2.5	3
5	REGN8023	15	7.5	4

Note: Results and discussion focus on PK/PD of REGN7999 and REGN8023 test articles (Groups 2 through 5) only.

[0259] The PK profile of anti-TMPRSS6 monoclonal antibodies (mAbs) REGN7999 or REGN8023 are described by nonlinear kinetics, with target-mediated clearance (TMC) at low concentrations and linear, dose-proportional kinetics consistent with saturation of the target-mediated elimination pathway at high mAb concentrations. Following a single-dose IV administration, the concentration time profiles of total REGN7999 and REGN8023 were characterized by initial brief distribution phase, followed by a brief linear beta elimination phase, and at low concentrations, a terminal nonlinear target-mediated elimination phase and a post target-

mediated elimination phase. No definitive evidence of anti-drug antibody- (ADA)-like impact on the profiles and no apparent impact on exposure were observed; thus, no concentration values were excluded from analysis.

[0260] Pharmacokinetic parameters are listed in Table 29. Following a single IV dose of 5 and 15 mg/kg of antibody, maximum serum concentration ($C_{\max}$) values of about 120 and about 410 $\mu\text{g/mL}$, respectively, were observed for REGN7999, and $C_{\max}$ values of about 162 and about 508, respectively, were observed for REGN8023. Corresponding dose-normalized $C_{\max}$ ($C_{\max}/\text{Dose}$) values were within about 1.4-fold, indicating a dose-proportional increase in $C_{\max}$ with increasing IV dose for anti-TMPRSS6 mAbs REGN7999 and REGN8023. A mean $t_{\max}$ (time required to reach $C_{\max}$) of about 0.5 h, the first measured time point, was observed for all dose groups.

[0261] Exposure (AUC_{inf}) to REGN7999 increased greater than dose-proportionally, with $\text{AUC}_{\text{inf}}/\text{Dose}$ following administration of 15 mg/kg REGN7999 about 1.6-fold greater than following the 5 mg/kg dose. Consistent with this, a greater total body clearance (CL) was observed at the 5 mg/kg dose due to a greater impact of TMC at lower concentrations. Exposure to REGN8023 increased proportionally with increasing dose, suggesting a more modest effect of TMC at the doses examined. The terminal half-life ($t_{1/2}$) representing TMC in all dose groups ranged between about 7-8 days.

Table 29: Mean Pharmacokinetic Parameters of Total REGN7999 and Total REGN8023 Following a Single Intravenous Bolus Administration to Female Cynomolgus Monkeys

Parameter	Unit	REGN7999				REGN8023			
		5 mg/kg (n=3)		15 mg/kg (n=4)		5 mg/kg (n=3)		15 mg/kg (n=4)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
$C_{\max}$	$\mu\text{g/mL}$	120	7.57	410	47.2	162	16.2	508	55.0
$C_{\max}/\text{Dose}$	$(\mu\text{g/mL}) / (\text{mg/kg})$	23.9	1.51	27.3	3.15	32.5	3.24	33.9	3.66
$t_{\max}$	h	0.500	0	0.500	0	0.500	0	0.500	0
AUC_{inf}	$\text{day} \cdot (\mu\text{g/mL})$	831	153	3950	617	1440	182	4500	951
$\text{AUC}_{\text{inf}}/\text{Dose}$	$\text{day} \cdot (\mu\text{g/mL}) / (\text{mg/kg})$	166	30.7	263	41.2	289	36.4	300	63.4
CL	mL/day/kg	6.17	1.25	3.88	0.680	3.50	0.446	3.46	0.792
V_{ss}	mL/kg	57.2	1.31	57.6	6.65	37.7	2.41	42.8	7.02
Terminal $t_{1/2}$	day	8.10	0.592	7.74	0.949	7.49	0.577	7.39	2.09

[0262] As shown in Table 30, the pharmacodynamic effects of REGN7999 and REGN8023 on serum hepcidin, serum iron, and transferrin saturation were followed over the course of the 57 day study. In groups treated with 5 mg/kg or 15 mg/kg REGN7999 or REGN8023, mean serum iron was reduced about 60% from baseline serum iron concentrations by Day 1 after dosing.

[0263] As shown in Table 31, transferrin saturation decreased to about 12% on Day 1 from about 35% at baseline, representing improvement in serum iron carrying capacity. The PD effect on Day 1 was independent of dose, suggesting saturation of TMPRSS6 binding at concentrations achieved on Day 1 at both low and high doses of REGN7999 and REGN8023. The reduction in serum iron and transferrin saturation was maintained through at least 6 weeks after REGN7999 dosing in both the 5 mg/kg and 15 mg/kg dose groups. In the 5 mg/kg REGN8023 dose group, the pharmacodynamic effect on serum iron began to return to baseline at approximately 1 week following IV administration and was not statistically different from isotype control treatment by week 4. In the 15 mg/kg REGN8023 dose group, the pharmacodynamic effects had a longer duration than in the 5 mg/kg REGN8023 group due to sustained higher drug concentrations. Both serum iron and transferrin saturation returned to approximately baseline values in most animals by Day 57.

[0264] As shown in Table 32, serum hepcidin concentrations increased slightly following dosing of REGN7999 or REGN8023, though large inter-animal variability was observed.

Table 30: Pharmacodynamics, mean (+/- SD) values for serum iron

Dose Group:	% Change in Serum Iron vs. Baseline		
	Day 1	Day 29	Day 57
Isotype Control Antibody (15 mg/kg)	-17.1 ± 18.0	-22.9 ± 6.8	-13.0 ± 9.6
REGN7999 (5 mg/kg)	-62.7 ± 4.8 ****	-62.5 ± 2.9 ***	-31.6 ± 11.3
REGN7999 (15 mg/kg)	-65.0 ± 9.6 ****	-67.2 ± 4.9 ****	-30.9 ± 20.6
REGN8023 (5 mg/kg)	-59.9 ± 7.3 ***	-41.4 ± 14.7	-16.4 ± 15.7
REGN8023 (15 mg/kg)	-62.7 ± 8.4 ****	-69.9 ± 11.5 ****	-39.0 ± 19.3 *

Table 31: Pharmacodynamics, mean (+/- SD) values transferrin saturation

Dose Group:	Transferrin Saturation (%)			
	Baseline (Day 0)	Day 1	Day 29	Day 57

Isotype Control Antibody (15 mg/kg)	34.6 ± 8.7	27.3 ± 9.2	24.5 ± 3.6	20.1 ± 0.4
REGN7999 (5 mg/kg)	34.9 ± 9.8	12.5 ± 4.8 ***	11.3 ± 2.4 **	18.8 ± 0.4
REGN7999 (15 mg/kg)	34.1 ± 1.8	12.3 ± 4.8 ***	10.4 ± 2.8 ***	17.3 ± 0.5
REGN8023 (5 mg/kg)	32.0 ± 3.4	12.9 ± 4.2 **	16.9 ± 3.8	15.8 ± 0.4
REGN8023 (15 mg/kg)	29.3 ± 7.7	11.7 ± 3.2 ***	9.1 ± 4.4 ***	14.3 ± 0.5

Table 32: Pharmacodynamics, mean (+/- SD) values for serum hepcidin

Dose Group:	% Change in Serum Hepcidin vs. Baseline		
	Day 1	Day 29	Day 57
Isotype Control Antibody (15 mg/kg)	-19.5	-36.4	-37.2
REGN7999 (5 mg/kg)	261.1	-32.1	-10.7
REGN7999 (15 mg/kg)	162.7**	50.4	4
REGN8023 (5 mg/kg)	52.2	-42.5	-18.8
REGN8023 (15 mg/kg)	132.2	7.9	-34.0

All values are mean ± SD, n=3-4 per group. Statistics – two way ANOVA with Dunnett's; *p<.05 vs. isotype **p<.01 vs. isotype control; ***p<.001 vs. isotype control; ****p<.0001 vs. isotype control

Example 15: Effect of REGN7999 on anaerobic capacity in mouse model of beta thalassemia

[0265] The effect of REGN7999 treatment on anaerobic capacity in a murine model of beta thalassemia was evaluated.

[0266] Mice were generated with a heterozygous deletion of $\beta 1$ and $\beta 2$ genes, which recapitulate beta thalassemia intermedia in humans (Ginzburg, *Blood* (2011) 118(16):4321-4330), and homozygous expression of human hemojuvelin (HJV) in place of mouse HJV (referred to as $Hbb^{th3/+} \times HJV^{HumIn}$) in a C57BL/6/129S6 background. $Hbb^{th3/+} \times HJV^{HumIn}$ mice (Example 12 for further details on the generation of these mice).

[0267] REGN7999 treatment in Hbb^{th3/+} mice appears to lead to an increase in red blood cells that appear smaller, healthier, and have a slower turnover (see, e.g. Example 13). However, it was not known whether this would translate to a functional improvement of red blood cells after REGN7999 treatment. For example, it was not known whether these smaller but healthier red blood cells are able deliver sufficient oxygen under anaerobic conditions, such as during exhaustion running.

[0268] To test this, Hbb^{th3/+} mice were subjected to forced running studies (see study plan depicted in Figure 1). Prior to all experiments, mice were trained for 2 weeks on the belt of a six-lane motorized treadmill (Series 8 Treadmill, IITC Life Science) supplied with shocker plates. For the exhaustion run, base speed (10 m/min) was increased every 5 min by 5 m/min. The maximum amount of distance was determined at 60 min. Mice that either remained inactive on the shocker unit or received 500 shocks were removed from the treadmill.

[0269] Hbb^{th3/+} mice were observed to run significantly less distance than wild-type C57BL/6 mice treated with isotype control antibody (REGN1945), suggesting these mice were faster exhausted (Figure 2A). Hbb^{th3/+} mice treated with 10 mg/kg REGN7999 were able to run a similar distance as wild-type animals (Figure 2A), indicating that the improvement of red blood cell health observed with REGN7999 treatment is reflected in improved anaerobic capacity. Wild-type C57BL/6 mice injected with REGN7999 showed no difference in running distance compared to isotype treated mice, indicating that REGN7999 does not improve running capacity in non-Hbb^{th3/+} mice (Figure 3).

[0270] Lactate measurements were also collected. To determine lactate production, 15 mL blood was collected from the tail-vein after the exhaustion run. Lactate levels were determined by using a lactometer (Nova Biomedical) as described in Summermatter et al. *Proc Natl Acad Sci USA*. 2013;110(21):8738-43. Control Hbb^{th3/+} mice produced more blood lactate despite running less distance than Hbb^{th3/+} mice treated with REGN7999, further suggesting improved red blood cell health (Figure 2B).

Example 16: Effects of REGN7999 and Acvr2b(L79D)-Fc on hepatic iron and erythropoiesis effectiveness

[0271] Acvr2b(L79D)-Fc has been shown to improve ineffective erythropoiesis and increase hemoglobin in mice and humans (see, e.g., Cappellini et al. *N Engl J Med*. 2020;382(13):1219-31; Suragani et al. *Blood*. 2014;123(25):3864-72). As shown herein, blockade of TMPRSS6 with REGN7999 appears to improve ineffective erythropoiesis and leads

to more and healthier red blood cells. The effect of REGN7999 treatment on hepatic iron and erythropoiesis effectiveness was evaluated and compared to treatment with Acvr2b(L79D)-Fc in a mouse model of beta thalassemia.

[0272] To compare the effects of REGN7999 treatment (10 mg/kg) and Acvr2b(L79D)-Fc treatment (10 mg/kg), Hbb^{th3/+} mice were administered one of the two molecules for 8 weeks. Serum hepcidin, serum iron, and liver iron content were measured as described in Example 10. After 8 weeks of treatment with either molecule, it was observed that REGN7999 treatment increased serum hepcidin, reduced serum iron, and liver iron content, but Acvr2b(L79D)-Fc treatment had no effect on these parameters (Figures 4A-C). Acvr2b(L79D)-Fc elevated body weights but had no effect on liver weight (Figure 5). REGN7999 treatment was shown to reduce spleen weight, but Acvr2b(L79D)-Fc treatment did not (Figure 6). Hbb^{th3/+} control mice have significant splenomegaly, which was reversed in part by treatment with REGN7999. Acvr2b(L79D)-Fc treatment did not provide any change in the Hbb^{th3/+} mice splenomegaly.

[0273] A blood count panel was also collected from the treated animals. As seen in the previous examples, the blood count panel shows that REGN7999 treatment increases red blood cell count significantly in Hbb^{th3/+} mice (Table 33). Acvr2b(L79D)-Fc treatment increased hemoglobin significantly, but not red blood cell count compared to Hbb^{th3/+} controls. REGN7999 treatment however showed a significant increase of RBC count (Table 33).

[0274] Flow cytometry was used to confirm this finding. Isolated red blood cells from spleens or bone marrow were incubated with murine Fc-blocker (anti-CD16/32, BD Biosciences) for 10 minutes followed by incubation with fluorescein isothiocyanate (FITC) anti-Ter119, phycoerythrin (PE) anti-CD71, allophycocyanine (APC) anti-CD44 and an APC-cyanine7 (APC-Cy7) anti-CD45/CD11b/Ly6G cocktail for 30 minutes (all antibodies BD Biosciences). To exclude dead cells from the analysis, the cells were stained with Sytox AADvanced for 30 minutes (ThermoFisher). All staining procedures were performed on ice. Compensation controls were performed using OneComp Beads (Invitrogen) and prepared at the time of cell staining. A minus-One control panel was prepared for correct gating of target populations. All samples were measured on a CytoFLEX Flow Cytometer (Beckman-Coulter).

[0275] The flow cytometry data showed increased splenic mature red blood cells only in REGN7999 treated mice, but not in Acvr2b(L79D)-Fc treated mice (**Figure 7**). REGN7999 treatment also showed a reduction in reticulocytes, which was not observed with Acvr2b(L79D)-Fc treatment (**Figure 8**).

Table 33: Blood count panel of Hbb^{th3/+} mice treated with REGN7999 or Acvr2b(L79D)-Fc

Group	WT REGN1945	Hbb^{th3/+} REGN1945	Hbb^{th3/+} REGN7999	Hbb^{th3/+} Acvr2b(L79D)-Fc
<i>n</i>	7	7	6	6
Red blood cells (M/mL)	10.25 ± 0.52	9.82 ± 1.47	11.65 ± 0.44**	11.08 ± 1.19
Reticulocytes (% of RBC)	0.001 ± 0.004	0.26 ± 0.41	0.00 ± 0.00	0.09 ± 0.21
Hematocrit (%)	42.64 ± 3.65	30.90 ± 3.95*	30.58 ± 1.76	31.82 ± 4.29
Hemoglobin (g/dL)	14.26 ± 0.63	10.11 ± 1.23*	10.37 ± 0.39	11.82 ± 1.24**
Mean Corpuscular Volume (fL)	41.7 ± 1.27	31.63 ± 2.55*	26.25 ± 0.83**	31.35 ± 2.09***

SEQUENCES

Table 34: Exemplary DNA and protein (PRT) sequences of the disclosure

SEQ ID NO:	DNA/PRT sequence	Sequence
1	DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGG AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAATAA CTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATCATATGATGGAAGTAATGAATATTATTTCAG ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACGGCT GTATATTACTGTGCGAGAGATCAGGACTACGGTGGTAACCTCTTTC ATTACTGGGGCCAGGGAACCCTGGTCACTGTCTCCTCA
2	PRT	QVQLVESGGGVVQPGRSLRLSCAASGFTFNNGMHVVRQAPGKGLE WVAIISYDGSNEYYSVSKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARDQDYGGNLFHYWGQGLTVTVSS
3	DNA	GGATTACCTTCAATAACTATGGC
4	PRT	GFTFNNG
5	DNA	ATATCATATGATGGAAGTAATGAA
6	PRT	ISYDGSNE
7	DNA	GCGAGAGATCAGGACTACGGTGGTAACCTCTTTCATTAC
8	PRT	ARDQDYGGNLFHY
9	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGC TATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCTAAGCTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCGTCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGT CTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACA GTACCCCTCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAA A
10	PRT	DIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFRSGSGSDFTLTISLQPEDFATYYCQSYSTPPITFG QGTRLEIK
11	DNA	CAGAGCATTAGCAGCTAT
12	PRT	QSISSY
13	DNA	GCTGCATCC
14	PRT	AAS
15	DNA	CAACAGAGTTACAGTACCCCTCCGATCACC
16	PRT	QQSYSTPPIT
17	DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGG AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAATAA CTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATCATATGATGGAAGTAATGAATATTATTTCAG ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTTCAAATGAACAGCCTGAGAGCTGAGGACACGGCT GTATATTACTGTGCGAGAGATCAGGACTACGGTGGTAACCTCTTTC ATTACTGGGGCCAGGGAACCCTGGTCACTGTCTCCTCAGCCTCCAC CAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC

		TCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGG CGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGAC CTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGA CAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCCTGCCCA GCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCAA AACCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCCGAGGTCCAGTTCAA CTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCTCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGC CCCCATCCCAGGAGGAGATGACCAAGAACCAGGTACGCCTGACCT GCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTCACCCTGG ACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGTCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACACAGAAGTCCCTCTCCCTG TCTCTGGGTAAATGA
18	PRT	QVQLVESGGGVVQPGRLRLSCAASGFTFNNYGMHWVRQAPGKGLE WVAIISYDGSNEYYSDSVKGRFTISRDN SKNTLYLQMSNLRAEDTAVY YCARDQDYGGNLFHYWGQGLVTVSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGKTYITCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK*
19	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGC TATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAGCTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCGTCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGT CTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACA GTACCCCTCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAA ACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATG AGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAA CTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCC CTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGC AAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAA GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATC AGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT GTTAG
20	PRT	DIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPITFG QGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC*

21	DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGG AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTAC CTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATCATATGATGGAAGTAATGACTACTATTTAG ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAACCTGAGAGCTGAGGACACGGCT GTGTATTACTGTGCGAAAGATCAGGGCTACGGTGGTAACCTCTTTG CCTCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
22	PRT	QVQLVESGGGVVQPRSLRLSCAASGFTFSTYGMHWVRQAPGKGLE WVAIISYDGSNDYYLDSVKGRFTISRDN SKNTLYLQMNNLRAEDTAV YYCAKDQGYGGNLFASWGQGLTVTVSS
23	DNA	GGATTACCTTCAGTACCTATGGC
24	PRT	GFTFSTYG
25	DNA	ATATCATATGATGGAAGTAATGAC
26	PRT	ISYDGSND
27	DNA	GCGAAAGATCAGGGCTACGGTGGTAACCTCTTTGCCTCC
28	PRT	AKDQGYGGNLFAS
29	DNA	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGG AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTAC CTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAG TGGGTGGCAATTATATCATATGATGGAAGTAATGACTACTATTTAG ACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAAATGAACAACCTGAGAGCTGAGGACACGGCT GTGTATTACTGTGCGAAAGATCAGGGCTACGGTGGTAACCTCTTTG CCTCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCAC CAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC TCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCTGTGGAAGTCAAGGCGCCCTGACCAGCGG CGTGACACCTTCCCGGTGTCTTACAGTCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGAC CTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGA CAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCTGCCCCA GCACCTGAGTTCCTGGGGGGACCATCAGTCTTCTGTTCCCCCAA AACCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAGTG CGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTCAA CTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTC ACCGTCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGGCCTCCCGTCTCCATCGAGAAAACCATCT CCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGC CCCCATCCCAGGAGGAGATGACCAAGAACCAGGTACAGCTGACCT GCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTACCGTGG ACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACACAGAAGTCCCTCTCCCTG TCTCTGGGTAAATGA
30	PRT	QVQLVESGGGVVQPRSLRLSCAASGFTFSTYGMHWVRQAPGKGLE WVAIISYDGSNDYYLDSVKGRFTISRDN SKNTLYLQMNNLRAEDTAV YYCAKDQGYGGNLFASWGQGLTVTVSSASTKGPSVFPLAPCSRSTSES TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV

		TVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLK*
31	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGTTAAAGCCGGGG GGGTCCCTTAGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAA CGCCTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA GTGGGTTGGCCGTATTAAGCAAACTGATAGTGGGACAACAGA CTACGCTGCACCCGTGAAAGGCAGATTCACCATCTCAAGAGATGAT TCAAAAAACACGCTGTATCTGCAATGAACAGCCTGAAAACCGAG GACACAGCCGTGTATTACTGTACCACAGTGGATAACGATTTTGGGA GTGGTTATTATAACTGGTTCGACCCCTGGGGCCAGGGAACCCCTGGT CACTGTCTCCTCA
32	PRT	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLE WVGRIKSKTDSGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTA VYYCTTVDNDFWSGYYNWFDPPWGQGLVTVSS
33	DNA	GGATTCACTTTCAGTAACGCCTGG
34	PRT	GFTFSNAW
35	DNA	ATTAAGCAAACTGATAGTGGGACAACA
36	PRT	IKSKTDSGTT
37	DNA	ACCACAGTGGATAACGATTTTGGAGTGGTTATTATAACTGGTTCG ACCC
38	PRT	TTVDNDFWSGYYNWFDPP
39	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGTTAAAGCCGGGG GGGTCCCTTAGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAA CGCCTGGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA GTGGGTTGGCCGTATTAAGCAAACTGATAGTGGGACAACAGA CTACGCTGCACCCGTGAAAGGCAGATTCACCATCTCAAGAGATGAT TCAAAAAACACGCTGTATCTGCAATGAACAGCCTGAAAACCGAG GACACAGCCGTGTATTACTGTACCACAGTGGATAACGATTTTGGGA GTGGTTATTATAACTGGTTCGACCCCTGGGGCCAGGGAACCCCTGGT CACTGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG CGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAC TCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTAC AGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC CAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGG TCCCCCATGCCCACCCTGCCAGCACCTGAGTTCCTGGGGGGACCA TCAGTCTTCTGTTCCTCCCCCAAAACCAAGGACACTCTCATGATCTC CCGGACCCCTGAGGTACGTGCGTGGTGGTGGACGTGAGCCAGGA AGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTG CATAATGCCAAGACAAAGCCGCGGAGGAGCAGTTCAACAGCACG TACCGTGTGGTCAGCGTCTCACCCTGCTGACACAGGACTGGCTGA ACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGT CCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGAGGAGGAGGAG AGCCACAGGTGTACACCTGCCCCCATCCAGGAGGAGATGACCA AGAACCAGGTGACCTGACCTGCTGCTGCAAGGCTTCTACCCAG CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA

		CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAGGCTCACCGTGGACAAGAGCAGGTGGCAGGAGGGGA ATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACACAGAAGTCCCTCTCCCTGTCTCTGGGTAAATGA
40	PRT	EVQLVESGGGLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLE WVGRIKSKTDSGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTA VYYCTTVDNDFWSGYYNWFDPWGQGLVTVSSASTKGPSVFPLAPCS RSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPCCPPCPAP EFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVF SCSVMHEALHNHYTQKSLSLGLK*
41	DNA	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTTAAGCCTGGG GGGTCCCTGAGAAATTCCTGTGCAGCCTCTGGATTTATTTTGTGTA TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAGTTGGAATAGTGGTAGTATAGGCTATGCGG ACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAA GTCCCTGTATCTGCAAATGAGTGGTCTGAGACCTGAGGACACGGCC TTGTATTATTGTGTAAGTGGGTTCTATTACGTTAGGTCTTACTT TGACAACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
42	PRT	EVQLVESGGGLVKPGGSLRLSCAASGFIFVDYAMHWVRQAPGKGLEW VSGISWNSGSIGYADSVKGRFTISRDNAKKSLYLQMSGLRPEDTALYY CVKSGFYVRSYFDNWGQGLVTVSS
43	DNA	GGATTTATTTTGTGATTATGCC
44	PRT	GFIFVDYA
45	DNA	ATTAGTTGGAATAGTGGTAGTATA
46	PRT	ISWNSGSI
47	DNA	GTAAAAAGTGGGTTCTATTACGTTAGGTCTTACTTTGACAAC
48	PRT	VKSGFYVRSYFDN
49	DNA	GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCCTCACCCCTGG AGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCTTGAT AGTGATGATGGAACACCTATTTGGACTGGTACCTGCGGAAGCCAG GGCAGTCTCCACAACCTCTGATCTATACGTTTCTATCGGGCCTCT GGAGTCCCAGACAGGTTCAAGTGGCAGTGGGTCAAGCACTGATTTCA CACTGAAAAATCAGTAGGGTGGAGGCTGACGATGTTGGAGTTTATTA CTGCATGCAACGTATAGAGTTTCCGCTCACTTTCCGCGGAGGGACC AAGGTGGAGATCAAA
50	PRT	DIVMTQSPLSLPVTPGEPASISCRSSQSLDSDDGNTYLDWYLRKPGQS PQLLIYTLSYRASGVPDRFSGSGSGTDFTLKISRVEADDDVGVYYCMQRI EFPLTFGGGTKVEIK
51	DNA	CAGAGCCTCTTGATAGTGATGATGGAACACCTAT
52	PRT	QSLDSDDGNTY
53	DNA	ACGCTTTC
54	PRT	TLS
55	DNA	ATGCAACGTATAGAGTTTCCGCTCACT
56	PRT	MQRIEFPLT
57	DNA	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTTAAGCCTGGG GGGTCCCTGAGAAATTCCTGTGCAGCCTCTGGATTTATTTTGTGTA

		TTATGCCATGCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAG TGGGTCTCAGGTATTAGTTGGAATAGTGGTAGTATAGGCTATGCGG ACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAA GTCCCTGTATCTGCAAATGAGTGGTCTGAGACCTGAGGACACGGCC TTGTATTATTGTGTAAAAAGTGGGTTCTATTACGTAGGTCTTACTT TGACAACTGGGGCCAGGGAACCTGGTCACCGTCTCCTCAGCCTCC ACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCA CCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTT CCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAG ACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTG GACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCACCTGCC CAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCTGTTCCTCCCCA AAACCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAGT GCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCA ACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGC CGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCT CACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTG CAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATC TCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTG CCCCCATCCAGGAGGAGATGACCAAGAACCAGGTGAGCCTGACC TGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGG AGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCG TGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTG GACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGA TGCATGAGGCTCTGCACAACCACTACACACAGAAGTCCCTCTCCCT GTCTCTGGGTAAATGA
58	PRT	EVQLVESGGGLVKPGGSLRISCAASGFIFVDYAMHWVRQAPGKGLEW VSGISWNSGSGYADSVKGRFTISRDNAKKSLYLQMSGLRPEDTALYY CVKSGFYVRSYFDNWQGTTLVTSSASTKGPSVFPLAPCSRSTSEST AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLGLK*
59	DNA	GATATTGTGATGACCCAGTCTCCACTCTCCCTGCCCGTCACCCCTGG AGAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCTTGAT AGTGATGATGGAAACACCTATTTGGACTGGTACCTGCGGAAGCCAG GGCAGTCTCCACAACCTCTGATCTATACGCTTTCCTATCGGGCCTCT GGAGTCCCAGACAGGTTCAAGTGGCAGTGGGTGAGGCACTGATTTCA CACTGAAAATCAGTAGGGTGGAGGCTGACGATGTTGGAGTTTATTA CTGCATGCAACGTATAGAGTTTCCGCTCACTTTCGGCGGAGGGACC AAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCT TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA AGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTAC AGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCT GACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTG

		CGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTC AACAGGGGAGAGTGTTAG
60	PRT	DIVMTQSPSLPVTPEPASISCRSSQSLLDSDDGNTYLDWYLRKPGQS PQLLIYTLASYRASGVDPDRFSGSGSGTDFTLKISRVEADDVGVYYCMQRI EFPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC*
61	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCAGGG CGGTCCCTGAGACTCTCCTGTATAGCTTCTGGATTCAACTTTGGTGA TTATGGTATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTAGGTTTCATTAGAAGCAAAGCTTATGGTGGGACAACAGAAT ACGCCGCGTCTGTGAAAGGCAGATTCACCATCTCAAGAGATGATTC CAAAAGCATCGCCTATCTGCAAATGAACAGCCTGAAAACCGAGGA CGCAGCCATGTATTACTGTTCTAGAGATTATTACGATATTTTGACTG GTTATTATGGCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTC CTCA
62	PRT	EVQLVESGGGLVQPGRSLRLSCIASGFNFGDYGMSWVRQAPGKGLEW VGFIRSKAYGGTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDAAM YYCSRYYDILTGYYGDYWGQGTLVTVSS
63	DNA	GGATTCAACTTTGGTGATTATGGT
64	PRT	GFNFGDYG
65	DNA	ATTAGAAGCAAAGCTTATGGTGGGACAACA
66	PRT	IRSKAYGGTT
67	DNA	TCTAGAGATTATTACGATATTTTGACTGGTTATTATGGCGACTAC
68	PRT	SRDYYDILTGYYGDY
69	DNA	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGG AGACAGAGTCACCATCACTTGCCGGGCCAGTCAGAGTATTAGTAGC TGGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCC TGATCTATCAGGCGTCTAGTTTAGAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACCATCAGCAGC CTGCAGCCTGAAGATTTTGCAACTTATTACTGCCAACAATATAATA GTTATTCTCGGACGTTCCGGCCAAGGGACCAAGGTGGAAATCAAA
70	PRT	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIY QASSLESQVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQYNSYSRTFG QGTKVEIK
71	DNA	CAGAGTATTAGTAGCTGG
72	PRT	QSISSW
73	DNA	CAGGCGTCT
74	PRT	QAS
75	DNA	CAACAATATAATAGTTATTCTCGGACG
76	PRT	QQYNSYSRT
77	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCAGGG CGGTCCCTGAGACTCTCCTGTATAGCTTCTGGATTCAACTTTGGTGA TTATGGTATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTAGGTTTCATTAGAAGCAAAGCTTATGGTGGGACAACAGAAT ACGCCGCGTCTGTGAAAGGCAGATTCACCATCTCAAGAGATGATTC CAAAAGCATCGCCTATCTGCAAATGAACAGCCTGAAAACCGAGGA CGCAGCCATGTATTACTGTTCTAGAGATTATTACGATATTTTGACTG GTTATTATGGCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTC CTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCT

		CCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCC CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTT GGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAA CACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGC CCACCCTGCCCAGCACCTGAGTTCCCTGGGGGGACCATCAGTCTTCC TGTTCCCCC AAAACCCAAGGACACTCTCATGATCTCCCGGACCCC TGAGGTACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGA GGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTG GTCAGCGTCCTACCGTCTGCACCAGGACTGGCTGAACGGCAAGG AGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCTCCATCGA GAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGT GTACACCCTGCCCCATCCCAGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAG GCTCACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG TCCCTCTCCCTGTCTCTGGGTAAATGA
78	PRT	EVQLVESGGGLVQGRSLRLSCIASGFNFGDYGMSWVRQAPGKGLEW VGFIRSKAYGGTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDAAM YYCSRYYDILTGYYGDYWGQGLVTVSSASTKGPSVFPLAPCSRSTS ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLK*
79	DNA	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGG AGACAGAGTCACCATCACTTGCCGGGCCAGTCAGAGTATTAGTAGC TGGTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCC TGATCTATCAGGCGTCTAGTTTAGAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGAAATCACTCTCACCATCAGCAGC CTGCAGCCTGAAGATTTTGCAACTTATTACTGCCAACAATATAATA GTTATTCTCGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACG AACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC AGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACTTC TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTC CAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAG GACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTT AG
80	PRT	DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIY QASSLESGVPSRFSGSGSGTEFTLTISLQPEDFATYYCQYNSYSRTFG QGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSPVTKSFNRGEC*

81	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAGCCTGGG GGGTCCCTGAGAGTCTCCTGTGCAGCCTCTGGAGTCACTTTCAGTA ACGTCTGGATGAGTTGGGTCCGCCAGGTTCCAGGGAAGGGGCTGGA ATGGGTTGGCCGTATTAAGCAAACTGATGGTGGGACAACAGA CTACGCTGCACCCGTGAAAGGCAGATTACCATCTCAAAAGATGAT TCAAAAAATACGTTGTTCTGCAAATGAACAGCCTGAAAACCGAAG ACACAGCCGTGTATTACTGTACCACAGAGACCGGCGATTTTTGGAG TGGTTATTATCCTCGCTTCCAGCACTGGGGCCAGGGCACCTGGTC ACCGTCTCCTCA
82	PRT	EVQLVESGGGLVKPGGSLRVSCAASGVTFSTNVWMSWVRQVPKGL WVGRIKSKTDGGTTDYAAPVKGRFTISKDDSKNTLFLQMNSLKTEDT AVYYCTTETGDFWSGYYPFQHWGQGLVTVSS
83	DNA	GGAGTCACTTTCAGTAACGTCTGG
84	PRT	GVTFSTNVW
85	DNA	ATTAAGCAAACTGATGGTGGGACAACA
86	PRT	IKSKTDGGTT
87	DNA	ACCACAGAGACCGGCGATTTTTGGAGTGGTTATTATCCTCGCTTCCA GCAC
88	PRT	TTETGDFWSGYYPFQH
89	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCAACATCACTTGCCTGGGCAAGTCAGTATATTAGCACC TATTTAAATTGGTATCAGCAGAAACCAGGGAAGCCCCCTAGCCTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGT CTGCAACCTGAAGATTTTGATCTTACTCCTGTCAACAGAGTTACAG TACCCCTCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA
90	PRT	DIQMTQSPSSLSASVGRVTITCRASQYISTYLNWYQKPKGAPSLLIY AASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFASYSQQSYSTPPYTF GQGTKLEIK
91	DNA	CAGTATATTAGCACCTAT
92	PRT	QYISTY
93	DNA	CAACAGAGTTACAGTACCCCTCCGTACACT
94	PRT	QQSYSTPPYT
95	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAGCCTGGG GGGTCCCTGAGAGTCTCCTGTGCAGCCTCTGGAGTCACTTTCAGTA ACGTCTGGATGAGTTGGGTCCGCCAGGTTCCAGGGAAGGGGCTGGA ATGGGTTGGCCGTATTAAGCAAACTGATGGTGGGACAACAGA CTACGCTGCACCCGTGAAAGGCAGATTACCATCTCAAAAGATGAT TCAAAAAATACGTTGTTCTGCAAATGAACAGCCTGAAAACCGAAG ACACAGCCGTGTATTACTGTACCACAGAGACCGGCGATTTTTGGAG TGGTTATTATCCTCGCTTCCAGCACTGGGGCCAGGGCACCTGGTC ACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGC GCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAC CAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCAACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGT CCCCATGCCCACCCTGCCAGCACCTGAGTTCTTGGGGGGACCAT CAGTCTTCTGTTCCTCCCAAAACCAAGGACACTCTCATGATCTCC CGGACCCCTGAGGTACAGTGCGTGGTGGTGGACGTGAGCCAGGAA

		GACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGC ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGT ACCGTGTGGTCAGCGTCCTCACCCTGCTGCACCAGGACTGGCTGAA CGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCC TCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCAGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCG ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC ACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCCTC TACAGCAGGCTCACCCTGGACAAGAGCAGGTGGCAGGAGGGGAAT GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC ACAGAAAGTCCCTCTCCCTGTCTCTGGGTAAATGA
96	PRT	EVQLVESGGGLVKPGGSLRVSCAASGVTFSTVWMSWVRQVPGKGLE WVGRIKSKTDGGTTDYAAPVKGRFTISKDDSKNTLFLQMNSLKTEDT AVYYCTTETGDFWSGYPRFQHWGQGLTVTVSSASTKGPSVFPLAPC SRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPCCPPCP APEFLGGPSVFLFPPKPKDITLMISRTPPEVTCVVDVVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVVS NKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGN VFSCSVMEALHNHYTQKSLSLGLK*
97	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCACCATCACTTGCCGGGCAAGTCAGTATATTAGCACC TATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAGCCTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGT CTGCAACCTGAAGATTTTGCATCTTACTCCTGTCAACAGAGTTACAG TACCCCTCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA GCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACT TCTATCCCAGAGAGGGCCAAAGTACAGTGGAAGGTGGATAACGCCCT CCAATCGGGTAACTCCAGGAGAGTGTACACAGAGCAGGACAGCAA GGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGC AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCA GGGCCTGAGCTCGCCCCTCACAAAGAGCTTCAACAGGGGAGAGTG TTAG
98	PRT	DIQMTQSPSSLSASVGRVTITCRASQYISTYLNWYQKPGKAPSLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFASYSQQSYSTPPYTF GQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSPVTKSFNRGEC*
99	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGG GGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGTAG CTATGCCATGACCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTCTCAGCTATTAGTGGTAGTGATACTAGCACATACTACGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTTTCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACATCAAGATTACGATTTTCTCTACTACTA CTCCGCTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC TCA

100	PRT	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMTWVRQAPGKGLEW VSAISGSDTSTYYADSVKGRFTISRDN SKNTLFLQMNSLRAEDTAVYY CAKHQDYDFSYYYSAMDVWGQGTTVTVSS
101	DNA	GGATTCACCTTTAGTAGCTATGCC
102	PRT	GFTFSSYA
103	DNA	ATTAGTGGTAGTGATACTAGCACA
104	PRT	ISGSDTST
105	DNA	GCGAAACATCAAGATTACGATTTTTCCTACTACTACTCCGCTATGGA CGTC
106	PRT	AKHQDYDFSYYYSAMDV
107	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCATCATCACTTGTCTGGGCGAGTCAGGATTCAACAGC TGGTTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAACTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGATTCTACTCTCACCATCAGCAGC CTGCAGCCTGAAGATTTTGCGACTTACTATTGTCAGCAGACTGACA GTTTCCCATTCACTTTTGCCCTGGGACCAAAGTGGATATCAAA
108	PRT	DIQMTQSPSSLSASVGDRIITCRASQDFNSWLAWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFLTITSLQPEDFATYYCQQTDSFPFTF GPGTKVDIK
109	DNA	CAGGATTTCAACAGCTGG
110	PRT	QDFNSW
111	DNA	CAGCAGACTGACAGTTTCCCATTCCT
112	PRT	QQTDSFPFT
113	DNA	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGG GGGTCCCTGAGACTCTCTGTGCAGCCTCTGGATTACCTTTAGTAG CTATGCCATGACCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGGTCTCAGCTATTAGTGGTAGTGATACTAGCACATACTACGCAG ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTTTCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCC GTATATTACTGTGCGAAACATCAAGATTACGATTTTTCTACTACTA CTCCGCTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC TCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTC CAGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAA GGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTACAGGCGCC CTGACCAGCGCGTGCACACCTTCCCGGCTGTCCTACAGTCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTT GGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAA CACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGC CCACCCTGCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCC TGTTCCCCCCTCAACCAAGGACACTCTCATGATCTCCCGGACCCC TGAGGTACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGA GGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTG GTCAGCGTCCTACCGTCTGCAACAGGACTGGCTGAACGGCAAGG AGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCTCCATCGA GAAAACCATCTCCAAGGCCAAAGGGCAGCCCCGAGAGCCACAGGT GTACACCCTGCCCCATCCCAGGAGGAGATGACCAAGAACCAGGT CAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAG

		GCTCACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG TCCCTCTCCCTGTCTCTGGGTAAATGA
114	PRT	EVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMTWVRQAPGKGLEW VSAISGSDTSTYYADSVKGRFTISRDNKNTLFLQMNSLRAEDTAVYY CAKHQDYDFSYYYSAMDVWGQGTITVTVSSASTKGPSVFPLAPCSRST SESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLK*
115	DNA	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGG AGACAGAGTCATCATCACTTGTCTGGGCGAGTCAGGATTTC AACAGC TGGTTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAACTCC TGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGATTTCACCTCACCATCAGCAGC CTGCAGCCTGAAGATTTTGC GACTTACTATTGT CAGCAGATGACA GTTTCCCATTCACTTTTGGCCCTGGGACCAAAGTGGATATCAAACG AACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC AGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTC TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTC CAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAG GACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCA GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTT AG
116	PRT	DIQMTQSPSSLSASVGDRIITCRASQDFNSWLAWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQTDSFPFTF GPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSPVTKSFNRGEC*
117	PRT	GYKAEVMVSQVYSGSLRVLNRFHSQDLTRRESSAFRSETAKAQKMLK ELITSTRLGTYNSSSVYSFGEGPLTCFFWFILQIPEHRRMLLSPEVVQA LLVEELLSTVNSSAAVPYRAEYVDPEGLVILEASVKDIAALNSTLGCY RYSYVGQGQVLRKGPDLHLASSCLWHLQGPDLMLKLRLEWTLAEC RDRLAMYDVAGPLEKRLITSVYGCSRQEPVVEVLASGAIMAVVWKKG LHSYYDPFVLSVQPVVFQACEVNLTLDNRLDSQGVLSPTYFSPSYSPQ THCSWHLTVPSLDYGLALWFDAYALRRQKYDLPCTQGQWTIQNRRL CGLRILQPYAERIPVVATAGITINFTSQISLTGPGVRVHYGLYNQSDPCP GEFLCSVNGLCVPACDGVKDCPNGLDERNCVCRAFQCKEDSTCISLP KVCDGQPDCLNGSDEEQCEGVPCGTFTFQCEDRSCVKKPNPQCDGR PDCRDGSDEEHCDCLGQPSSRIVGGAVSSEGEWPWQASLQVRGRHI CGGALIADRWWITA AHCFQEDSMASVLTWTVFLGK VWQNSRWPGEV SFKVSRLLLHPYHEEDSHDYDVALQLDHPVVRSAAVRPVCLPARSHF FEPGLHCWITGWGALREGGPISNALQKVDVQLIPQDLCEVYRYQVTP RMLCAGYRKGGKDACQGDAGGPLVCKALSGRWFLAGLVSWGLGCG RPNYFGVYTRITGVISWQQVVTQKLISEEDLGGEQKLISEEDLHHHH HH

118	PRT	KAEVTVSQVYSGSLRVLNRHFSQDLGRRESIAFRSESAKAQKMLQELV ASTRLGTYYNSSSVYSFGEGPLTCFFWFILDIPEYQRLTLSPEVVRELLV DELLSNSSTLASYTEYEVDPPEGLVILEASVNDIVVLNSTLGCYRYSYV NPGQVLPLKGPDQQTTSCLWHLQGPEDLMIKVRLEWTRVDCRDRVA MYDAAGPLEKRLITSVYGCSRQEPVMEVLASGSVMVAVVWKKGMHSY YDPFLLSVKSVAFQDCQVNLTLLEGRLDTQGFLRTPYYPSPSYSPSTHCS WHLTVPSLDYGLALWFDAYALRRQKYNRLCTQGQWMIQNRRLCGFR TLQPYAERIPMVASDGVTFINFTSQISLTGPGVQVYYSLYNQSDPCPGEF LCSVNGLCVPACDGIKDCPNGLDERNCVCRAMFQCQEDSTCISLPRVC DRQPDCLNGSDEEQCQEGVPCGTFTFQCEDRSCVKKPNPECDGQSDC RDGSDEQHCDCLQGLSSRIVGGTVSSEGEWPWQASLQIRGRHICGA LIADRWVITAAHCFQEDSMASPKLWTVFLGKMRQNSRWPGEVSFKVS RLFLHPYHEEDSHDYDVALQLDHPVVYSATVRPVCLPARSHFFEPGQ HCWITGWGAQREGGPVSNTLQKVDVQLVPQDLCSEAYRYQVSPRML CAGYRKGGKDACQGDAGGPLVCREPSGRWFLAGLVSWGLGCGRPNF FGVYTRVTRVINWQQVLTQKLISEEDLGGEQKLISEEDLHHHHHH
119	PRT	GYKAEVTVSQVYSGSLRVLNRHFSQDLTRRESSAFRSETAKAQKMLK ELIASTRLGTYYNSSSVYSFGEGPLTCFFWFILQIPEHRRMLLSPEVVQA LLVEELLSTVNSSAAVPYRAEYEVDPPEGLVILEASVKDIAALNSTLGCY RYSYVGQGVRLRLKGPDLASSCLWHLQGPEDLMMLKRLLEWTLAEC RDRLAMVDVAGPLEKRLITSVYGCSRQEPVVEVLASGAIMAVVWKKG LHSYYDPFMLSQSVVFQACEVNLTLDDRLDSQGVLPSTPYFSPSYSPR THCSWHLTVPSLDYGLALWFDAYALRRQKYDLPCTQGQWTIQNRR CGLRILQPYAERIPVVATAGITINFTSQISLTGPGVRVHYGLYNQSDPCP GEFLCSVNGLCVPACDGVKDCPNGLDERNCVCRA TFQCQEDSTCISLL KVCDGQPDCLNGSDEERCQEGVPCGTFTFQCEDQSCVKKPNPQCDGR PDCRDGSDEQHCDCLQGPPSRIVGGA VSSEGEWPWQASLQVRGRHI CGGALIADRWVITAAHCFQEDSMASPALWTVFLGKVWQNSRWPGEV SFKVSRLLLHPYHEEDSHDYDVALQLDHPVVRSAAVRPVCLPARSHF FEPGLHCWITGWGALREGGPTSINALQKVDVQLIPQDLCSEAYRYQVTP RMLCAGYRKGGKDACQGDAGGPLVCKALSGRWFLAGLVSWGLGCG RPNYFGVYTRITGVIGWQQVVTQKLISEEDLGGEQKLISEEDLHHHH HH
120	PRT	SHFFEPGLHCW
121	PRT	LVSWGLGCGRPNYFGVYTRITGVISW
122	PRT	LITSTRLGT
123	PRT	GEWPWQASL
124	PRT	STVLWTVFLGKVWQNSRWPGEVSFK
125	PRT	ITGWGALREGGPISINALQKVD
126	PRT	MLLLFHSKRMPVAEAPQVAGGQGDGGDGEEAEPEGMFKACEDSKRK ARGYLRLVPLFVLLALLVLASAGVLLWYFLGYKAEVMVSQVYSGSLR VLNRHFSQDLTRRESSAFRSETAKAQKMLKELITSTRLGTYYNSSSVYS FGEGPLTCFFWFILQIPEHRRMLLSPEVVQALLVEELLSTVNSSAAVPY RAEYEVDPPEGLVILEASVKDIAALNSTLGCYRYSYVGQGVRLRLKGP DLASSCLWHLQGPDLMLKRLLEWTLAECRDRLAMVDVAGPLEKRLI TSVYGCSRQEPVVEVLASGAIMAVVWKKGLHSYYDPFVLSQPVVFQ ACEVNLTLDNRLDSQGVLPSTPYFSPSYSPQTHCSWHLTVPSLDYGLAL WFDAYALRRQKYDLPCTQGQWTIQNRRLCGLRILQPYAERIPVVATA GITINFTSQISLTGPGVRVHYGLYNQSDPCPGEFLCSVNGLCVPACDGV KDCPNGLDERNCVCRA TFQCKEDSTCISLPKVCDGQPDCLNGSDEEQC QEGVPCGTFTFQCEDRSCVKKPNPQCDGRPDGRDGSDEEHCDCLQG

		PSSRIVGGAVSSEGEWPWQASLQVRGRHICGGALIADRWVITA AHCFQ EDSMAS TVLWTVFLGK V WQNSRWPGEVSFKVSRLL LHPYHEEDSHD YDVALLQLDHPVVRSA A VRPVCLPARSHFFEPGLHCWITGWGALREG GPISNALQKVDVQLIPQDL CSEVYRYQVTPRMLCAGYRK GK K DACQG DSGGPLVCKALSGRWFLAGLVSWGLGCGRPNYFGVYTRITGVISW IQ QVVT
127	DNA	CTTGAGCCAGACCCAGTCCAGCTCTGGTGCCCTCTGGTGCGA GCTGACCTGAGATGCACTTCCCTCCTCTGTGAGCTGTCTCGGCACCC ACTTGCACTGACTGCCGCCTGATGTTGTTACTCTTCCACTCCAAAAG GATGCCCCGTGGCCGAGGCCCCCAGGTGGCTGGCGGGCAGGGGGA CGGAGGTGATGGCGAGGAAGCGGAGCCGGAGGGGATGTTCAAGGC CTGTGAGGACTCCAAGAGAAAAGCCCGGGGCTACCTCCGCCTGGTG CCCCTGTTTGTGCTGCTGGCCCTGCTCGTGCTGGCTTCGGCGGGGT GCTACTCTGGTATTTCTAGGGTACAAGGCGGAGGTGATGGTCAGC CAGGTGTACTCAGGCAGTCTGCGTGTACTCAATCGCCACTTCTCCCA GGATCTTACCCGCCGGGAATCTAGTGCCTTCCGCAGTGAAACCGCC AAAGCCCAGAAGATGCTCAAGGAGCTCATCACCAGACCCCGCTG GGAACCTACTACAACCTCCAGCTCCGTCTATTCTTTGGGAGGGAC CCCTCACCTGCTTCTTCTGGTTCATTCTCCAAATCCCCGAGCACCGC CGGCTGATGCTGAGCCCCGAGGTGGTGCAGGCACTGCTGGTGGAGG AGCTGCTGTCCACAGTCAACAGCTCGGCTGCCGTCCCTACAGGGC CGAGTACGAAGTGGACCCCGAGGGCCTAGTGATCCTGGAAGCCAG TGTGAAAGACATAGCTGCATTGAATTCCACGCTGGGTGTTACCGC TACAGCTACGTGGGCCAGGGCCAGGTCTCCGGCTGAAGGGGCCTG ACCACCTGGCCTCCAGCTGCCTGTGGCACCTGCAGGGCCCCAAGGA CCTCATGCTCAAACTCCGGCTGGAGTGGACGCTGGCAGAGTGCCGG GACCGACTGGCCATGTATGACGTGGCCGGGCCCCCTGGAGAAGAGG CTCATCACCTCGGTGTACGGCTGCAGCCGCCAGGAGCCCGTGGTGG AGGTTCTGGCGTCGGGGGCCATCATGGCGGTCTGTGGAAGAAGGG CCTGCACAGCTACTACGACCCCTTCGTGCTCTCCGTGCAGCCGGTG GTCTTCCAGGCCTGTGAAGTGAACCTGACGCTGACACAACAGGCTCG ACTCCAGGGCGTCTCTCAGCACCCCGTACTTCCCACTACTACTC GCCCCAAACCCACTGCTCCTGGCACCTCACGGTGGCCTCTCTGGAC TACGGCTTGGCCCTCTGGTTTGATGCCTATGCACTGAGGAGGCAGA AGTATGATTTGCCGTGCACCCAGGGCCAGTGGACGATCCAGA ACAGGAGGCTGTGTGGCTTGCATCCTGCAGCCCTACGCCGAGAG GATCCCCGTGGTGGCCACGGCCGGGATCACCATCAACTTCACCTCC CAGATCTCCCTCACCGGGCCCCGGTGTGCGGGTGCACTATGGCTTGT ACAACCAGTCGGACCCCTGCCCTGGAGAGTTCTCTGTTCTGTGAA TGGACTCTGTGTCCCTGCCTGTGATGGGGTCAAGGACTGCCCCAAC GGCCTGGATGAGAGAACTGCGTTTGCAGAGCCACATTCCAGTGCA AAGAGGACAGCACATGCATCTCACTGCCCAAGGTCTGTGATGGGCA GCCTGATTGTCTCAACGGCAGCGACGAAGAGCAGTGCCAGGAAGG GGTGCCATGTGGGACATTACCTTCCAGTGTGAGGACCGGAGCTGC GTGAAGAAGCCCAACCCGCA GTGTGATGGGCGGCCGACTGCAGG GACGGCTCGGATGAGGAGCACTGTGACTGTGGCCTCCAGGGCCCCCT CCAGCCGCATTGTTGGTGGAGCTGTGTCTCCGAGGGTGAGTGGCC ATGGCAGGCCAGCCTCCAGGTTCCGGGTCGACACATCTGTGGGGGG GCCCTCATCGCTGACCGCTGGGTGATAACAGCTGCCCACTGCTTCC AGGAGGACAGCATGGCCTCCACGGTGTGTGGACCGTGTTCTCTGGG CAAGGTGTGGCAGAACTCGCGCTGGCCTGGAGAGGTGTCCTTCAAG

		GTGAGCCGCCTGCTCCTGCACCCGTACCACGAAGAGGACAGCCATG ACTACGACGTGGCGCTGCTGCAGCTCGACCACCCGGTGGTGCGCTC GGCCGCCGTGCGCCCCGTCTGCCTGCCCGCGCGCTCCCACTTCTTCG AGCCCGGCCTGCACTGCTGGATTACGGGCTGGGGCGCCTTGCGCGA GGGCGGCCCCATCAGCAACGCTCTGCAGAAAGTGGATGTGCAGTTG ATCCACAGGACCTGTGCAGCGAGGTCTATCGCTACCAGGTGACGC CACGCATGCTGTGTGCCGGCTACCGCAAGGGCAAGAAGGATGCCTG TCAGGGTGACTCAGGTGGTCCGCTGGTGTGCAAGGCACTCAGTGGC CGCTGGTTCCTGGCGGGGCTGGTCAGCTGGGGCCTGGGCTGTGGCC GGCCTAACTACTTCGGCGTCTACACCCGCATCACAGGTGTGATCAG CTGGATCCAGCAAGTGGTGACCTGAGGAACTGCCCCCCTGCAAAGC AGGGCCCACCTCCTGGACTCAGAGAGCCCAGGGCAACTGCCAAGC AGGGGGACAAGTATTCTGGCGGGGGGTGGGGGAGAGAGCAGGCCC TGTGGTGGCAGGAGGTGGCATCTTGTCTCGTCCCTGATGTCTGCTCC AGTGATGGCAGGAGGATGGAGAAGTGCCAGCAGCTGGGGGTCAAG ACGTCCCCTGAGGACCCAGGCCCACACCCAGCCCTTCTGCCTCCCA ATTCTCTCTCCTCCGTCCCCTTCCCTCACTGCTGCCTAATGCAAGGC AGTGGCTCAGCAGCAAGAATGCTG GTTCTACATCCCGAGGAGTGTCTGAGGTGCGCCCCACTCTGTACAG AGGCTGTTTGGGCAGCCTTGCCTCCAGAGAGCAGATTCCAGCTTCG GAAGCCCCTGGTCTAACTTGGGATCTGGGAATGGAAGGTGCTCCCA TCGGAGGGGACCCTCAGAGCCCTGGAGACTGCCAGGTGGGCCTGCT GCCACTGTAAGCCAAAAGGTGGGGAAGTCTGACTCCAGGGTCCTT GCCCCACCCCTGCCTGCCACCTGGGCCCTCACAGCCAGACCCTCA CTGGGAGGTGAGCTCAGCTGCCCTTTGGAATAAAGCTGCCTGATCC AAAAAAAAAAAAAAAAAAAA
128	PRT	MPVAKAPQVAGGQGDGGDGEEAEPEGMFEACEDSKRKARGYLRLAP LWLTLVVLTsvGVLLWYFLGYKAEVTVSqvYSGSLRVLNRHFSQDLT RRESSAFRSETAKAQKMLKELIASTRLGTYNsssvYSFGEGLTCFFW FILQIPEHRRMLLSPEVVQALLVEELLSTVNSSAAVPYRAEYEVDPGL VILEASVKDIAALNSTLGCYRYSYVGQGVRLKGPDLHLASSCLWHL QGPEDLMLKLRLLEWTLAECDRLAMYDVAGPLEKRLITSVYGCSRQE PVVEVLASGAIMAVVWKKGLHSYYPFMLSvQSVVFQACEVNLTLTD DRLDSQGVLTSPYFSPSYSPRTHCSWHLTVPSLDYGLALWFDAYALR RQKYDLPCTQGQWTIQNRRLCGLRILQPYAERIPVVATAGITINFTSQIS LTGPGVRVHYGLYNQSDPCPGEFLCSVNGLCVPACDGVKDCPNGLDE RNCVCRA TFQCQEDSTCISLLKVC DGPDC LNGSDEERCQEGVPCGTF TFQCEDQSCVKKPNPQC DGRPD CRDGSDEQHCDGLQGPSSRIVGGA VSSEGEWPWQASLQVRGRHICGGALIA DRWVITAAHCFQEDSMASPA LWTVFLGKVWQNSRWPGEVSFKVSRLLLHPYHEEDSHDYDVALLQL DHPVVRSAAVRPVCLPARSHFFEPGLHCWITGWGALREGGPTSNA LQ KVDVQLIPQDLCSEAYRYQVTPRMLCAGYRK GKKDACQGDSSGGLV CKALSGRWFLAGLVSWGLGCGRPNYFGVYTRITGVIGWIIQQVVT
129	DNA	CTCAGTCACAGTGCCGTCCTGGGCCCAGCAGGAGGAGCCCCATGGA GCCTGGGGCCACAGGCCACAGGGGACAAGGGCCAGACACCCTGGC CATGGCTCTAGGCCATTGATCCAGGCCGGGCTGGCACGGTGGGGGT AGGGAGGCCTTGGCCTGGACAAACAAAGGCTTCTGAGGCCTGCGTG CAGGCCCAGCACCTATCCGCCACTCCCAAAGGATGCCTGTGGCCAA GGCCCCCAGGTGGCTGGTGGGCAGGGGGACGGAGGTGATGGCGA GGAAGCGGAGCCAGAGGGGATGTTTCGAGGCCTGTGAGGACTCCAA GAGAAAAGCCCGGGGCTACCTCCGCCTGGCGCCCCCTGTGGCTGACC

	CTGGTTGTGCTGACTTCAGTGGGGGTGCTACTCTGGTATTTCTAGG GTACAAGGCGGAGGTGACGGTCAGCCAGGTGTACTCAGGCAGCCTT CGCGTGCTCAATCGCCACTTCTCCCAGGATCTTACCCGCCGGAAT CCAGTGCCTTCCGCAGTGAAACCGCCAAAGCCCAGAAGATGCTCAA GGAGCTCATCGCCAGCACCCGCTGGGAACTTATTACAACCTCCAGC TCCGTCTATTCTTTGGGGAGGGACCGCTCACCTGCTTCTTCTGGTT CATTCTCCAAATCCCCGAGCACCGCCGGCTGATGCTGAGCCCCGAG GTGGTGCAGGCACTGCTGGTGGAGGAGCTGCTGTCCACAGTCAACA GCTCGGCGGCCGTCCCCTACAGGGCCGAGTACGAAGTGGACCCCG AGGGCCTAGTGATCCTAGAAGCCAGTGTGAAAGACATAGCTGCACT GAATTCCACGCTGGGTTGTTACCGCTACAGCTACGTGGGCCAGGGT CAGGTCTCCGCTGAAGGGACCCGACCACCTGGCCTCCAGCTGCC TGTGGCACCTGCAGGGCCCCGAAGACCTCATGCTGAAACTCCGGCT GGAGTGGACGCTGGCCGAGTGCCGGGACCGACTGGCCATGTATGA CGTGGCTGGGCCCCCTGGAGAAGAGGCTCATCACCTCGGTGTATGGC TGCAGCCGCCAGGAGCCTGTGGTGGAGTCTTGGCATCGGGGGCCA TCATGGCGGTGGTCTGGAAGAAGGGCCTGCACAGCTACTACGACCC CTTATGCTCTCCGTGCAGTCGGTGGTCTTCCAGGCTGCGAGTTAA ACCTGACGCTGGATGACAGGCTGGACTCCCAGGGCGTCTCAGCAC CCCGTACTTCCCCAGCTACTACTCGCCCCGAACCCACTGCTCCTGGC ACCTCACGGTGCCTTCTCTGGACTACGGCTTGGCCCTCTGGTT TGACGCCTACGCACTGCGGAGGCAGAAAGTATGATTTGCCGTGCACC CAGGGCCAGTGGACGATCCAGAACAGGAGGCTGTGTGGCCTGCGC ATCCTGCAGCCTTACGCTGAGAGGATCCCCGTGGTGGCCACGGCCG GCATCACCATCAATTTACCTCCCAGATCTCCCTCACAGGGCCTGGT GTGCGGGTGCATATGGCTTGTACAACCAAGTCGGACCCCTGCCCTG GAGAGTTCCTCTGCTCTGTGAACGGAAGTCTGCGTCCCTGCCTGTGAT GGGGTCAAGGACTGCCCCAACGGCCTGGATGAGAGAACTGCGTTT GCAGAGCCACATTCCAGTGCCAAGAGGACAGCACGTGCATCTCACT GCTTAAGGTCTGTGACGGGCAGCCTGACTGTCTCAACGGCAGCGAT GAAGAGCGGTGCCAGGAAGGGGTGCCCTGCGGGACATTACCTTCC AGTGTGAGGACCAGAGCTGCGTGAAGAAGCCCAACCCACAGTGTG ATGGGCGGCCCGACTGCAGGGACGGCTCAGACGAGCAGCACTGTG ACTGTGGCCTCCAGGGCCCCCTCCAGTCGCATTGTTGGTGGGGCCGT GTCCTCCGAGGGTGAGTGGCCATGGCAGGCCAGCCTCCAGGTTCCG GGTCGACACATCTGTGGGGGCGCCCTCATCGCTGACCGCTGGGTGA TAACAGCTGCCCATTGCTTCCAGGAGGACAGCATGGCCTCCCCGGC GCTGTGGACGGTGTTCTTGGGCAAGGTGTGGCAGAACTCGCGCTGG CCTGGAGAGGTGTCCTTCAAGGTGAGCCGCCTACTCCTGCATCCGT ATCACGAAGAGGACAGCCACGACTACGACGTGGCGCTGTTGCAGCT CGACCACCCGGTGGTGCCTCGGCCGCCGTGCGTCCAGTCTGCCTG CCCGCGCGCTCCCACTTCTTCGAACCCGGCCTGCACTGCTGGATCA CTGGCTGGGGCGCCCTGCGCGAAGGCGGCCCCACCAGCAATGCTCT GCAGAAAGTGGACGTGCAGTTGATCCCACAGGACCTGTGCAGCGA GGCCTATCGCTACCAGGTGACGCCACGCATGCTGTGTGCCGGCTAC CGCAAGGGCAAGAAGGATGCCAGGGTGACTCGGGTGGTCCG CTGGTATGCAAGGCACTCAGTGGCCGCTGGTTCCTGGCAGGGCTGG TCAGCTGGGGCCTGGGCTGTGGCCGACCTAACTACTTCCGGCTCTA CACCCGCATCACAGGTGTGATCGGCTGGATCCAGCAAGTGGTGACC TGAGGAACTGCCCCCTGCAGAGCAGGTCCACCTCTTGACTCAG AGAGCCCAGGGCAATTGCCAAGCAGGGGGACAAGTATTCTGGGGG
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		GAGGGGGGCGCGAGCAGGCCCTGTGGTGGCAGGAGGTGGCATCTT GTCTTGTCCTGATGTCTGCTCCAGTGATGGCAGGAGGATGGAGGA GTGCCAGCAGCTGGGGGTCAAGACGTCCCCTGGGGACCCAGGCC ACACCCAGCCCTTCTGCCTCCCGATTCTCTCTCCTCCGTCCTTCTCCT CCACTGCTGCCTATTGCAAGGAAGTGGCTCAGCAGCAAGAATGCTG GCTCTACGTCCCCAGGAGTGTCTGAGCTGTGCCCCGCTCTGTACAG AGGCTGCTTGGGCAGCCTTGCCTCTAGAGAGCAGATGCCAGCTTCG GAAGCCCCTGGTCTAACTTGGGATCTGGGAATGGAAGGTGCCCCCA TAGGAGGGGACCCTCACAGCCCTGGGGACTGCCAGGTGGGCCGGC TGCCACCGTAAGCCAAAAAAGGTGGGGAAGCCCTGACTCCAAGGT CCTTGCCCCACCCCTGCCTGCCACCTGGCCCCCTCACAGCCAGACC CTCACCGGCAGGTGAGCTCAGCTGCCCTTTGGAATAAAGCTGCCTG ATCCAA
130	PRT	MPRCFQLPCSTRMPTTEVPQAADGQGDAGDGEEAAEPEGKFKPPKNT KRKNRDYVRFTPLLLVLAALVSAGVMLWYFLGYKAEVTVSQVYSGS LRVLNRHFSQDLGRRESIAFRSESAKAQKMLQELVASTRLGTYYNSSS VYSFGEGLTCFFWFILDIPEYQRLTSPVRELLVDELLSNSSTLAS KTEYEVDPGLVILEASVNDIVVLNSTLGCYRYSYVNPQVLPKGPD QQTTSCLWHLQGPEDLMIKVRLEWTRVDCRDRVAMYDAAGPLEKRLI TSVYGCSRQEPVMEVLASGSVMAVVWKKGMHSYDPLLSVKSVA QDCQVNLTLLEGRDTPYPSYSPSTHCSWHLTVPSLDYGLA LWFDAYALRRQKYNRLCTQGQWMIQNRRLCGFRTLQPYAERIPMAS DGVTFINFTSQISLTGPGVQVYYSLYNQSDPCPGEFLCSVNLGCV PACDGIKDCPNGLDERNCVCRAFMQCQEDSTCISLPRVCDRQPDCL NGSDEEQCQEGVPCGTFTFQCEDRSCVKKNPECDGQSDCRDGSDE QHCDCGLQLSSRIVGGTVSSEGEWPWQASLQIRGRHICGGALIA DRWVITAAHCFQEDSMASPKLWTVFLGKMRQNSRWPGEVSFKVS RFLHPYHEEDSHDYDVALQLDHPVVYSATVRPVCLPARSHFFEP GQHCWITGWGAQREGGPVSNTLQKVDVQLVPQDLCSEAYRYQV SPRMLCAGYRKGGKDACQGDSGGPLVCREPSGRWFLAGLVSWGL GCGRPNFFGVYTRVTRVINWQQVLT
131	DNA	AGTTTCATTGTCGCCCTGGACCTGACAGGAGAGGCCCATGGAAC TTGGGGCCACAGGCCACAAGGGACAAGGGCCAGACACCCAGCCAT GCTCCAGGCCATTGATCCAACCTAAGCTGGCCAGTTGGGGGTGGAA AGACCTTGGCCTGGATAAACAGAGGCCTCCAGGCCTGTGTGCAGGC CCGGCACCTACCTTCCACTCTTGAAGATGCCGAGATGTTTCCAGCTC CCCTGTTCTACCAGGATGCCACCACCGAGGTCCCCCAAGCGGCTG ATGGTCAGGGCGATGCGGGTGATGGAGAGGAAGCTGCTGAGCCAG AGGGGAAGTTCAAGCCCCCAAAAAACACCAAGAGAAAAAACCGG GACTACGTCCGCTTCACGCCACTGTTGCTGGTCTTGGCTGCGCTGG TCTCAGCAGGGGTCATGCTTTGGTATTTCTAGGGTACAAAGCGGAAG TGACCGTAAGCCAGGTGTACTCTGGCAGCCTCCGGGTGCTCAACCG TCATTTCTCCCAGGACCTGGGCCGACGGGAGTCTATTGCTTTCCGCA GTGAATCTGCCAAAGCCCAGAAGATGCTCCAAGAACTGGTTGCCAG CACCCGCCTGGGTACTTACTACAACCTCTAGTTCTGTCTACTCTTTG GGGAGGGACCCCTCACCTGCTTCTTCTGGTTTATCCTTGACATCCCT GAGTACCAGCGACTGACCCTGAGCCCTGAAGTAGTGCGCGAGCTCC TGGTGGATGAGCTACTGTCCAACAGCTCAACCCTGGCTTCCTATAA GACCGAATATGAGGTGGACCCGGAAGGCCTGGTGATCCTGGAAGC CAGTGTGAACGACATAGTCGTAAGTGAATTCACGCTGGGCTGTTAT CGCTACAGCTATGTGAACCCAGGCCAGGTCTCTCCATTGAAGGGGC

	CTGACCAGCAGACCACAAGCTGCCTGTGGCATCTGCAAGGGCCCCGA AGACCTCATGATCAAAGTGCGGCTGGAGTGGACCCGGGTCGATTGC AGAGACAGGGTGGCGATGTACGACGCAGCTGGGCCCCTGGAGAAG AGACTTATCACCTCGGTCTATGGGTGCAGCCGCCAGGAACCTGTGA TGGAGGTGCTGGCATCGGGCTCCGTCATGGCCGTGGTGTGAAAAA GGGCATGCATAGCTACTATGACCCTTTCCTGCTCTCAGTGAAGTCTG TGGCCTTCCAGGACTGCCAGGTGAACCTGACACTGGAGGGCCGGCT GGACACACAGGGCTTCCCTCCGTACACCCTACTACCCAGTTACTAC TCTCCCAGTACCCACTGCTCCTGGCATCTCACGGTACCCTCTCTGGA CTACGGCTTGGCGCTCTGGTTCGATGCCTACGCACTGAGGAGGCAG AAGTACAACCGACTGTGTACTCAGGGCCAGTGGATGATCCAGAACA GGAGGCTGTGTGGCTTCCGTACCCTGCAGCCATATGCTGAGAGGAT CCCCATGGTGGCCTCAGATGGTGTACCATCAACTTCACCTCCCAG ATCTCCCTCACAGGCCCCGGGTGTGCAAGTGTACTACAGCTTGTACA ACCAATCAGACCCCTGCCCTGGTGAGTTCCCTCTGCTCTGTGAAT GGACTGTGTGTCCCTGCGTGTGACGGGATCAAGGACTGCCCAATG GCCTGGATGAGAGAACTGTGTCTGCAGAGCCATGTTCCAGTGCCA AGAGGACAGCACGTGCATTTCACTGCCTAGAGTCTGTGACCGGACG CCCGACTGTCTCAATGGCAGTGACGAAGAACAGTGCCAAGAAGGA GTGCCCTGTGGGACATTCACTTTCAGTGTGAGGACCGGAGCTGTG TGAAGAAGCCCAACCCAGAGTGTGACGGCCAGTCAGATTGCAGAG ACGGCTCAGATGAGCAACACTGTGACTGTGGCCTCCAGGGCCTCTC CAGCCGTATTGTGGGCGGGACCGTGTCTCCGAGGGTGAGTGGCCA TGGCAGGCCAGCCTCCAGATTCGGGGTCGACACATCTGTGGGGGGG CTCTCATCGCTGACCGCTGGGTCATAACGGCCGCCCACTGCTTCCA GGAGGACAGCATGGCCTCCCCGAAGCTGTGGACCGTGTTCCTGGGA AAGATGCGGCAGAACTCGCGCTGGCCAGGCGAGGTGTCTTCAAG GTGAGCCGTCTGTTCCCTGCACCCGTACCACGAGGAGGACAGCCATG ACTACGACGTGGCCCTGCTGCAGCTCGACCACCCCGTGGTGTACTC GGCCACTGTGCGCCCCGTCTGCCTGCCTGCCCGTCCCACTTCTTTG AGCCAGGCCAGCACTGCTGGATCACAGGCTGGGGAGCCAGCGAG AGGGTGGTCCGGTGAGCAACACCCTGCAGAAGGTGGACGTACAGC TGGTCCCTCAGGACCTCTGCAGTGAGGCCTACCGCTACCAGGTGTC CCCACGCATGCTCTGTGCTGGCTACCGCAAGGGCAAGAAAGATGCC TGCCAGGGTGACTCTGGAGGCCCACTGGTTTGCAGGGAGCCAGTG GCCGCTGGTTCCTGGCAGGGTTGGTTAGCTGGGGCCTGGGCTGTGG CCGACCCAATTTCTTTGGCGTCTACACCCGTGTACACGTGTGATCA ACTGGATCCAGCAGGTGCTGACCTGAGGGCTGTTCTACAGAGCTGG ACCTGCCTCCAGGCCAAGTTCAGGGTGTCCACCCAGCCAGGACACA AGTATTCTGGGGCAAGTGACCCTGCTAAGGCCTGTTTCCCTCAGGC CTACCCCACTGACAGTACAGAGAAGGATGTGAGCTGGTGGTTAGGA TGCCTCCTGAGGTCCAGGGGCCAGCCTCGGCTAGGTTTCACTTCTA ACCTTTTCTTATTCTAGTCCTTTCCCTCCCTGCTCCTACCACTGTTT TGGAGTGGGGTCTGGCGGCCATGACCTTGGCCTCCGGGTCTCTGTA GGAAAGAAAGAATCCTTCCCCTTGCAAAAGCCTCTTGGGGGAACTG CACAGAGAAAGAAGGTGCCTCTATCAAGGCTCTATCAGAGCCCTTG AGTCTGCCAAGTGGGCTGTACTCTAAGCCAAATCACCGGGCAGCCT CAGCTGCAGATGCCTGCTGAAGCTCTGCCTGCTACAGGGGCCTCCC TGCCATTCACTGGAGGCCCACTGTCTGTTCTGGGAATAAAGCACTT GACCAAGCCCTGACACTGAAAAAAAAAAAAAAAAA
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CLAIMS

What is claimed is:

1. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof binds to the catalytic domain of TMPRSS6 but does not bind to the catalytic triad of TMPRSS6.
2. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment thereof further binds at a site comprised within the LDLRa domain 2 of TMPRSS6.
3. The isolated antibody or antigen-binding fragment thereof of claim 1 or 2, wherein binding of the antibody or antigen-binding fragment thereof to TMPRSS6 does not directly occlude the catalytic triad of TMPRSS6.
4. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment thereof to TMPRSS6 mediates allosteric regulation of TMPRSS6.
5. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein binding of the antibody or antigen-binding fragment thereof to TMPRSS6 mediates allosteric inhibition of TMPRSS6.
6. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof is an allosteric inhibitor of TMPRSS6.
7. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment maintains TMPRSS6 in an inactive conformation that is capable of binding to but not cleaving a TMPRSS6 substrate.

8. The isolated antibody or antigen-binding fragment thereof of claim 7, wherein the TMPRSS6 substrate is hemojuvelin (HJV).

9. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment binds within the catalytic domain of TMPRSS6 at a site that sterically occludes the catalytic triad of TMPRSS6 .

10. The isolated antibody or antigen-binding fragment thereof of claim 9, wherein the antibody or antigen-binding fragment thereof competes with hemojuvelin (HHV) for binding to TMPRSS6.

11. An isolated antibody or antigen-binding fragment thereof that binds specifically to human Transmembrane Protease Serine 6 (hTMPRSS6) protein (SEQ ID NO:126), wherein the antibody or antigen-binding fragment thereof interacts with one or more amino acids contained within the extracellular domain of hTMPRSS6, as determined by hydrogen/deuterium exchange.

12. The isolated antibody or antigen-binding fragment thereof of claim 11, wherein the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 125 to 133 of SEQ ID NO: 126; (b) amino acids 586-594 of SEQ ID NO: 126; (c) amino acids 626 to 650 of SEQ ID NO: 126; (d) amino acids 693 to 703 of SEQ ID NO: 126; (e) amino acids 704 to 724 of SEQ ID NO: 126; and/or (f) amino acids 780 to 805 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange.

13. The isolated antibody or antigen-binding fragment thereof of claim 12, wherein the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 693 to 703 of SEQ ID NO: 126; and/or (b) amino acids 780 to 805 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange.

14. The isolated antibody or antigen-binding fragment thereof of claim 12, wherein the antibody or antigen-binding fragment thereof interacts with an amino acid sequence selected from: (a) amino acids 125 to 133 of SEQ ID NO: 126; (b) amino acids 586-594 of SEQ ID NO: 126; (c) amino acids 626 to 650 of SEQ ID NO: 126; and/or (d) amino acids 704 to 724 of SEQ ID NO: 126, as determined by hydrogen/deuterium exchange.

15. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof interacts with residues G699, H701, D724, Q726, L727, I728, P729, L732, E735, G749, Y750, R751, K752, and N791 of human TMPRSS6 (SEQ ID NO:126), as determined by cryoelectron microscopy structural modeling.

16. The antibody or antigen-binding fragment thereof of claim 15, wherein the antibody or antigen-binding fragment thereof further interacts with residues V490, S501, T502, C503, I504, S505, and K508 of human TMPRSS6 (SEQ ID NO:126), as determined by cryoelectron microscopy structural modeling.

17. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof interacts with residues R597, R599, I601, D622, L710, R711, E712, G713, G714, P715, and I716 of human TMPRSS6, as determined by cryoelectron microscopy structural modeling.

18. An isolated antibody or antigen-binding fragment thereof that binds specifically to Transmembrane Protease Serine 6 (TMPRSS6) protein, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR); and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR), wherein the HCDR3 has an amino acid sequence selected from a HCDR3 sequence listed in Table 1.

19. The antibody or antigen-binding fragment thereof of claim 18 wherein the LCDR3 sequence has an amino acid sequence selected from a LCDR3 sequence listed in Table 1.

20. The antibody or antigen binding fragment of claim 18 or 19, which comprises a HCDR1, HCDR2, and HCDR3 sequence listed in Table 1.

21. The antibody or antigen binding fragment of any of claims 18-20, which comprising a LCDR1, LCDR2, and LCDR3 sequence listed in Table 1.

22. The antibody or antigen-binding fragment thereof of any of claim 18-21, wherein:

- (a) the HCDR1 domain has an amino acid sequence selected from SEQ ID NOs: 4, 24, 34, 44, 64, 84, or 102;
- (b) the HCDR2 domain has an amino acid sequence selected from SEQ ID NOs: 6, 26, 36, 46, 66, 86, or 104;
- (c) the HCDR3 domain has an amino acid sequence selected from SEQ ID NOs: 8, 28, 38, 48, 68, 88, or 106;
- (d) the LCDR1 domain has an amino acid sequence selected from SEQ ID NOs: 12, 52, 72, 92, or 110;
- (e) the LCDR2 domain has an amino acid sequence selected from SEQ ID NOs: 14, 54, or 74; and
- (f) the LCDR3 domain has an amino acid sequence selected from SEQ ID NOs: 16, 56, 76, 94, or 112.

23. The antibody or antigen-binding fragment thereof of any one of claims 18-22 wherein the three heavy chain CDRs and three light chain CDRs comprise one of the following sets of CDRs:

- (a) SEQ ID NO: 4 (HCDR1), SEQ ID NO:6 (HCDR2), SEQ ID NO:8 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3);
- (b) SEQ ID NO: 24 (HCDR1), SEQ ID NO:26 (HCDR2), SEQ ID NO:28 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3);
- (c) SEQ ID NO: 34 (HCDR1), SEQ ID NO:36 (HCDR2), SEQ ID NO:38 (HCDR3), SEQ ID NO:12 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:16 (LCDR3);
- (d) SEQ ID NO: 44 (HCDR1), SEQ ID NO:46 (HCDR2), SEQ ID NO:48 (HCDR3), SEQ ID NO:52 (LCDR1), SEQ ID NO:54 (LCDR2), and SEQ ID NO:56 (LCDR3);
- (e) SEQ ID NO: 64 (HCDR1), SEQ ID NO:66 (HCDR2), SEQ ID NO:68 (HCDR3), SEQ ID NO:72 (LCDR1), SEQ ID NO:74 (LCDR2), and SEQ ID NO:76 (LCDR3);
- (f) SEQ ID NO: 84 (HCDR1), SEQ ID NO:86 (HCDR2), SEQ ID NO:88 (HCDR3), SEQ ID NO:92 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:94 (LCDR3); or
- (g) SEQ ID NO: 102 (HCDR1), SEQ ID NO:104 (HCDR2), SEQ ID NO:106 (HCDR3), SEQ ID NO:110 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:112 (LCDR3).

24. The antibody or antigen-binding fragment thereof of any one of claims 18-23 wherein the three heavy chain CDRs and three light chain CDRs comprise the following set of CDRs:

SEQ ID NO: 44 (HCDR1), SEQ ID NO:46 (HCDR2), SEQ ID NO:48 (HCDR3), SEQ ID NO:52 (LCDR1), SEQ ID NO:54 (LCDR2), and SEQ ID NO:56 (LCDR3).

25. The antibody or antigen-binding fragment thereof of any one of claims 18-23 wherein the three heavy chain CDRs and three light chain CDRs comprise the following set of CDRs:

SEQ ID NO: 102 (HCDR1), SEQ ID NO:104 (HCDR2), SEQ ID NO:106 (HCDR3), SEQ ID NO:110 (LCDR1), SEQ ID NO:14 (LCDR2), and SEQ ID NO:112 (LCDR3).

26. An antibody or antigen-binding fragment thereof that binds to TMPRSS6, wherein the antibody or antigen-binding fragment thereof comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within a HCVR and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within a LCVR; wherein the HCVR comprises:

- (i) an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100;
- (ii) an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100;
- (iii) an amino acid sequence having at least 95% identity to an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100; or
- (iv) an amino acid sequence having no more than 12 amino acid substitutions with respect to a sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100.

27. The antibody or antigen-binding fragment of claim 26, wherein the LCVR comprises:

- (a) an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108;
- (b) an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108;
- (c) an amino acid sequence having at least 95% identity to an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108; or
- (d) an amino acid sequence having no more than 10 amino acid substitutions with respect to a sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108.

28. The antibody or antigen-binding fragment thereof of claim 26 or 27, comprising a HCVR having an amino acid sequence selected from SEQ ID NOs: 2, 22, 32, 42, 62, 82, or 100.

29. The antibody or antigen-binding fragment thereof of any of claims 26-28, comprising a LCVR having an amino acid sequence selected from SEQ ID NOs: 10, 50, 70, 90, or 108.

30. The antibody or antigen-binding fragment thereof of any one of claims 26-29 comprising a HCVR/LCVR amino acid sequence pair selected from SEQ ID NOs: 2/10, 22/10, 32/10, 42/50, 62/70, 82/90, or 100/108.

31. The antibody or antigen-binding fragment thereof of claim 30 comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 42/50.

32. The antibody or antigen-binding fragment thereof claim 30 comprising the HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 100/108.

33. An antibody that binds to TMPRSS6, wherein the antibody comprises a heavy chain (HC) and a light chain (LC), wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 30, 40, 58, 78, 96 and 114.

34. The antibody of claim 33, wherein the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 60, 80, 98 and 116.

35. The antibody of claim 33 or 34 comprising a HC/LC amino acid sequence pair selected from SEQ ID NOs: 18/20, 30/20, 40/20, 58/60, 78/80, 96/98 or 114/116.

36. The antibody of claim 35, comprising the HC/LC amino acid sequence pair of 58/60.

37. The antibody of claim 35, comprising the HC/LC amino acid sequence pair of 114/116.

38. The antibody or antigen-binding fragment thereof of any one of claims 1-37, wherein

the antibody has one or more properties selected from:

- (a) is a fully human monoclonal antibody;
- (b) binds to human TMPRSS6 at 25°C and at 37°C with a dissociation constant (K_D) of less than about 21.2 nM, as measured in a surface plasmon resonance assay;
- (c) binds to monkey TMPRSS6 at 25°C and at 37°C with a K_D of less than about 25.7 nM, as measured in a surface plasmon resonance assay;
- (d) binds to mouse TMPRSS6 at 25°C and at 37°C with a K_D of less than about 703 nM, as measured in a surface plasmon resonance assay;
- (e) binds to cells expressing human TMPRSS6 with EC_{50} less than about 2.7 nM;
- (f) binds to cells expressing monkey TMPRSS6 with EC_{50} less than about 3.6 nM;
- (g) binds to cells expressing mouse TMPRSS6 with EC_{50} less than about 35 nM;
- (h) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6, with percent inhibition greater than about 90%;
- (i) inhibits protease-dependent release of cell surface hemojuvelin in the presence of human TMPRSS6, e.g., inhibits human TMPRSS6 and exhibits an IC_{50} less than about 200 pM;
- (j) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6, with percent inhibition greater than about 42%;
- (k) inhibits protease-dependent release of cell surface hemojuvelin in the presence of mouse TMPRSS6, e.g., inhibits mouse TMPRSS6 and exhibits an IC_{50} less than about 274 pM;
- (l) inhibits human TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 65%;
- (m) inhibits human TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 10 nM;
- (n) inhibits monkey TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 66%;
- (o) inhibits monkey TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 50 nM;
- (p) inhibits mouse TMPRSS6 at 25°C and at 37°C with percent inhibition greater than about 58%;
- (q) inhibits mouse TMPRSS6 at 25°C and at 37°C and exhibits an IC_{50} less than about 35 nM;
- (r) when administered to a subject in need thereof, decreases serum iron levels of the

subject;

(s) when administered to a subject in need thereof, increases serum hepcidin levels of the subject;

(t) when administered to a subject in need thereof, increases mature red blood cell levels of the subject;

(u) when administered to a subject in need thereof, increases mature red blood cell levels in the spleen and/or bone marrow of the subject;

(v) when administered to a subject in need thereof, increases hemoglobin levels of the subject; or

(w) when administered to a subject in need thereof, decreases transferrin saturation levels of the subject.

39. The antibody or antigen-binding fragment thereof of any one of claims 1-38, wherein the antibody is a fully human monoclonal antibody.

40. An antibody or antigen-binding fragment thereof that competes for binding to TMPRSS6 with an antibody or antigen-binding fragment thereof of any one of claims 1-38.

41. An antibody or antigen-binding fragment thereof that binds to the same epitope as an antibody or antigen-binding fragment thereof of any one of claims 1-38.

42. A pharmaceutical composition comprising an isolated antibody or antigen-binding fragment thereof that binds to TMPRSS6 according to any one of claims 1-41 and a pharmaceutically acceptable carrier or diluent.

43. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a HCVR of an antibody or antigen-binding fragment thereof as set forth in any one of claims 1-41.

44. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes a LCVR of an antibody or antigen-binding fragment thereof as set forth in any one of claims 1-41.

45. A vector comprising the polynucleotide sequence of claim 43 and/or the polynucleotide sequence of claim 44.

46. A set of vectors comprising a first vector comprising the polynucleotide of claim 43 and a second vector comprising the polynucleotide of claim 44.

47. A host cell comprising the vector of claim 45 or the set of vectors of claim 46.

48. A method of producing an antibody or antigen-binding fragment thereof that binds specifically to TMPRSS6, comprising culturing the host cell of claim 47 under conditions permitting production of the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof so produced.

49. The method of claim 48, further comprising formulating the antibody or antigen-binding fragment thereof as a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

50. A method of treating, preventing, or ameliorating at least one symptom or indication of a disease or disorder associated with iron overload, the method comprising administering a pharmaceutical composition comprising a therapeutically effective amount of an antibody or antigen-binding fragment thereof of any one of claims 1-41 to a subject in need thereof.

51. The method of claim 50, wherein disease or disorder is a congenital dyserythropoietic anemia, Diamond-Blackfan anemia, alpha thalassemia, beta thalassemia, a transfusion-dependent hemolytic anemia, myelodysplastic syndrome, sickle cell disease, polycythemia vera, hereditary hemochromatosis, or chronic liver disease.

52. The method of claim 51, wherein the disease or disorder is beta thalassemia.

53. The method of claim 52, wherein the disease or disorder is beta thalassemia major or beta thalassemia intermedia.

54. The method of claim 51, wherein the disease or disorder is myelodysplastic syndrome.

55. The method of claim 54, wherein the disease or disorder is myelodysplastic syndrome with ring sideroblasts.

56. The method of claim 51, wherein the disease or disorder is a transfusion-dependent hemolytic anemia.

57. The method of claim 56, wherein the disease or disorder is a transfusion-dependent hemolytic anemia due to pyruvate kinase deficiency, or a sideroblastic transfusion-dependent hemolytic anemia.

58. The method of claim 51, wherein the disease or disorder is a chronic liver disease.

59. The method of claim 58, wherein the disease or disorder is an alcohol related chronic liver disease, hepatitis C, or autoimmune hepatitis.

60. The method of any one of claims 50-59, wherein the pharmaceutical composition is administered prophylactically or therapeutically to the subject in need thereof.

61. The method of any one of claims 50-60, wherein the pharmaceutical composition is administered subcutaneously, intravenously, intradermally, intraperitoneally, or intramuscularly.