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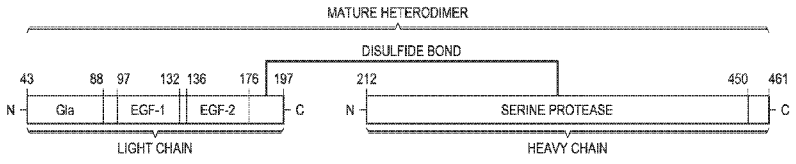
(54) **Title**: MONOCLONAL ANTIBODIES AGAISNT ACTIVATED PROTEIN C (aPC)

Figure 1



(57) **Abstract**: Provided herein are antibodies, antigen-binding antibody fragments (Fabs), and other protein scaffolds, directed against human activated Protein C (aPC) with minimal binding to its zymogen Protein C (PC). Moreover, these aPC binding proteins could potentially block the anti-coagulant activity of aPC to induce coagulation. Therapeutic uses of these binders are described herein as are methods of panning and screening specific antibodies.

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DESCRIPTION

MONOCLONAL ANTIBODIES AGAINST ACTIVATED PROTEIN C (aPC)

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/731,294 filed November 29, 2012 and to U.S. Provisional Patent Application No. 61/786,472 filed March 15, 2013, the disclosures of which are hereby incorporated by reference herein in their entirety.

Sequence listing submission

[0002] The Sequence Listing associated with this application is filed in electronic format *via* EFS-Web and hereby incorporated by reference into the specification in its entirety.

Field of the embodiments

[0003] Provided are isolated monoclonal antibodies and fragments thereof that preferentially bind activated form of human protein C (aPC).

Background

[0004] Human Protein C (PC) zymogen is synthesized in the liver as a 461-amino acid residue precursor and secreted into the blood (as shown in SEQ ID NO: 1). Prior to secretion, the single-chain polypeptide precursor is converted into a heterodimer by removal of a dipeptide (Lys156-Arg157) and a 42-aa residues preproleader. The heterodimeric form (417 residues) consists of the light chain (155aa, 21 kDa) and the heavy chain (262aa, 41 kDa) linked by a disulfide bridge (as shown in SEQ ID NO: 2). PC zymogen contains the thrombin cleavage site, leading to removal of the "activation peptide" and activation of PC to activated PC (aPC) form (405 residues) shown in SEQ ID NO: 3. Figure 1 provides a cartoon depiction of human PC and its activated form, aPC. Human PC contains 9 Gla-residues and 4 potential sites for N-linked glycosylation. The light chain contains the Gla domain and 2 EGF-like domains. The heavy chain harbors an active serine protease domain.

[0005] PC normally circulates at 3-5ug/ml (~65 nM) in healthy human blood and its half-life is 6-8 hours. The predominant form of circulating PC zymogen is the heterodimeric form. The light chain of PC contains one gamma-carboxy glutamic acid (Gla) – rich domain (45aa), two EGF-like domains (46aa) and the linker sequences. The heavy chain of PC harbors a 12-aa highly polar "activation peptide" and a catalytic domain with a typical serine protease catalytic triad.

[0006] Human PC undergoes extensive post-translational modifications including glycosylation, vitamin K-dependent gamma-carboxylation, and gamma-hydroxylation (1-2). It contains 23% carbohydrate (by weight) and 4 potential N-linked glycosylation sites (one in the light chain Asn97 and three in the heavy chain Asn248/313/329). Its Gla domain contains 9 Gla residues and is responsible for the calcium-dependent binding of PC to negatively-charged phospholipid membranes. The Gla domain can also bind to the endothelial protein C receptor (EPCR), which aligns thrombin and thrombomodulin on the endothelial membrane during PC activation.

[0007] Protein C zymogen is typically converted to its active enzyme --- activated protein C (aPC) to have biological potency. The activity of the PC pathway is controlled by the rate of PC activation and aPC inactivation. PC activation occurs on the surface of endothelial cells in a two-step process. It requires binding of PC (via Gla domain) to the EPCR on endothelial cells, followed by proteolytic activation of PC through thrombin/thrombomodulin complexes. A single cleavage at Arg12 of the heavy chain of human PC, which is catalyzed by thrombin/thrombomodulin on the endothelial cell surface, liberates the 12-aa AP and converts the zymogen PC into aPC, an active serine protease. Thus, the primary difference between the amino acid sequences of PC and aPC is the presence of a 12-aa activation peptide in PC that is absent in APC. Activation of PC into aPC also induces conformational changes; consequently only aPC, not PC, can be labeled by benzamidine or with chloromethylketone (CMK) peptide inhibitor in its enzymatic active site. The crystal structure of Gla-domainless aPC in complex with CMK-inhibitor was recently resolved. The major aPC inactivator in human plasma is the protein C inhibitor (PCI) present at 100nM in human plasma, a member of the serpin superfamily. Under physiological conditions, aPC circulates at very low concentration (1-2 ng/ml or 40 pM) in human blood with a half-life of 20-30 min.

[0008] The protein C pathway serves as a natural defense mechanism against thrombosis. It differs from other anticoagulants in that it is an on-demand system that can amplify the

anticoagulant response as the coagulant response increases. Upon injury, thrombin is generated for coagulation. At the same time, thrombin also triggers an anti-coagulant response by binding to thrombomodulin lined on the vascular surface, and this promotes protein C activation. Thus, aPC generation is roughly proportional to thrombin concentration and PC levels.

[0009] The physiological importance of the protein C pathway as a key regulator of coagulation process is shown by 3 clinical findings: (a) Severe thrombotic complications associated with protein C deficiency and the ability to correct the defect by protein C supplement (b) familial thrombophilia associated with deficiencies in protein C cofactor (protein S); and (c) thrombotic risk associated with the inherited mutations in its substrate (Factor V Leiden R506Q) which make it resistant to cleavage by aPC (Bernard, GR et.al. N Engl J Med 2001, 344:699-709 review).

[00010] In contrast to the other vitamin K-dependent coagulation factors, aPC functions as an anticoagulant by proteolytic inactivation of two coagulation cofactors, Factor Va and VIIIa, thereby inhibiting the generation of thrombin. As a result of decreased thrombin levels, the inflammatory, pro-coagulant and anti-fibrinolytic responses, induced by thrombin, are reduced. aPC also directly contributes to the enhanced fibrinolytic response by complex formation with plasminogen activator inhibitors (PAI).

[00011] In addition to its anti-coagulant functions, aPC induces cytoprotective effects, including anti-inflammatory and anti-apoptotic activities, and protection of endothelial barrier function. These direct cytoprotective effects of aPC on cells require EPCR and the G-protein-coupled receptor, protease activated receptor-1 (PAR-1). Thus, aPC promotes fibrinolysis and inhibits thrombosis and inflammation. The anti-coagulant and cytoprotective functions of aPC appear to be separable. Most of the cytoprotective effects are primarily independent of the anticoagulant activity of aPC and aPC mutants with minimal anti-coagulant activity and normal cytoprotective activity have been generated. Likewise, hyper-anticoagulant but non-cytoprotective aPC mutants have also been reported.

[00012] The C-terminus of aPC light chain is also a highly charged region that comprises residue Gly142-Leu155 on the opposite side of the active site in the protease domain. E149A-aPC had amidolytic activity that is indistinguishable from wild-typeaPC, but had more than a 3-fold increase in anti-coagulant activity in the activated partial thromboplastin time (aPTT) clotting assays due to increased sensitivity to protein S cofactor activity. E149A-aPC showed

hyperactive anticoagulant activity in plasma-clotting assays as well as hyperactive anti-thrombotic potency in vivo. This mutant also had reduced cytoprotective and mortality reduction activities in a LPS-induced lethal endotoxemia murine model. This suggests that aPC's cytoprotective activity is required to reduce mortality in the murine model. In contrast, aPC's anticoagulant activity is neither necessary nor sufficient for mortality reduction. aPC has been used to treat sepsis, a life-threatening condition associated with hypercoagulation and generalized inflammatory reactions. A severe side effect of aPC therapy in sepsis is major bleeding that occurs in 2% of patients. This severe side effect limits its clinical use.

Summary

[00013] Monoclonal antibodies to human activated Protein C (aPC) are provided. In at least one embodiment, the anti-aPC monoclonal antibodies exhibit minimal binding to Protein C, which is the zymogen of aPC.

[00014] In some embodiments, the monoclonal antibodies to aPC provided have been optimized, for example to increase affinity, to increase functional activity or to reduce divergence from a germline sequence.

[00015] Also provided are specific epitopes on human aPC bound by isolated monoclonal antibody. Further provided are the isolated nucleic acid molecules encoding the same.

[00016] Pharmaceutical compositions comprising the anti-aPC monoclonal antibodies and methods of treatment of genetic and acquired deficiencies or defects in coagulation such as hemophilia A and B are also provided. Also provided are methods for shortening the bleeding time by administering an anti-aPC monoclonal antibody to a patient in need thereof. Methods for producing a monoclonal antibody that binds human aPC are also provided.

Brief description of the drawings

[00017] The skilled artisan will understand that the drawings , described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[00018] **Figure 1** shows a cartoon drawing of human activated Protein C in its mature heterodimer form.

[00019] **Figure 2** shows an amino acid sequence alignment of heavy and light chain CDRs is shown among 10 anti-aPC Fabs identified from the human Fab antibody library.

[0010] **Figure 3** depicts a graph characterizing anti-APC Fabs by direct ELISA. An ELISA plate was coated with human PC (hPC), human aPC (hAPC), dog aPC (dAPC), mouse aPC (mAPC) at 100 ng per well. Purified Fabs designate on the X-axis were added to the plate at 20 nM (1 ug/ml). Bound Fab was detected by the secondary antibody (anti-human Fab-HRP) followed by HRP substrate AmplexRed. The purified Fabs preferentially bind to human aPC and, with the exception of Fab R41C17, show little to no binding to human PC. One Fab T46J23 also showed some binding to mouse aPC.

[0011] **Figure 4** shows binding selectivity of anti-aPC Fabs by ELISA.

[0012] **Figure 5** depicts a graph showing inhibition of clot formation of normal human plasma in a dose-dependent manner by aPTT by spiking in human aPC. 50% pooled human normal plasma formed clots in 52 seconds. Preincubation of human aPC at 100, 200, 400, 800, or 1600 ng/ml with the plasma prolonged the clotting time in a dose-dependent manner. Nearly identical potency for recombinant human aPC (rh-APC) and plasma-derived human aPC (pdh-APC) was observed.

[0013] **Figure 6** depicts graphs showing anti-aPC Fabs inhibit human aPC and induce clot formation in human normal plasma. Human aPC at 400ng/ml extended the plasma clotting time from 52 seconds to 180 seconds. Incubation of control antibody (Control) or its Fab (Control-Fab) or select Fabs at 0, 0.5, 1, 2, 5, 10, or 20ug/ml with aPC reduced the clotting time in a dose-dependent manner (top panel). Three Fabs (R41E3, C22J13, Control-Fab) were also tested at 40 ug/ml for a greater effect (bottom panel).

[0014] **Figure 7** shows anti-aPC Fabs inhibit dog aPC and induce clot formation in aPTT.

[0015] **Figure 8** shows the effect of anti-aPC Fabs on the amidolytic activity of aPC. Human aPC protein (20 nM) was first preincubated with an equal volume of anti-aPC Fab (1-3000 nM) at room temperature for 20 min before the chromogenic substrate SPECTROZYME PCa was added to the reaction mixture up to 1 mM. The amidolytic activity of human aPC at a final concentration of 10 nM was measured in the presence of Fabs. Hydrolysis rates were inhibited in the presence of the Fabs, reaching a maximum reduction of 80%.

[0016] **Figure 9** shows the effect of anti-aPC Fabs on the Factor Va (FVa) inactivation activity of aPC.

[0017] *Figure 10* shows binding specificity of anti-aPC human IgG1s and shows species cross-reactivity of anti-aPC human IgG1s by ELISA. An ELISA plate was coated with human PC (hPC), human aPC (hAPC), dog aPC, mouse aPC, rabbit aPC at 1 ug/ml. Purified IgGs (20nM) were added to the plate. Bound IgG was detected by the secondary antibody (anti-human IgG-HRP) followed by HRP substrate AmplexRed. Five anti-aPC human IgG1s cross-react with dog and rabbit aPCs and one IgG1 also binds mouse aPC.

[0018] **Figure 11** shows the effect of anti-aPC IgGs on amidolytic activity of species aPCs – (a) human, (b) rabbit, (c) dog, and (d) mouse. aPC protein (20 nM) was first preincubated with an equal volume of anti-aPC-hIgG1 (1-1000 nM) at room temperature for 20 min before the chromogenic substrate SPECTROZYME PCa was added to the reaction mixture up to 1 mM. The amidolytic activity of aPC at a final concentration of 10 nM was measured in the presence of Fabs. Hydrolysis rates were inhibited in the presence of the IgGs. A negative control antibody (anti-CTX-hIgG1) was used.

[0019] Figure 12 shows anti-aPC-hIgG1s shorten clotting time and induce coagulation in human plasma clotting assays (aPTT).

[0020] **Figure 13** shows the effect of anti-aPC-IgG1 on severe hemophilic patient plasma. In the presence of endothelial cells and thrombomodulin, PC is activated to aPC and reduces thrombin generation. Unlike control Ab, anti-aPC-antibody rapidly inhibits this newly generated aPC and increase thrombin generation by 5-10x. Enhanced thrombin generation will lead to improved coagulation in patients with coagulopathy.

[0021] **Figure 14** shows activity profile of anti-aPC-antibody variants. Similar to the parental antibody, C25K23, such variants (a) bind to aPC with high affinity, (b) potently inhibit aPC activity in purified system, and (c) shorten clotting time leading to coagulation in human plasma clotting assay.

[0022] **Figure 15** shows a cartoon depicting the complex structure was refined to a final Rwork= 0.201, Rfree = 0.241. The left and right panels show the same complex structure with a rotation change of 90°. The HCDR3 loop from the Fab C25K23 has extensive interactions with the heavy chain of aPC.

[0023] **Figure 16** shows in the left panel shows a zoomed view of interactions around the residue Trp104 in the CDR3 loop of Fab C25K23 heavy chain. It blocks the accessibility of active site of aPC (catalytically important residues His57, Asp102, and Ser195). The right

panel shows that the Fab C25K23 inhibits the activity of aPC in a way similar to the PPACK inhibitor because Trp104 and PPACK occupy the same region at the active site.

[0024] **Figure 17** shows a graph depicting anti-aPC antibodies, in both Fab and IgG forms, binding or not binding to active-site-blocked aPC by ELISA.

Detailed description

[0025] As discussed above, the present disclosure provides antibodies, including monoclonal antibodies, and other binding proteins that specifically bind to the activated form of human Protein C (aPC), but exhibit comparatively little or no reactivity against the zymogen form of human Protein C (PC).

[0026] For purposes of this patent document, the following terminology will be used with the definitions set out below.

Definitions

[0027] Whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with the usage of that word in any other document, including any document incorporated herein by reference, the definition set forth below shall always control for purposes of interpreting this specification and its associated claims unless a contrary meaning is clearly intended (for example in the document where the term is originally used). The use of "or" means "and/or" unless stated otherwise. The use of “a” herein means “one or more” unless stated otherwise or where the use of “one or more” is clearly inappropriate. The use of “comprise,” “comprises,” “comprising,” “include,” “includes,” and “including” are interchangeable and are not limiting. For example, the term “including” shall mean “including, but not limited to.”

[0028] The term “Protein C” or “PC” as used herein refers to any variant, isoform, and/or species homolog of Protein C in its zymogen form that is naturally expressed by cells and present in plasma and is distinct from the activated form of Protein C.

[0029] The term “activated Protein C” or “aPC” as used herein refers to an activated form of Protein C that is characterized by the absence of a 12 amino acid activation peptide present in Protein C.

[0030] As used herein, an “antibody” refers to a whole antibody and any antigen binding fragment (i.e., “antigen-binding portion”) or single chain thereof. The term includes a full-

length immunoglobulin molecule (e.g., an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes, or an immunologically active portion of an immunoglobulin molecule, such as an antibody fragment, that retains the specific binding activity. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the full-length antibody. For example, an anti-aPC monoclonal antibody fragment binds to an epitope of aPC. The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; (vi) an isolated complementarity determining region (CDR); (vii) minibodies, diaboidies, triabodies, tetrabodies, and kappa bodies (see, e.g. Ill et al., Protein Eng 1997;10:949-57); (viii) camel IgG; and (ix) IgNAR . Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are analyzed for utility in the same manner as are intact antibodies.

[0031] Furthermore, it is contemplated that an antigen binding fragment can be encompassed in an antibody mimetic. The term "antibody mimetic" or "mimetic" as used herein is meant a protein that exhibits binding similar to an antibody but is a smaller alternative antibody or a non-antibody protein. Such antibody mimetic can be comprised in a scaffold. The term "scaffold" refers to a polypeptide platform for the engineering of new products with tailored functions and characteristics.

[0032] As used herein, the term "anti-aPC antibody" refers to an antibody that specifically binds to an epitope of aPC. When bound in vivo to an epitope of aPC, the anti-aPC antibodies disclosed herein augment one or more aspects of the blood clotting cascade.

[0033] As used herein, the terms "inhibits binding" and "blocks binding" (e.g., referring to inhibition/blocking of binding of aPC substrate to aPC) are used interchangeably and encompass both partial and complete inhibition or blocking of a protein with its substrate, such as an inhibition or blocking by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about100%. As used herein, "about" means +/- 10% of the numerical value indicated.

[0034] In reference to the inhibition and/or blocking of binding of aPC substrate to aPC, the terms inhibition and blocking also include any measurable decrease in the binding affinity of aPC to a physiological substrate when in contact with an anti-aPC antibody as compared to aPC not in contact with an anti-aPC antibody, e.g., the blocking of the interaction of aPC with its substrates, including Factor Va or with Factor VIIIa, by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

[0035] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity that have variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies can 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).

[0036] An "isolated antibody," as used herein, is intended to refer to an antibody which is substantially free of other biological molecules, including antibodies having different antigenic specificities (e.g., an isolated antibody that binds to aPC is substantially free of antibodies that bind antigens other than aPC). . In some embodiments, the isolated antibody is at least about 75%, about 80%, about 90%, about 95%, about 97%, about 99%, about 99.9% or about 100% pure by dry weight. In some embodiments, purity can be measured by a method such as column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. An isolated antibody that binds to an epitope, isoform or variant of human aPC can, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., aPC species homologs). Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals. As used herein, "specific binding" refers to antibody binding to a

predetermined antigen. Typically, an antibody that exhibits "specific binding" binds to an antigen with an affinity of at least about 105 M-1 and binds to that antigen with an affinity that is higher, for example at least two-fold greater, than its binding affinity for an irrelevant antigen (e.g., BSA, casein). The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

[0037] As used herein, the term "minimal binding" refers to an antibody that does not bind to and/or exhibits low affinity to a specified antigen. Typically, an antibody having minimal binding to an antigen binds to that antigen with an affinity that is lower than about 102 M-1 and does not bind to a predetermined antigen with higher affinity than it binds to an irrelevant antigen.

[0038] As used herein, the term "high affinity" for an antibody, such as an IgG antibody refers to a binding affinity of at least about 107M-1, in at least one embodiment at least about 108M-1, in some embodiments at least about 109M-1, 1010M-1, 1011M-1 or greater, e.g., up to 1013M-1 or greater. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to a binding affinity of at least about 107M-1. As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

[0039] "Complementarity-determining region" or "CDR" refers to one of three hypervariable regions within the variable region of the heavy chain or the variable region of the light chain of an antibody molecule that form the N-terminal antigen-binding surface that is complementary to the three-dimensional structure of the bound antigen. Proceeding from the N-terminus of a heavy or light chain, these complementarity-determining regions are denoted as "CDR1," "CDR2," and "CDR3," respectively [Wu TT, Kabat EA, Bilofsky H, Proc Natl Acad Sci U S A. 1975 Dec;72(12):5107 and Wu TT, Kabat EA, J Exp Med. 1970 Aug 1;132(2):211]. CDRs are involved in antigen-antibody binding, and the CDR3 comprises a unique region specific for antigen-antibody binding. An antigen-binding site, therefore, can include six CDRs, comprising the CDR regions from each of a heavy and a light chain V region.

[0040] The term "epitope" refers to the area or region of an antigen to which an antibody specifically binds or interacts, which in some embodiments indicates where the antigen is in physical contact with the antibody. Conversely, the term "paratope" refers to the area or

region of the antibody on which the antigen specifically binds. Epitopes characterized by competition binding are said to be overlapping if the binding of the corresponding antibodies are mutually exclusive, i.e. binding of one antibody excludes simultaneous binding of another antibody. The epitopes are said to be separate (unique) if the antigen is able to accommodate binding of both corresponding antibodies simultaneously.

[0041] The term "competing antibodies," as used herein, refers to antibodies that bind to about, substantially or essentially the same, or even the same, epitope as an antibody against aPC as described herein. "Competing antibodies" include antibodies with overlapping epitope specificities. Competing antibodies are thus able to effectively compete with an antibody as described herein for binding to aPC. In some embodiments, the competing antibody can bind to the same epitope as the antibody described herein. Alternatively viewed, the competing antibody has the same epitope specificity as the antibody described herein.

[0042] As used herein, "conservative substitutions" refers to modifications of a polypeptide that involve the substitution of one or more amino acids for amino acids having similar biochemical properties that do not result in loss of a biological or biochemical function of the polypeptide. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Antibodies of the present disclosure can have one or more conservative amino acid substitutions yet retain antigen binding activity.

[0043] For nucleic acids and polypeptides, the term "substantial homology" indicates that two nucleic acids or two polypeptides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide or amino acid insertions or deletions, in at least about 80% of the nucleotides or amino acids, usually at least about 85%, in some embodiments about 90%, 91%, 92%, 93%, 94%, or 95%, in at least one embodiment at least about 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, or 99.5% of the nucleotides or amino acids. Alternatively, substantial homology for nucleic acids exists when the segments will hybridize under selective hybridization conditions to the complement of the

strand. Also included are nucleic acid sequences and polypeptide sequences having substantial homology to the specific nucleic acid sequences and amino acid sequences recited herein.

[0044] The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions / total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, such as without limitation the AlignX™ module of VectorNTI™ (Invitrogen Corp., Carlsbad, CA). For AlignX™, the default parameters of multiple alignment are: gap opening penalty: 10; gap extension penalty: 0.05; gap separation penalty range: 8; % identity for alignment delay: 40. (further details found at http://www.invitrogen.com/site/us/en/home/LINNEA-Online-Guides/LINNEA-Communities/Vector-NTI-Community/Sequence-analysis-and-data-management-software-for-PCs/AlignX-Module-for-Vector-NTI-Advance.reg.us.html).

[0045] Another method for determining the best overall match between a query sequence (a sequence of the present disclosure) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson et al., Nucleic Acids Research, 1994, 2(22): 4673-4680), which is based on the algorithm of Higgins et al., (Computer Applications in the Biosciences (CABIOS), 1992, 8(2): 189-191). In a sequence alignment the query and subject sequences are both DNA sequences. The result of said global sequence alignment is in percent identity. Parameters that can be used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix = IUB, k-tuple = 1, Number of Top Diagonals = 5, Gap Penalty = 3, Gap Open Penalty = 10, Gap Extension Penalty = 0.1. For multiple alignments, the following CLUSTALW parameters can be used: Gap Opening Penalty = 10, Gap Extension Parameter = 0.05; Gap Separation Penalty Range = 8; % Identity for Alignment Delay = 40.

[0046] The nucleic acids can be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is “isolated” or “rendered substantially pure” when purified away from other cellular components with which it is normally associated in the natural environment. To isolate a nucleic acid, standard techniques such as

the following can be used: alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art.

Monoclonal Antibodies against Activated Protein C

[0047] aPC is known for its anti-coagulant properties. Bleeding disorders where homeostasis is deregulated in hemophilia or in trauma patients where the wound results in a temporary loss of hemostatsis, can be treated by aPC inhibitors. Antibodies, antigen-binding fragments thereof, and other aPC-specific protein scaffolds can be used to provide targeting specificity to inhibit a subset of aPC protein functions while preserving the rest. Given the at least1000-fold difference in plasma concentration of aPC (<4 ng/ml) versus PC (4 ug/ml), increased specificity of any potential aPC inhibitor therapeutics is helpful to block aPC function in the presence of a high circulating excess of PC.

[0048] aPC specific antibodies that block the anti-coagulant function of aPC can be used as therapeutics for patients with bleeding disorders, including, for example, hemophilia, hemophilia patients with inhibitors, trauma-induced coagulopathy, severe bleeding patients during sepsis treatment by aPC, bleeding resulting from elective surgery such as transplantation, cardiac surgery, orthopedic surgery, or excessive bleeding from Menorrhagia.

[0049] Anti-aPC antibodies having long circulating half-live can be useful in treating chronic diseases like hemophilia. aPC antibody fragments or aPC-binding protein scaffolds with shorter half-lives can be more effective for acute use (e.g. therapeutic use in trauma). As aPC is a multi-function protein, selective aPC function blockers (SAFB) including antibodies, antigen-binding antibody fragments, aPC-specific protein scaffolds with increased affinity and targeting specificity can selectively block only one aPC function without affecting other aPC functions.

[0050] aPC-binding antibodies were identified by panning and screening human antibody libraries against human aPC. The identified antibodies exhibited no or minimal binding to human PC. The heavy chain variable region and light chain variable region of each monoclonal antibody isolated was sequenced and its CDR regions were identified. The sequence identifier numbers ("SEQ ID NO") that correspond to the heavy and light chain regions of each of the aPC-specific monoclonal antibodies are summarized in Table 1.

Table 1. Human anti-aPC antibodies

Clone	Light Chain Variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
C7A23	QSVLTQPPSASGTPGQRVT ISCSGSSSNIGNNYVSWYQ QLPGTAPKLLIYRNNQRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCQSYDSD LSGPYVLFGGGTKLTVLG	SEQ ID NO: 4	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTS	SEQ ID NO: 14
C7I7	QSVLTQPPSASGTPGQRVT ISCTGSSSNIGAGYDVHWY QQLPGTAPKLLIYGNSNRP SGVPDRFSGSKSGTSASLAI SGLRSEDEAAYYCSSYVGS DLVVFGGGTKLTVLG	SEQ ID NO: 5	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTS	SEQ ID NO: 15
O3E7	QSVLTQPPSTSGTPGQRVTI SCTGSSSNIGAGFDVHWY QQLPGTAPKLLIYGNSNRP SGVPDRFSGSKSGTSASLAI SGLRSEDEADYYCATWQD TLTGWMFGGGTKLTVLG	SEQ ID NO: 6	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYSMNWVRQ APGKGLEWVSAISGSGGSTY YADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCAR DRRVRGIYDAFDMWGQGTL VTVTS	SEQ ID NO: 16
C22J13	QSVLTQPPSASGTPGQRVT ISCSGSDSNIGSNAVNWYQ QLPGTAPKLLIYDNNKRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCSSYTSSN TVVFGGGTKLTVLG	SEQ ID NO: 7	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSNYMSWVRQ APGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAMYYCA LTGRSGWMRFPNWFDPWG QGTLVTVTS	SEQ ID NO: 17
C25K23	QSVLTQPPSASGTPGQRVT ISCTGSSSNIGAAYDVHWY QQLPGTAPKLLIYGNNKRP SGVPDRFSGSKSGTSASLAI SGLRSEDEADYYCQSYDSS LSGSVFGGGTKLTVLG	SEQ ID NO: 8	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYWMSWVRQ APGKGLEWVSGVSWNGSRT HYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA LTGRSGWMRFPNWFDPWG QGTLVTVTS	SEQ ID NO: 18
C26B9	QSVLTQPPSASGTPGQRVT ISCSGSSSNIRSNTVNWYQ QLPGTAPKLLIYGNSNRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCQSYDSSL SGDVVFGGGTKLTVLG	SEQ ID NO: 9	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYGMHWVRQ APGKGLEWVSVIYSGGSTY YADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCAR MGRAFDIWGQGTLVTVTS	SEQ ID NO: 19
R41C17	LTQPPSASGTPGQRVTISCT GSSSNIGAGYVVHWYQQL PGTAPKLLIYRNNHRPSGV PDRFSGSKSGTSASLAISGL RSEDEADYYCAAWDDSLN GRVFGGGTKLTVLGQPKA APSVTLFP	SEQ ID NO: 10	EVQLLESGGGLVQPGGSLRL SCAASGFTFSNYAMSWVRQ SPGKGLEWVAVISYDGREK YYSDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA RDRGRTFDYWGQGTLVTVT SASTKGPSVF	SEQ ID NO: 20
R41E3	LTQPPSASGTPGQRVTISCS GSSSNIGNNAVNWYQQLP GTAPKLLIYSNNQRPSGVP DRFSGSKSGTSASLAISGLR	SEQ ID NO: 11	EVQLLESGGGLVQPGGSLRL SCAASGFTFNNYAMTWVRQ APGKGLEWVSGVSWNGSRT HYADSVKGRFTISRDNSKNT	SEQ ID NO: 21

Clone	Light Chain Variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
	SEDEADYYCSSYTSSSTHV VFGGGTKLTVLGQPKAAP SVTL		LYLQMNSLRAEDTAVYYCA RADSSSAGRWAGSLDYWG QGTLVTVTSASTKGPSVF	
T46J23	LTQPPSASGTPGQRVTISCT GTSSNIGAGYDVHWYQQL PGTAPKLLIYDNNNRPSGV PDRFSGSKSGTSASLAISGL RSEDEADYYCAAWDDSLN GVVFGGGTKLTVLGQPKA APSVTLFP	SEQ ID NO: 12	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTSASTKGPSVF	SEQ ID NO: 22
T46P19	LTQPPSASGTPGQRVTISCT GSSSNIGAGYDVHWYQQL PGTAPKLLIYGNINRPSGVP DRFSGSKSGTSASLAISGLR SEDEADYYCSSYTRSATLV FGGGTKLTVLGQPKAAPS VTLFP	SEQ ID NO: 13	EVQLLESGGGLVQPGGSLRL SCAASGFTFSGYGMHWVRQ APGKGLEWVSGINWNGGST GYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA RNRATRSGYYYFDSWGQGT LVTVTSASTKGPSVF	SEQ ID NO: 23

[0051] In one embodiment, provided is an isolated monoclonal antibody that binds to human activated protein C (aPC) and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14-23.

[0052] In another embodiment, provided is an isolated monoclonal antibody that binds to human activated protein C (aPC) and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4-13.

[0053] In another embodiment, provided is an isolated monoclonal antibody that binds to human activated protein C (aPC) and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14-23 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4-13.

[0054] In other embodiments, the antibody comprises heavy and light chain variable regions comprising:

[0055] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 4;

[0056] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 5;

[0057] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 16 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 6;

[0058] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 17 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 7;

[0059] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 18 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 8;

[0060] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 19 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 9;

[0061] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 10;

[0062] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 21 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 11;

[0063] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 22 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 12; and

[0064] a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 23 and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 13.

[0065] Shown in Table 2 is a summary of the SEQ ID Nos for the CDR regions ("CDR1", "CDR2", and "CDR3") of each heavy and light chain of the monoclonal antibodies binding to human aPC.

Table 2. Sequence Identifiers for CDR regions of Human Anti-aPC antibodies

Clone	Light Chain Variable Region			Heavy Chain Variable Region		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
C7A23	44	54	64	74	84	94
C7I7	45	55	65	75	85	95
O3E7	46	56	66	76	86	96
C22J13	47	57	67	77	87	97
C25K23	48	58	68	78	88	98
C26B9	49	59	69	79	89	99
R41C17	50	60	70	80	90	100

Clone	Light Chain Variable Region			Heavy Chain Variable Region		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
R41E3	51	61	71	81	91	101
T46J23	52	62	72	82	92	102
T46P19	53	63	73	83	93	103

[0066] In one embodiment, provided is an isolated monoclonal antibody that binds to human activated protein C (aPC), wherein the antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 94-103. These CDR3s are identified from the heavy chains of the antibodies identified during panning and screening. In a further embodiment, this antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-83, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-93, or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-83 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-93.

[0067] In another embodiment, provided are antibodies that share a CDR3 from one of the light chains of the antibodies identified during panning and screening. Thus, also provided is an isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64-73. In further embodiments, the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44-53, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54-63, or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44-53 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54-63.

[0068] In another embodiment, the antibody comprises a CDR3 from a heavy chain and a light chain of the antibodies identified from screening and panning. Provided is an isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group

consisting of SEQ ID NOs: 94-103 and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64-73. In a further embodiment, the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74-83, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-93, (c) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44-53, and/or (d) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54-63.

[0069] In some embodiments, the antibody comprises heavy and light chain variable regions comprising:

[0070] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 44, 54, and 64 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 74, 84, and 94;

[0071] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 45, 55, and 65 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 75, 85, and 95;

[0072] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 46, 56, and 66 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 76, 86, and 96;

[0073] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 47, 57, and 67 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 77, 87, and 97;

[0074] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 48, 58, and 68 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 78, 88, and 98;

[0075] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 49, 59, and 69 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 79, 89, and 99;

[0076] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 50, 60, and 70 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 80, 90, and 100;

[0077] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 51, 61, and 71 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 81, 91, and 101;

[0078] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 52, 62, and 72 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 82, 92, and 102; and

[0079] a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 53, 63, and 73 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 83, 93, and 103.

[0080] Also provided is an isolated monoclonal antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises an amino acid sequence having at least 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NO: 4-13.

[0081] Also provided is an isolated monoclonal antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises an amino acid sequence having at least 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NO: 14-23.

[0082] The antibody can be species specific or can cross react with multiple species. In some embodiments, the antibody can specifically react or cross react with aPC of human, mouse, rat, rabbit, guinea pig, monkey, pig, dog, cat or other mammalian species.

[0083] The antibody can be of any of the various classes of antibodies, such as without limitation an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, a secretory IgA, an IgD, and an IgE antibody.

[0084] In one embodiment, provided is an isolated fully human monoclonal antibody to human activated protein C.

Optimized variants of anti-aPC antibodies

[0085] In some embodiments, the antibodies panned and screened can be optimized, for example to increase affinity to aPC, to further decrease any affinity to PC, to improve cross-reactivity to different species, or to improve blocking activity of aPC. Such optimization can

be performed for example by utilizing site saturation mutagenesis of the CDRs or amino acid residues in close proximity to the CDRs, i.e. about 3 or 4 residues adjacent to the CDRs, of the antibodies.

[0086] Also provided are monoclonal antibodies having increased or high affinity to aPC. In some embodiments, the anti-aPC antibodies have a binding affinity of at least about 107M-1, in some embodiments at least about 108M-1, in some embodiments at least about 109M-1, 1010M-1, 1011M-1 or greater, e.g., up to 1013M-1 or greater.

[0087] In some embodiments, additional amino acid modifications can be introduced to reduce divergence from the germline sequence. In other embodiments, amino acid modifications can be introduced to facilitate antibody production for large scale production processes.

[0088] In some embodiments, provided are isolated anti-aPC monoclonal antibodies that specifically bind to human activated Protein C, which antibodies comprise one or more amino acid modifications. In some embodiments, the antibody comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more modifications.

[0089] Accordingly, in some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain comprising the amino acid sequence shown in SEQ ID NO: 8, wherein the amino acid sequence comprises one or more amino acid modifications. In some embodiments, the modification of the light chain is a substitution, an insertion or a deletion. In some embodiments, the modifications are located in the CDRs of the light chain. In other embodiments, the modifications are located outside the CDRs of the light chain.

[0090] In some embodiments, the modification of the light chain of SEQ ID NO:8 is at a position selected from G52, N53, N54, R56, P57, S58, Q91, Y93, S95, S96, L97, S98, G99, S100 and V101. The modification can be for example one of the following substitutions: G52S, G52Y, G52H, G52F, N53G, N54K, N54R, R56K, P57G, P57W, P57N, S58V, S58F, S58R, Q91R, Q91G, Y93W, S95F, S95Y, S95G, S95W, S95E, S96G, S96A, S96Y, S96W, S96R, L97M, L97G, L97R, L97V, S98L, S98W, S98V, S98R, G99A, G99E, S100A, S100V, V101Y, V101L or V101E. Further, in some embodiments, the antibody may comprise two or more substitutions from G52S, G52Y, G52H, G52F, N53G, N54K, N54R, R56K, P57G, P57W, P57N, S58V, S58F, S58R, Q91R, Q91G, Y93W, S95F, S95Y, S95G, S95W, S95E,

S96G, S96A, S96Y, S96W, S96R, L97M, L97G, L97R, L97V, S98L, S98W, S98V, S98R, G99A, G99E, S100A, S100V, V101Y, V101L or V101E.

[0091] In some embodiments, the light chain of SEQ ID NO:8 further comprises a modification at one or more of the positions selected from A10, T13, S78, R81 and S82. In some embodiments, the modification at position A10 in the light chain is A10V. In some embodiments, the modification at position T13 in the light chain is T13A. In some embodiments, the modification at position S78 in the light chain is S78T. In some embodiments, the modification at position R81 in the light chain is R81Q. In some embodiments, the modification at position S82 in the light chain is S82A. In some embodiments, the light chain of SEQ ID NO:8 comprises two or more of the modifications A10V, T13A, S78T, R81Q and S82A. In some embodiments, the light chain of SEQ ID NO:8 comprises all the modifications A10V, T13A, S78T, R81Q and S82A.

[0092] In other embodiments, provided is an isolated monoclonal antibody that specifically binds to human activated form of Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 18, wherein the amino acid sequence comprises one or more amino acid modifications. In some embodiments, the modification of the light chain is a substitution, an insertion or a deletion.

[0093] In some embodiments, the heavy chain of SEQ ID NO:18 further comprises a modification at positions N54 or S56. In some embodiments, the modification at position N54 of the heavy chain is N54G, N54Q or N54A. In some embodiments, modification at position S56 of the heavy chain is S56A or S56G.

[0094] In some embodiments, amino acid modifications can be made in order to in order to facilitate antibody production for large scale production processes. For example, in some embodiments, modifications can be made to reduce the hydrophobic surface region of antibodies for improved biophysical properties (e.g. minimal aggregation/stickiness). In some embodiments, additional modifications are made in the light chain of SEQ ID NO: 8. In some embodiments, the modification of the light chain of SEQ ID NO:8 is at position Y33. In some embodiments, the modification and Y33 in the light chain is Y33A, Y33K or Y33D. In some embodiments, additional modifications are made in the heavy chain of SEQ ID NO:18. In some embodiments, the modifications of the heavy chain of SEQ ID NO:18 are at one or more of the positions Y32, W33, W53 or W110. In some embodiments, the

modification in the heavy chain of SEQ ID NO:18 is selected from Y32A, Y32K, Y32D, W33A, W33K, W33D, W53A, W53K, W53D, W110A, W110K, or W110D.

[0095] In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 108. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 110. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 112. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 114. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 116. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 118.

[0096] In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 109. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 111. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 113. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 115. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 117. In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 119.

[0097] In some embodiments, provided is an isolated monoclonal antibody that binds to human activated Protein C, wherein the antibody comprises a light chain having the amino acid sequence shown in SEQ ID NO: 12, wherein the amino acid sequence comprises one or more amino acid modifications. In some embodiments, the modification of the light chain is a substitution, an insertion or a deletion. In some embodiments, the modifications are located in the CDRs of the light chain. In other embodiments, the modifications are located outside the CDRs of the light chain.

[0098] In some embodiments, the modification of the light chain of SEQ ID NO:12 is at a position selected from T25, D52, N53, N54, N55, D95, N98 or G99. The modification can be for example the one of the following substitutions: T25S, D52Y, D52F, D52L, D52G, N53C, N53K, N53G, N54S, N55K, D95G, N98S, G99H, G99L or G99F. Further, in some embodiments, the antibody may comprise two or more substitutions from T25S, D52Y, D52F, D52L, D52G, N53C, N53K, N53G, N54S, N55K, D95G, N98S, G99H, G99L or G99F.

[0099] In a further embodiment, provided is an isolated anti-aPC monoclonal antibody that binds to the human activated form of Protein C, wherein the antibody comprises a heavy chain having the amino acid sequence shown in SEQ ID NO: 22, wherein the amino acid sequence comprises one or more amino acid modifications. In some embodiments, the modification of the light chain is a substitution, an insertion or a deletion.

Epitopes

[00100] Also provided is an isolated monoclonal antibody that bind to an epitope of human activated Protein C, wherein the epitope comprises one or more of residues from the heavy chain of human aPC shown in SEQ ID NO:3.

[00101] In some embodiments, the epitope can include the active site of human aPC. In some embodiments, the active site can comprise amino acid residue S195 of human aPC.

[00102] In some embodiments, the epitope can comprises one or more residues selected from D60, K96, S97, T98, T99, E170, V171, M172, S173, M175, A190, S195, W215, G216, E217, G218, and G218 of human activated Protein C shown in SEQ ID NO:3.

[00103] Also provided are antibodies which can compete with any of the antibodies described herein for binding to human activated Protein C. For example, such a competing antibody can bind to one or more epitopes described above.

Nucleic Acids, Vectors and Host Cells

[00104] Also provided are isolated nucleic acid molecules encoding any of the monoclonal antibodies described above.

[00105] Thus, provided is an isolated nucleic acid molecule encoding an antibody that binds to human activated Protein C.

[00106] In some embodiments, provided are isolated nucleic acid molecules encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 34-43.

[00107] In some embodiments, provided are isolated nucleic acid molecules encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a light chain variable region comprising a nucleic acid sequence selected from the group consisting of SEQ ID Nos: 24-33.

[00108] In some embodiments, provided are isolated nucleic acid molecules encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14-23.

[00109] In some embodiments, provided are isolated nucleic acid molecules encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4-13.

[00110] In another embodiment, provided are isolated nucleic acid molecules encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14-23 or a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4-13, and one or more amino acid modifications in the heavy chain variable region or light chain variable region.

[00111] Further, also provided are vectors comprising the isolated nucleic acid molecules encoding any of the monoclonal antibodies described above and host cells comprising such vectors.

Methods of Preparing Antibodies to aPC

[00112] The monoclonal antibody can be produced recombinantly by expressing a nucleotide sequence encoding the variable regions of the monoclonal antibody according to one of the present embodiments in a host cell. With the aid of an expression vector, a nucleic acid containing the nucleotide sequence can be transfected and expressed in a host cell suitable for the production. Accordingly, also provided is a method for producing a monoclonal antibody that binds with human aPC comprising:

[00113] (a) transfecting a nucleic acid molecule encoding a monoclonal antibody into a host cell,

[00114] (b) culturing the host cell so to express the monoclonal antibody in the host cell, and optionally isolating and purifying the produced monoclonal antibody, wherein the nucleic acid molecule comprises a nucleotide sequence encoding a monoclonal antibody.

[00115] In one example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains obtained by standard molecular biology techniques are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the VH segment is

operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[00116] In addition to the antibody chain encoding genes, the recombinant expression vectors carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Examples of regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. Alternatively, nonviral regulatory sequences can be used, such as the ubiquitin promoter or ß-globin promoter.

[00117] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al.). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Examples of selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[00118] For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, includingmammalian host cells, is typical because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[00119] Examples of mammalian host cells for expressing the recombinant antibodies include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) Mol. Biol. 159:601-621), NSO myeloma cells, COS cells, HKB11 cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods, such as ultrafiltration, size exclusion chromatography, ion exchange chromatography and centrifugation.

Use of Partial Antibody Sequences to Express Intact Antibodies

[00120] Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain CDRs. For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. et al., 1998, Nature 332:323-327; Jones, P. et al., 1986, Nature 321:522-525; and Queen, C. et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:10029-10033). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline

sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V(D)J joining during B cell maturation. It is not necessary to obtain the entire DNA sequence of a particular antibody in order to recreate an intact recombinant antibody having binding properties similar to those of the original antibody (see WO 99/45962). Partial heavy and light chain sequence spanning the CDR regions is typically sufficient for this purpose. The partial sequence is used to determine which germline variable and joining gene segments contributed to the recombined antibody variable genes. The germline sequence is then used to fill in missing portions of the variable regions. Heavy and light chain leader sequences are cleaved during protein maturation and do not contribute to the properties of the final antibody. For this reason, it is necessary to use the corresponding germline leader sequence for expression constructs. To add missing sequences, cloned cDNA sequences can be combined with synthetic oligonucleotides by ligation or PCR amplification. Alternatively, the entire variable region can be synthesized as a set of short, overlapping, oligonucleotides and combined by PCR amplification to create an entirely synthetic variable region clone. This process has certain advantages such as elimination or inclusion or particular restriction sites, or optimization of particular codons.

[00121] The nucleotide sequences of heavy and light chain transcripts are used to design an overlapping set of synthetic oligonucleotides to create synthetic V sequences with identical amino acid coding capacities as the natural sequences. The synthetic heavy and light chain sequences can differ from the natural sequences. For example: strings of repeated nucleotide bases are interrupted to facilitate oligonucleotide synthesis and PCR amplification; optimal translation initiation sites are incorporated according to Kozak's rules (Kozak, 1991, J. Biol. Chem. 266:19867-19870); and restriction sites are engineered upstream or downstream of the translation initiation sites.

[00122] For both the heavy and light chain variable regions, the optimized coding, and corresponding non-coding, strand sequences are broken down into 30-50 nucleotide sections at approximately the midpoint of the corresponding non-coding oligonucleotide. Thus, for each chain, the oligonucleotides can be assembled into overlapping double stranded sets that span segments of 150-400 nucleotides. The pools are then used as templates to produce PCR amplification products of 150-400 nucleotides. Typically, a single variable region oligonucleotide set will be broken down into two pools which are separately amplified to generate two overlapping PCR products. These overlapping products are then combined by

PCR amplification to form the complete variable region. It can also be desirable to include an overlapping fragment of the heavy or light chain constant region in the PCR amplification to generate fragments that can easily be cloned into the expression vector constructs.

[00123] The reconstructed heavy and light chain variable regions are then combined with cloned promoter, translation initiation, constant region, 3' untranslated, polyadenylation, and transcription termination sequences to form expression vector constructs. The heavy and light chain expression constructs can be combined into a single vector, co-transfected, serially transfected, or separately transfected into host cells which are then fused to form a host cell expressing both chains.

[00124] Thus, in another aspect, the structural features of a human anti-aPC antibody are used to create structurally related human anti-aPC antibodies that retain the function of binding to aPC. More specifically, one or more CDRs of the specifically identified heavy and light chain regions of the monoclonal antibodies can be combined recombinantly with known human framework regions and CDRs to create additional, recombinantly-engineered, human anti-aPC antibodies.

Pharmaceutical Compositions

[00125] Also provided are pharmaceutical compositions comprising therapeutically effective amounts of anti-aPC monoclonal antibody and a pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" is a substance that can be added to the active ingredient to help formulate or stabilize the preparation and causes no significant adverse toxicological effects to the patient. Examples of such carriers are well known to those skilled in the art and include water, sugars such as maltose or sucrose, albumin, salts such as sodium chloride, etc. Other carriers are described for example in Remington's Pharmaceutical Sciences by E. W. Martin. Such compositions will contain a therapeutically effective amount of at least one anti-TFPI monoclonal antibody.

[00126] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. The composition is in some embodiments formulated for parenteral injection. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example,

glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. In some cases, it will include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

[00127] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, some methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Pharmaceutical Uses

[00128] The monoclonal antibody can be used for therapeutic purposes for treating genetic and acquired deficiencies or defects in coagulation. For example, the monoclonal antibodies in the embodiments described above can be used to block the interaction of aPC with its substrate, which can include Factor Va or Factor VIIIa.

[00129] The monoclonal antibodies have therapeutic use in the treatment of disorders of hemostasis such as thrombocytopenia, platelet disorders and bleeding disorders (e.g., hemophilia A, hemophilia B and hemophilia C). Such disorders can be treated by administering a therapeutically effective amount of the anti-aPC monoclonal antibody to a patient in need thereof. The monoclonal antibodies also have therapeutic use in the treatment of uncontrolled bleeds in indications such as trauma and hemorrhagic stroke. Thus, also provided is a method for shortening the bleeding time comprising administering a therapeutically effective amount of an anti-aPC monoclonal antibody to a patient in need thereof.

[00130] In another embodiment, the anti-aPC antibody can be useful as an antidote for aPC-treated patients, including for example wherein aPC is used for the treatment of sepsis or bleeding disorder.

[00131] The antibodies can be used as monotherapy or in combination with other therapies to address a hemostatic disorder. For example, co-administration of one or more antibodies with a clotting factor such as factor VIIa, factor VIII or factor IX is believed useful for

treating hemophilia. In one embodiment, provided is a method for treating genetic and acquired deficiencies or defects in coagulation comprising administering (a) a first amount of a monoclonal antibody that binds to human tissue factor pathway inhibitor and (b) a second amount of factor VIII or factor IX, wherein said first and second amounts together are effective for treating said deficiencies or defects. In another embodiment, provided is a method for treating genetic and acquired deficiencies or defects in coagulation comprising administering (a) a first amount of a monoclonal antibody that binds to human tissue factor pathway inhibitor and (b) a second amount of factor VIII or factor IX, wherein said first and second amounts together are effective for treating said deficiencies or defects, and further wherein factor VII is not coadministered. Also included is a pharmaceutical composition comprising a therapeutically effective amount of the combination of a monoclonal antibody and factor VIII or factor IX, wherein the composition does not contain factor VII. "Factor VII" includes factor VII and factor VIIa. These combination therapies are likely to reduce the necessary infusion frequency of the clotting factor. By co-administration or combination therapy is meant administration of the two therapeutic drugs each formulated separately or formulated together in one composition, and, when formulated separately, administered either at approximately the same time or at different times, but over the same therapeutic period.

[00132] In some embodiments, one or more antibodies described herein can be used in combination to address a hemostatic disorder. For example, co-administration of two or more of the antibodies described herein is believed useful for treating hemophilia or other hemostatic disorder.

[00133] The pharmaceutical compositions can be parenterally administered to subjects suffering from hemophilia A or B at a dosage and frequency that can vary with the severity of the bleeding episode or, in the case of prophylactic therapy, can vary with the severity of the patient's clotting deficiency.

[00134] The compositions can be administered to patients in need as a bolus or by continuous infusion. For example, a bolus administration of an inventive antibody present as a Fab fragment can be in an amount of from 0.0025 to 100 mg/kg body weight, 0.025 to 0.25 mg/kg, 0.010 to 0.10 mg/kg or 0.10-0.50 mg/kg. For continuous infusion, an inventive antibody present as an Fab fragment can be administered at 0.001 to 100 mg/kg body weight/minute, 0.0125 to 1.25 mg/kg/min., 0.010 to 0.75 mg/kg/min., 0.010 to 1.0 mg/kg/min. or 0.10-0.50 mg/kg/min. for a period of 1-24 hours, 1-12 hours, 2-12 hours, 6-12 hours, 2-8 hours, or 1-2 hours. For administration of an inventive antibody present as a full-length

antibody (with full constant regions), dosage amounts can be about 1-10 mg/kg body weight, 2-8 mg/kg, or 5-6 mg/kg. Such full-length antibodies would typically be administered by infusion extending for a period of thirty minutes to three hours. The frequency of the administration would depend upon the severity of the condition. Frequency could range from three times per week to once every two weeks to six months.

[00135] Additionally, the compositions can be administered to patients via subcutaneous injection. For example, a dose of 10 to 100 mg anti-aPC antibody can be administered to patients via subcutaneous injection weekly, biweekly or monthly.

[00136] As used herein, "therapeutically effective amount" means an amount of an anti-aPC monoclonal antibody or of a combination of such antibody and factor VIII or factor IX that is needed to effectively increase the clotting time in vivo or otherwise cause a measurable benefit in vivo to a patient in need. The precise amount will depend upon numerous factors, including, but not limited to the components and physical characteristics of the therapeutic composition, intended patient population, individual patient considerations, and the like, and can readily be determined by one skilled in the art.

Examples

[00137] Aspects of the present disclosure may be further understood in light of the following examples, which should not be construed as limiting the scope of the present teachings in any way.

Example 1. Materials and Methods

Screening of human aPC-specific binders

[00138] Preparation of Master Plates: Master plates were produced by picking 1880 clones per panning strategy into 384 well plates (ThermoFisher Scientific, Weltham, MA USA) containing growth media (2XYT/1%glucose/100 μg/ml Carbenicillin) using the Qpix2 (Genetix, Boston, MA USA) colony picker. Plates were grown overnight at 37 °C with shaking.

[00139] Production of Expression plates: Using the Evolution P3 liquid handler (Perkin Elmer, Waltham, MA, USA) 5 μl of media from the master plates were transferred to 384 well plates containing expression media (2XYT/0.1%glucose/100 ug/ml Carb) and incubated

at 30 °C. When the cultures reach an OD 600 of 0.5, IPTG is added at a final concentration of 0.5 mM. Plates are then returned to 30 °C for overnight growth.

[00140] Primary ELISA: Maxisorp 384 well plates (ThermoFisher Scientific, Rochester, NY USA) were coated with recombinant human aPC or human PC (Mol. Innovation) at 1 µg/ml in DPBS with Ca/Mg and incubated overnight at 4 °C. Coated ELISA plates were washed three times with DPBST (PBS+0.05% TWEEN) and blocked with MDPBST (PBS+0.05%TWEEN+5%Milk) for 1hr at RT. Blocked plates were aspirated and 15 µl expression media and 30 µl MDPBST were transferred to each well. ELISA plates were incubated at room temperature for 1 hr, followed by 5 times of wash with DPBST. Anti-hFab-HRP (Jackson ImmunoResearch, 1:10,000 dilution in DPBST) was added to each well and incubated for 1hr at room temperature. Plates were then washed 5 times with DPBST. Amplex Red (Invitrogen) substrate was added and plates were read at an excitation of 485 nm and emission of 595 nm.

[00141] Confirming ELISA: Using the Qpix2 colony picker, putative positive clones were rearrayed from the master plates into 96 deep-well plates (Qiagen) containing 1 ml growth media and grown overnight at 37 °C. Expression plates were inoculated from the master plates and induced with IPTG at 0.5 mM final concentration when the cultures reached an OD600 of 0.5. ELISA was then performed on the expression media as outlined above.

Library selections with biotinylated aPC (in-solution panning)

[00142] Two methods were carried out: depletion of PC binders and non-depletion for total PC and aPC binders. Dynabeads M280 Streptavidin was coupled to100 nM biotin-TF (tissue factor, for non-depletion) or 100 nM biotin-PC (depletion) and captured by magnetic device. 1-7.5x1012 cfu Fab library phage, pre-blocked with DPBS/3%BSA/0.05% TWEEN 20, was incubated with biotin-TF or biotin-PC coupled Strepavidin beads on a rotator at room temperature for 2 hours. The biotin-TF (non-depletion) or biotin-PC (depletion)/Streptavidin beads were captured and discarded. The resulting phage supernatants were incubated with 100 nM (first round), 50 nM (second round) or 10 nM (third round) biotin-aPC in 1ml DPBS/3%BSA/0.05% TWEEN 20/1mM CaCl2 for 2 hours at RT or 40 °C overnight. 100ul of Streptavidin-coupled magnetic beads were added to the phage-aPC solution and incubated for 30 minutes at room temperature. The phage-aPC complex beads was captured on magnetic device and washed with various times of DPBS with 3%BSA or 0.05% TWEEN 20

depending upon the panning rounds. The bound phage was eluted with 1mg/ml trypsin and neutralized with aprotinin. The eluted phage was then used to infect 10 ml exponentially growing E. coli HB101F' and amplified for the next round of selection. The phage stock was also analyzed in a CFU titration (panning output).

Library selections with immobilized aPC (solid-phase panning)

[00143] Five wells of Maxi-sorp 96-well plate was coated with 400 ng/well recombinant aPC in DPBS at 4 °C overnight. The same as in-solution panning, the phage library was pre-treated with biotin-TF for non-depletion or biotin-PC for depletion. The resulting phage then was added to the aPC coated wells and incubated on a shaker for 1-2 hours at room temperature. Unbound phage was washed away by washing with various times of DPBS with 3% BSA or 0.05% TWEEN 20 depending upon the panning rounds. The bound phage was eluted with 1 mg/ml trypsin and neutralized with aprotinin. The eluted phage was then used to infect 10 ml exponentially growing E. coli HB101F' and amplified for the next round of selection. The phage stock was also analyzed in a CFU titration (panning output).

Amplification of selected phage pools: Eluted phage stocks were amplified in HB101F' using helper phage M13K07 for selection round 2, 3 and 4.

[00144] A volume of 10ml of exponentially growing HB101F' was infected with eluted phage from each round of selection and incubated at 37 °C for 45 minutes, 50 rpm. The bacteria were then resuspended in 2xYT medium and spread on two 15cm agar plates containing 100 μg/ml carbocinin, 15 μg/ml tetracycline and 1% glucose followed by overnight incubation at 30 °C. The lawn of bacteria from the plates were collected with total of 8 ml 2xYT/carb/tet.

[00145] About 10 μl of cells were resuspended in 10 ml of 2xYT/carb/tet (OD600 is around 0.1-0.2) and incubated at 37 °C until OD600 reached 0.5-0.7. 5x1010cfu of M13K07 helper phage was added to the cells and incubated for 45 minutes at 37 °C. The infected cells were then resuspended in 15 ml of fresh 2xYT/carb/kanamycin (50 μg/ml)/tet and shaking overnight at 30 °C to produce phage. The phage supernatant was collected by centrifugation and filtration through 0.45 μm filter. 900 μl of the supernatant was used for next round of selection.

DNA Sequencing Analysis of aPC Antibodies

[00146] Plasmid was prepared using standard molecular biology techniques. The following primers were used for DNA sequencing of selected antibody clones.

a) Primer A: 5' GAAACAGCTATGAAATACCTATTGC 3'

b) Primer B: 5' GCCTGAGCAGTGGAAGTCC 3'

c) Primer C: 5' TAGGTATTTCATTATGACTGTCTC 3'

d) Primer D: 5' CCCAGTCACGACGTTGTAAAACG 3'

Purification of Protein C from plasma.

[00147] One liter of dog or rabbit plasma was purchased as 20x50ml frozen stocks with heparin included as anticoagulant (Bioreclamation, Inc., Westbury, NY). The purification method was described by Esmon's lab (12) with modifications. Plasma was thawed at 4C, and diluted 1:1 with 0.02M Tris-HCl, pH7.5, heparin 1U/ml final, benzamidine HCl 10mM final, at RT before loading onto a Q-Sepharose column for capturing protein C and other vitamin K-dependent proteins. The column was washed with buffered 0.15M NaCl, and protein C was eluted with buffered 0.5M NaCl. Eluents were recalcified with 10mM Ca++ and 100U/ml heparin and then loaded onto HCP4-Affigel-10 affinity column. The column was washed with Ca-containing buffer and eluted with EDTA-containing buffer. Purified PC was dialyzed overnight into PBS buffer, flash frozen and stored at -80 as 0.5ml aliquots. The purification yield was 1.75mg from one liter dog plasma. The Purified PC had 98% purity as determined by SDS-PAGE and analytical SEC.

Fab expression and purification

[00148] For Fab expression, 5 • 1 sFab E. coli glycerol stock was inoculated into 1 ml growth media (LB, 1% glucose, 100 • g/ml ampicillin), and the culture grew at 37 °C overnight with shaking at 250 rpm. The overnight culture 500 • 1 was then inoculated into 10 ml prewarmed (37 °C) induction media (LB, 0.1% glucose, 100 • g/ml ampicillin) and grew at 37 °C to OD500 0.6-0.7 at 250 rpm. IPTG was added to the culture to 0.5 mM final concentration for Fab expression, and the culture grew overnight at 30C with shaking at 250 rpm. Next day, the overnight culture was centrifuged at 3, 000g for 15 min at 4 °C to

separate the media from cells. Both supernate and pellet were saved for Fab purification. Fab expression in both supernate and pellet can be confirmed by western blot analysis using anti-His antibody.

[00149] For Fab purification, Protein A column (MabSure) was used as recommended by the BioInvent protocol. Supernate was filtered through a 0.45um filter to remove debris and mixed with a tablet of complete protease inhibitors (Roche 11873580001) before loading onto a buffer-equilibrated protein A column. Fab was eluted with pH 2-3 buffer then buffer-exchanged to PBS, pH 7.0. In order to liberate Fab from cell pellets, 1 ml lysis buffer was added to pellet. The mixture was incubated for 1h for lysis at 4 °C on a rocking platform then centrifuged at 3,000 g for 30 min at 4 °C. Clear supernate was transferred to a new tube and loaded onto Protein A column. Lysis buffer contains freshly prepared 1 mg/ml lysozyme (Sigma L-6876) in cold sucrose solution (20% sucrose (w/v), 30 mM TRIS-HCL, 1 mM EDTA, pH 8.0), 2.5 U/ml benzonase (Sigma E1014) (25 KU/ml, stock solution 1/10.000), and 1 tablet of complete protease inhibitors (Roche 11873580001). Purity of the purified Fab was confirmed by SDS-PAGE and analytical size-exclusion chromatography (SEC). Endotoxin levels were also monitored.

Western blot analysis of PC and aPC.

[00150] Purified protein (100ng/lane) was mixed with 4x SDS-PAGE loading dye with DTT (reducing) or without DTT (non-reducing), heated at 95 °C for 5 min then loaded onto 4-12% NuPAGE gels. Proteins were transferred to nitrocellulose membranes by i-Blot (Life technologies, Carlsbad, CA). Probing steps were done with SNAP-id (Millipore). After blocking with 5% milk/PBS for 10 min, the membranes were incubated with various reagents (e.g. Streptavidin-HRP for detection of biotinylated aPC, the mouse anti-human PC monoclonal antibody HCP-4 and anti-PC goat polyclonal antibody for detection of dog aPC). The probing was followed by incubation with HRP secondary antibody for 10 minutes at room temperature. After washing the blots with PBS with 0.1% TWEEN-20, the signal from HRP was detected using a chemiluminescent substrate (ECL) (Pierce, Rockford, IL) and exposure to x-ray film.

Fab ELISA

[00151] Antigen proteins (human PC, human PC, mouse APC, dog APC) were coated to an ELISA plate at 100ng/100ul/well in PBS/Ca buffer (Life technologies) overnight at 4 °C. The next day, the plate was washed 3x and blocked with 5% PBS/Ca/BSA/Tween20 for 1h at RT. Soluble Fab was added to each well and incubated for 1 h at RT. After adding the anti-human lambda-antibody-HRP as detection antibody, the plate was incubated at room temperature for 1 hr, washed extensively and then developed using Amplex Red substrate as described by the kit manufacturer. The signal was measured as RFU using a fluorescent plate reader (SpectraMax 340pc, Molecular Devices, Sunnyvale, CA). The standard curve was fitted to a four-parameter model, and the values of the unknowns were extrapolated from the curve.

Example 2. Panning of aPC antibody from library

[00152] Panning and screening of a fully human Fab antibody library against human activated Protein C was performed using the methods as described in Example 1. DNA sequencing was performed on the positive antibody clones resulting in 10 unique antibody sequences. An alignment of the heavy chain and light chains of the antibodies is shown in Figure 2. Identical heavy chain CDR3 sequences are found in 5 Fabs (C7I7, C7A23, T46J23, C22J13, C25K23).

[00153] The purified Fabs were characterized by a panel of functional assays to assess: a) their binding specificity (aPC vs. PC); binding affinity (by ELISA and Biacore); and species cross-reactivity (ie. Binding to aPCs of different species origins including human, dog and mouse). Rabbit aPC was also used later for IgG format; b) their binding selectivity against other vitamin K-dependent coagulation factors (e.g. FIIa, FVIIa, FIXa, FXa); c) their potency of inhibiting aPC's anti-coagulant activity in the plasma clotting assay aPTT; and d) their effect on aPC's protease enzymatic activity in buffer using amidolytic activity assay (on a small peptide substrate) and FVa inactivation assay (on the protein substrate FVa).

Example 3. Binding affinity of aPC-specific antibodies and cross-species reactivity

[00154] Antigen-binding activities of these purified anti-aPC Fabs were determined by direct ELISA as shown in Figure 3. Antigens were coated directly on ELISA plates. Coating

antigens included human PC (plasma-derived), human aPC (recombinant), dog aPC (plasma-derived), and mouse aPC (recombinant) at 100ng/well in PBS/Ca buffer. After blocking the plate with 5% milk/PBS and washing the plate with PBS-Tween20, soluble Fabs (1 ug/ml, 20 nM) were added to the plate and incubated for 1 h at RT with shaking. Fab binding was detected with anti-human Fab (lambda) antibody-HRP and Amplex red as substrate. ELISA data showed that all Fabs specifically bind to human aPC but not to human PC. One Fab, R41C17, showed minimal binding to human PC. In contrast, R41C17 binds to both human APC and human PC. Also shown in Figure 3 is cross-species reactivity of Fabs by ELISA. Among 8 aPC-specific binders, 4 of them (C7I7, C7A23, C25K23, T46J23) also showed cross-reactivity with dog aPC. Further, one Fab, T46J23, showed some binding mouse aPC.

[00155] Shown in Table 3 is the EC_{50} as measured by ELISA of anti-aPC antibodies to human aPC and dog aPC.

Table 3. ELISA analysis of anti-aPC Fabs

Fab/ EC_{50} (nM)	C7A23	C7I7	C22J13	C25K23	C26B9	R41C17	R41E3	T46J23	T46P19
Human aPC	2.4	6.3	10.6	4.3	10.3	8.6	10.4	6.7	10.3
Dog aPC	6	3.9		8.2				16.6	

[00156] The affinity of the anti-aPC Fabs was determined by Biacore and is shown in Table 4.

Table 4. ELISA analysis of anti-aPC Fabs

Fab/ KD (nM)	C7A23	C7I7	C22J13	C25K23	C26B9	R41C17	R41E3	T46J23	T46P19
Human aPC	1.9	1.3	16	2	7.9	4.8	11	4.1	7.2
Dog aPC	1.3	1.3	300	5.2				21	

Example 4. Binding selectivity of anti-aPC Fabs

[00157] To determine the binding selectivity of these fabs, their binding activities to the proenzyme human PC, to thrombin (FIIa), and to the activated Factor II (FIIa, thrombin), Factor VII (FVIIa), Factor IX (FIXa), and Factor X (FXa) were also assessed by ELISA. Briefly, an ELISA plate was coated with human aPC at 1 ug/ml, mouse PC at 10ug/ml, dog PC at 10 ug/ml, other coagulation factors (FIIa, FVIIa, FIXa, FXa) at 5-10 ug/ml. Anti-aPC Fabs were added to the wells at 20 nM (1 ug/ml). Bound Fabs were detected by the secondary antibody (anti-human Fab-HRP) followed by HRP substrate AmplexRed. As positive control, a control antibody specific for each antigen was used to demonstrate that coating antigen is present.

[00158] As shown in Figure 4, up to a concentration of 20 nM, none of the Fabs showed binding to the factors IIa, VIIa, IXa, or Xa. Binding to the proenzyme mouse PC or dog PC was also not detectable.

Example 5. Anti-aPC Fabs inihibit aPC and induce clot formation in normal human plasma

[00159] Human aPC is a potent anti-coagulant, and this function can be easily demonstrated by the plasma clotting assay (aPTT) as shown in Figure 5. In aPTT assays, 50% normal human pooled plasma formed clots in 52 seconds upon adding CaCls (initiator) to the mixture of plasma and phospholipids. Preincubation of human aPC at 100, 200, 400, 800, or 1600 ng/ml with plasma prolonged the clotting time in a dose-dependent manner. As shown in Figure 5, nearly identical potency was obtained for plasma-derived aPC and the recombinant aPC. Since the maximal setting of clotting time for the Stago instrument was 240 seconds, the anti-coagulant activity of human aPC in this functional assay reached its maximum at 800 ng/ml of aPC.

[00160] To evaluate potential inhibitory effects of anti-aPC Fabs on the anti-coagulant activity of aPC, 400 ng/ml aPC was used in aPTT assays for a good assay range (Figure 6). The plasma clotting time extended from 52 seconds to 180 seconds due to the anti-coagulant activity of administered aPC. Incubation of a tool mouse anti-human APC antibody (control) or its Fab (control-Fab) or Fab C7A23 at 0, 0.5, 1, 2, 5, 10, or 20ug/ml with aPC (i.e. 1.5x to 60x fold excess of Fab over aPC) reduced the clotting time in a dose-dependent manner. Fab C7A23 was 4-5-fold more potent than contrl-Fab in reversing the anti-coagulant activity of

human aPC. In contrast, the negative control Fab (human Fab lambda) had no effect on the clotting time. In Figure 6, the full-length control antibody (bivalent) was 10-fold more potent than control-Fab (monovalent) in aPTT assay. This result was consistent with their EC50 values [control (0.56nM) vs. control-Fab (6.56 nM)] in direct ELISA for aPC binding (data not shown). Thus, suggesting a more potent molecule when the anti-aPC Fabs are converted into IgG format. The aPTT results suggest that the anti-aPC Fabs significantly inhibited the anti-coagulant activity of aPC and shortened the clotting time. All the tested Fabs were evaluated in plasma clotting assay aPTT in comparison with the control-Fab (Figure 6). In the upper graph of Figure 6, a non-specific human Fab was used as negative control, and it did not affect clotting time as expected. Positive controls (control and control-Fab) shortened the clotting time in a dose-dependent manner.

[00161] Fabs C7A23, C7I7, C25K23, T46J23, and T46P19 at 5 ug/ml (15-fold molar excess over spiked-in aPC) caused 80-93% inhibition of human aPC activity and enhanced clot formation. They were clearly more potent than control-Fab. In contrast, Fab R41E3 only produced 30-40% inhibition of aPC activity under identical conditions. The weak activity of R41E3 in aPTT likely resulted from its lower affinity of aPC binding as determined by ELISA and Biacore. An increase in the R41E3 Fab concentration to 40ug/ml (100-fold molar excess over aPC) indeed caused 80% inhibition of human aPC as shown in lower graph of Figure 6. Likewise, a high dose (40ug/ml) of C22J13 Fab produced 80% inhibition of human aPC. Fab C26B9 was more potent than control-Fab in this assay. In the lower graph, Fab R41C17 had no effect on aPC activity, because it binds both PC and aPC and there are over 1000-fold more abundant PC than aPC in human plasma. This data also indicates that Fab R41C17 has a different binding epitope from the other Fabs.

[00162] As indicated by species aPC ELISA data, 4 Fabs (C7A23, C7I7, C25K23, T46J23) also bind to dog aPC at nanomolar affinity, these Fabs were evaluated by aPTT using dog aPC spiked into 50% pooled human normal plasma as shown in Figure 7. Dog aPC exhibited identical anti-coagulant activity as human aPC by aPTT (data not shown). The dog aPC at 300 ng/ml increased the clotting time from 47 seconds to 117 seconds. Incubation of the control antibody or control-Fab at 0, 0.5, 1, 2, 5, 10, or 20 ug/ml with dog aPC did not affect the clotting time because they do not cross-react with dog aPC by ELISA. However, the Fab C7A23 significantly reduced clotting time in a dose-dependent manner and inhibited the dog aPC activity up to 80% at 5 ug/ml or 85% at 20 ug/ml. Moreover, C7A23 showed comparable potency in blocking human aPC and dog aPC in aPTT assays. Fabs C7A23, C717, C25K23

clearly inhibited dog aPC activity in a dose-dependent manner. At 20 ug/ml Fab concentration, these 3 Fabs cause 80-90% inhibition of aPC and shorten the clotting time. Fab T46J23 gave only 40% inhibition at high dose, in consistent with its weaker binding to dog aPC (KD=22 nM) than C7A23, C7I7, C25K23 (KD=1-5nM) by ELISA and Biacore. In contrast, Fabs T46P19 and R41E3 had no effect on dog aPC in APTT as expected since they could not bind to dog-aPC by ELISA.

Example 6. Effect of anti-aPC Fabs on enzymatic activity of aPC

[00163] Activated Protein C is a serine protease. Its catalytic activity can be measured by two methods: a) amidolytic activity assay using a small peptide substrate, and b) FVa degradation assay using a physiological protein substrate FVa.

[00164] Amidolytic activity of human aPC was investigated by using a chromogenic peptide substrate of aPC in buffer. Purified aPC protein at 10 nM was incubated with the chromogenic substrate SPECTROZYME Pca (Lys-Pro-Arg-pNA, MW 773.9 Da) at 1 mM for 30 min. The conversion of substrate to colorimetric product (ie. Enzyme activity of aPC) was monitored by kinetically reading OD450 every 5 minutes. A standard curve was generated with recombinant human aPC. To test the effect of anti-aPC Fabs on aPC's amidolytic activity (Figure 8), purified aPC protein (20 nM) was first preincubated with an equal volume of anti-aPC Fab (1-1000 nM) at RT for 20 min before the chromogenic substrate SPECTROZYME Pca was added to the reaction mixture up to 1 mM. The amidolytic activity of human aPC at a final concentration of 10 nM was measured in the presence of Fabs. Hydrolysis rates at a final substrate concentration of 1 mM were partially inhibited in the presence of the Fabs, reaching a maximum reduction of 80%. All Fabs except R41C17 inhibited aPC in a dose- dependent manner. IC50 correlated with EC50 in ELISA binding assay, as high-affinity binders (C7I7, C7A23, T46P19, T46J23, C25K23) showed much faster inhibition in this assay than the rest of weaker binders (R41E3, C22J13, C26B9). However, increasing concentration of Fabs for weaker binders also produced maximal inhibition. For example, R41E3 at 3,000 nM produced about 80% inhibition of aPC activity, and the same extent of inhibition was achieved by high affinity binders at 100 nM. Thus, most binders interacted with the active site of aPC causing the inhibition of its amidolytic activity. Interestingly, the control antibody caused partial inhibition of aPC (40%) and reached a plateau at concentrations greater than 100 nM. No inhibitory effect was

observed when using increasing concentrations of R41C17 Fab. Since its binding affinity for human aPC is comparable to high affinity binders with a KD value of 4.8 nM by Biacore, these data indicate that R41C17 has a binding epitope far away from the enzymatic active site of aPC.

[00165] The FVa inactivation activity of human aPC can be measured by incubating human aPC (180 pM) with its physiological protein substrate FVa (1.25 nM), then adding FXa and prothrombin to the reaction mixture to form prothrombinase complex. Chromogenic peptide substrate of thrombin was added to detect the production of thrombin (Figure 9). The readout is thrombin production (FIIa/sec). Purified factors Va (1.25nM) were incubated with aPC (180pM) in the presence of range of concentrations of the Fabs (1-500 nM), and the FVa activities were evaluated in the prothrombinase/tenase assay.

[00166] The influence of the Fabs on the aPC activity toward the biological substrate FVa was measured by an FXa- and a thrombin-generation assay utilizing purified FVa. In this assay, FVa at 0.16 U/ml (1.25 nM) was incubated with aPC 180 pM in assay buffer (20 mM TrisHCl, 137 nM NaCl, 10 ug/ml phospholipids, 5 mM CaCl2, 1 mg/ml BSA) in the presence or absence of antibodies. After incubation for 30 min, 25 ul mixture was transferred to wells. Subsequently, 50 ul human FXa and prothrombin in assay buffer was added to the wells and the kinetics of thrombin–mediated substrate hydrolysis monitored at 30 °C by using plate reader. As the baseline of aPC activity, in the absence of added Fab, incubation of aPC changed the readout from 0.0022 nM FIIa/sec to 0.0015 nM FIIa/sec.

[00167] Addition of the Fabs to the reaction mixtures resulted in a nearly complete inhibition of aPC-mediated proteolysis of FVa and a rapid increase in thrombin generation in a dose-dependent manner. As shown in Figure 9, IC50 values for the inhibition of proteolysis of FVa by aPC were in the nanomolar range and were comparable for all Fabs tested. Most Fabs were more potent than the positive control Fab. R41E3 had a slower increase due to its weaker binding to human aPC. R41C17 surprisingly showed some activity in this assay. This Fab had no effect on aPC's anti-coagulant activity by aPTT or on aPC's amidolytic activity when small peptide substrate was used. These data indicate that R4117 binding epitope differs significantly from those of other Fabs.

Example 7. Expression and Purification of anti-aPC IgGs

[00168] All 10 anti-aPC Fabs were converted to human IgG1 by cloning Fv sequences into human IgG1 expression vectors. Plasmids were transfected into HEK293 cells for transient expression. Antibodies were secreted into the culture medium and purified by protein A column. One high-yield antibody T46J23-hIgG1 produced 10.3 mg per 200 ml culture. Some antibodies only produced 1 mg per 200 ml. Endotoxin levels were also monitored (less than 0.01 EU/mg).

[00169] Similar to purified Fabs, all purified IgGs were characterized by a panel of functional assays to assess a) their binding specificity and binding affinity; b) their species cross-reactivity (binding to aPCs of different species origins including rabbit aPC); c) their effects on the enzymatic activity of species aPC's using amidolytic activity assay; and d) their potency of inhibiting aPC's anti-coagulant activity in the plasma clotting assay aPTT using human plasma and mouse plasma.

Example 8. Binding specificity and binding affinity of anti-aPC IgGs

[00170] As shown in Figure 10, ELISA revealed that most IgG antibodies retain their binding specificity like Fabs as they preferentially bind to human aPC over human PC. On the other hand, R41C17 and O3E7 bind both human aPC and human PC. Surprisingly, T46J23 gained human PC binding after its conversion of Fab to IgG. Titration experiment by ELISA also revealed that, in general, the binding affinity of these bivalent IgG1 was increased 2-50-fold as compared to the corresponding monovalent Fabs as shown in Table 5. In particular, the low-affinity Fab R41E3 increased binding affinity almost 50-fold after Fab-IgG conversion with EC50 value of 104 nM for Fab vs. 1.76 nM for IgG. All IgGs showed high-affinity binding to human APC with EC50 values of subnanomolar and low nanomolar range. O3E7-IgG is the weakest IgG with EC50 of 16.9 nM.

Table 5. ELISA analysis of anti-aPC IgGs

EC_{50} (nM)	**Direct ELISA (aPC coating)**			
hIgG1	**Human aPC**	**Rabbit aPC**	**Dog aPC**	**Mouse aPC**
C7A23	3.29	7	10.9	
C7I7	2.66	2	2.3	
C22J13	0.49	0.6	2.0	

EC_{50} (nM)	Direct ELISA (aPC coating)			
hIgG1	Human aPC	Rabbit aPC	Dog aPC	Mouse aPC
C25K23	1.25	2	3.4	
C26B9	0.65			
R41C17	0.55			
R41E3	1.76			
T46J23	2.18	7	1.68	4.6
T46P19	0.37			
O3E7	16.9			

[00171] Also shown in Figure 10, species cross-reactivity of these IgG was investigated using (a) human, (b) rabbit, (c) dog, (d) mouse aPCs and PCs. Among 10 anti-human aPC IgGs, 5 IgGs bind to rabbit aPC with high affinity (EC_{50} = 0.6 – 7 nM) without detectable binding to rabbit PC. These 5 IgGs also bind to dog APC with high affinity (EC_{50}= 1.7 – 10 nM) and they did not bind to dog PC. One antibody among the 5 IgGs, T46J23, also binds to mouse aPC with EC_{50} value of 6 nM. T46J23 did not bind to mouse PC.

Example 9. Effect of anti-APC IgGs on the enzymatic activity of species APC's in buffer using amidolytic activity assay

[00172] The 5 species cross-reactive IgGs were then evaluated for their effect on the amidolytic activity of species APCs (Figure 11). In human aPC amidolytic activity assays, the negative control IgG (anti-CTX antibody) had no inhibitory effect. The 5 IgGs all inhibited human aPC in a dose-dependent manner. Their IC50 values are 18 nM for T46J23-IgG; 27nM for C22J13; 64nM for C7I7; 78 nM for C7A23, and 131 nM for C25K23.

[00173] In rabbit aPC amidolytic activity assays, the negative control IgG (anti-CTX antibody) had no inhibitory effect. The 5 IgGs all inhibited rabbit aPC in a dose-dependent manner. Their IC50 values are 17 nM for T46J23-IgG; 24nM for C22J13; 29nM for C7I7; 25 nM for C7A23, and 74 nM for C25K23.

[00174] In dog aPC amidolytic activity assays, the negative control IgG (anti-CTX antibody) had no inhibitory effect. The 5 IgGs weakly inhibited dog aPC in a dose-dependent manner. Their IC50 values are 625 nM for T46J23-IgG; 1300 nM for C22J13; 147 nM for C7I7; 49 nM for C7A23, and 692 nM for C25K23.

[00175] In mouse aPC amidolytic activity assays, only T46J23 could inhibit mouse aPC although it needs high dose (1000 nM). C7I7 and other IgGs had no effect on mouse aPC. The inhibitory effects of these antibodies on species APC activity are summarized in Table 6.

Table 6. ELISA and Amidolytic activity

	Anti-aPC EC_{50} (nM)			Anti-aPC IC_{50} (nM)		
	ELISA			Amidolytic activity		
hIgG1	**Human aPC**	**Rabbit aPC**	**Dog aPC**	**Human aPC**	**Rabbit aPC**	**Dog aPC**
C7A23	3.3	7	10.9	78	25	49
C7I7	2.7	2	2.3	64	29	147
C22J13	0.5	0.6	--	27	24	1300
C25K23	1.2	2	3.4	131	74	692
T46J23	2.2	1.7	1.7	18	17	625

[00176] Shown in Figure 14(b), in human aPC amidolytic activity assays, two variants of C25K23 IgG1 referred to as 2310-IgG2 and 2312-IgG2 show potent inhibition of aPC in a purified system. C25K23 IgG1 has a light chain as shown in SEQ ID NO:108 and heavy chain as shown in SEQ ID NO:109. TPP-2031 is a modified C25K23 IgG with a heavy chain comprising the modification N54G. Variant 2310 is a modified C25K23 IgG with a light chain comprising the modifications A10V, T13A, S78T, R81Q and S82A as shown in SEQ ID NO: 112 and heavy chain comprising the modification N54Q as shown in SEQ ID NO:113. Variant 2312 is a modified C25K23 IgG with a light chain comprising the modifications A10V, T13A, S78T, R81Q and S82A as shown in SEQ ID NO: 116 and heavy chain comprising the modification S56A as shown in SEQ ID NO:117. Such variants also display a high affinity to aPC as shown in Figure 14(a). TPP-2309 is a modified C25K23 IgG1 with a light chain comprising the modifications A10V, T13A, S78T, R81Q and S82A as shown in SEQ ID NO: 110 and heavy chain comprising the modification N54G as shown in SEQ ID NO:111.

Example 10. Anti-aPC IgGs inihibit aPC and induce clot formation in normal human plasma

[00177] The effect of anti-aPC IgGs on aPC's anti-coagulant activity was first investigated in human plasma clotting assays (aPTT) and is shown in Figure 12. Fifty percent (50%)

human plasma had a baseline clotting time of 50-52 sec in the absence of aPC. Addition of human aPC to plasma increased clotting time to 190 sec as expected, since aPC is a well-known anti-coagulant. Pre-incubation of aPC with the negative control IgG1 (anti-CTX antibody) did not change clotting time. In contrast, pre-incubation of aPC with anti-aPC specific IgG significantly shortened the clotting time in a dose-dependent manner. At 1:1 molar ratio, both T46J23-IgG and C7I7-IgG at 1 ug/ml inhibited ~50% activity of aPC (at 400ng/ml) and shortened the clotting time from 190 to 114 sec. At 20ug/ml, all three antibodies (T46J23, C7I7, C26B9) completely reverse the anti-coagulant activity of aPC and restored the clotting to normal. R41E3-IgG was less potent than these 3 IgGs in inhibiting aPC. R41E3 partially restored the clotting time to 75 sec and inhibited ~80% activity of aPC at 163-fold molar excess.

[00178] The effect of modified variants of anti-aPC IgGs was also investigated in an aPTT assay as shown in Figure 14(c). Again similar to the results in Figure 12, pre-incubation of aPC with the modified anti-aPC specific IgG significantly shortened the clotting time in a dose-dependent manner.

[00179] **Example 11. Anti-aPC IgGs inhibit aPC and induce clot formation in severe hemophilic patient plasma.** The effect of anti-APC IgGs on aPC's anti-coagulant activity was further investigated using Hemophilic patient plasma in thrombin generation assay (TGA) as shown in Figure 13. Damages on the cells lining blood vessel (endothelial cells) results in exposure of tissue factor leading to limited amount of thrombin generation, known as extrinsic coagulation pathway. Thrombomodulin on the endothelial cells contribute to generation of aPC and its anti-coagulant activity. Severe hemophilic plasma generated only ~50 nM total thrombin. Adding anti-aPC-antibody to the hemophilic plasma increased thrombin generation in dose dependent manner.

Example 12. Co-crystal studies

Antibody Preparation and QC

[00180] Recombinant anti-aPC human Fabs (C25K23 and T46J23) were expressed in *E.coli* and purified to homogeneity by Protein A chromatography. Purified Fabs were showed to have a >90% purity and are lack of aggregation by SDS-PAGE and by analytical size exclusion chromatography. Their functions were characterized by aPC-binding assay

(ELISA). Both C25K23Fab and T46J23Fab bind human aPC full-length and the Gla-domainless aPC at comparable EC_{50} values of 2-4 nM as measured by ELISA. Ten milligrams of these Fabs were produced.

Antigen Preparation and QC

[00181] Plasma-derived human aPC-Gla-domain-less (aPC-GD) was purchased from Enzyme Research Lab and characterized by ELISA to confirm that it can be recognized by both C25K23Fab and T46J23Fab.

Complex Formation

[00182] For complex formation, 0.9mg aPC-GD was mixed with 1.05 mg C25K23Fab and the reaction mixture was incubated at 4 °C for 5 hours. The mixture was loaded onto a gel filtration column to separate free Fab or free aPC-GD from the aPC-GD-Fab complex. Each fraction was collected and analyzed by SDS-PAGE under a non-reducing condition. This process was repeated three times, and the fractions containing the aPC-GD-Fab complex were pooled and concentrated to 10 mg/ml.

[00183] Crystallization of aPC-Fab complexes under different crystal growth conditions were performed to produce crystals suitable for structure determination (max. resolution < 3 Å). High throughput crystallization screening kits were utilized and 2 hits were identified:

a) 0.1% n-Octyl-β-D-glucoside,0.1M sodium citrate tribasic dihydrate PH5.5, 22% PEG 3350

b) 18% 2-propanol, 0.1M sodium citrate tribasic dihydrate PH5.5, 20% PEG 4000

Data Collection

[00184] Structure determination at 2.2 angstrom resolution was successful from aPC-GD-C25K23Fab crystal diffraction image by Molecular Replacement with reported aPC and Fab X-ray structures as models (e.g. pdb code 1aut by Mather et al., 1996), followed by model building and refinement. Shown in Figure 15 is a cartoon representation of the aPC and C25K23 Fab structure. As shown in Figure 15, the C25K23 utilizes the CDR3 loop of its heavy chain to contact the aPC catalytic domain. Very significantly, as shown in Figure 16,

the side chain of W104 from the C25K23 inserts into the catalytic pocket of aPC, having steric overlap with a previously reported aPC inhibitor (tri-peptide inhibitor PPACK).

[00185] From this structure, it was determined that the epitope of aPC bound by the antibody is in the heavy chain of aPC. Contacting residues between the aPC heavy chain and Fab include aPC residues D60, K96, S97, T98, T99, E170, V171, M172, S173, M175, A190, S195, W215, G216, E217, G218, and G218.

[00186] Specifically for Fab C25K23, it was determined that the paratope comprises residues S31, Y32, W53, R57, R101, W104, R106, F107, W110 of the heavy chain shown in SEQ ID NO:18 and K55 of the light chain shown in SEQ ID NO:8.

Example 13. Active-Site Binding

[00187] An irreversible active-site inhibitor, biotin-PPACK, was used to occupy the active site of human aPC, see Figure 16. Biotin-PPACK-hAPC or human aPC was coated onto a maxisorp 96-well plate. Anti-aPC antibodies (Fab and IgG) were serial-diluted at 1:3 from 20 nM to 0.007 nM and added to coated wells and incubated for 1h at room temperature. The bound anti-aPC-Fab or anti-aPC IgG was detected by HRP-conjugated anti-human or anti-mouse Fab antibody followed by incubation with fluorogenic substrates (amplex red and H2O2) to produce fluorescent signals (RFU). The plate was read by Gemini EM fluorescence microplate reader (Molecular Devices, Sunnyvale, CA). The RFUs at 20 nM antibody concentration were presented as mean of triplicate wells (+/-SD) in the bar graph.

[00188] As shown in Figure 17, at least two types of antibodies were identified from the library. First those that are active-site directed including T46J23 (Fab and hIgG) and C25K23 (Fab and hIgG) which no longer bound to biotin-PPACK-hAPC (the active site-blocked hAPC). Second, those that are non-active-site directed including R41C17 which is believed to be an anti-Gla-domain antibody. These data provide solid evidence for active-site binding of T46J23 and C25K23 on human aPC and explain the functional characteristics of these antibodies, i.e. complete blockade of hAPC activities.

[00189] While the present embodiments have been described with reference to the specific embodiments and examples, it should be understood that various modifications and changes can be made and equivalents can be substituted without departing from the true spirit and scope of the claims appended hereto. The specification and examples are, accordingly, to be regarded in an illustrative rather than in a restrictive sense. Furthermore, the disclosure of all

articles, books, patent applications and patents referred to herein are incorporated herein by reference in their entireties.

CLAIMS

1. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14, 15, 17, 18, 19, 21, 22, 23, 109, 111, 113, 115, 117 and 119.

2. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4, 5, 7, 8, 9, 11, 12, 13, 108, 110, 112, 114, 116 and 118.

3. The isolated monoclonal antibody of claim 1 further comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4, 5, 7, 8, 9, 11, 12, 13, 108, 110, 112, 114, 116 and 118.

4. The isolated monoclonal antibody of claim 3, wherein the antibody comprises heavy and light chain variable regions comprising:

   a) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 14 and a light chain variable region having an amino acid sequence of SEQ ID NO: 4;

   b) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 15 and a light chain variable region having an amino acid sequence of SEQ ID NO: 5;

   c) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 17 and a light chain variable region having an amino acid sequence of SEQ ID NO: 7;

   d) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 18 and a light chain variable region having an amino acid sequence of SEQ ID NO: 8;

   e) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 19 and a light chain variable region having an amino acid sequence of SEQ ID NO: 9;

   f) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 21 and a light chain variable region having an amino acid sequence of SEQ ID NO: 11;

g) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 22 and a light chain variable region having an amino acid sequence of SEQ ID NO: 12;

h) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 23 and a light chain variable region having an amino acid sequence of SEQ ID NO: 13;

i) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 109 and a light chain variable region having an amino acid sequence of SEQ ID NO: 108;

j) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 111 and a light chain variable region having an amino acid sequence of SEQ ID NO: 110;

k) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 113 and a light chain variable region having an amino acid sequence of SEQ ID NO: 112;

l) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 115 and a light chain variable region having an amino acid sequence of SEQ ID NO: 114;

m) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 117 and a light chain variable region having an amino acid sequence of SEQ ID NO: 116; and

n) a heavy chain variable region having an amino acid sequence of SEQ ID NO: 119 and a light chain variable region having an amino acid sequence of SEQ ID NO: 118.

5. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 94, 95, 97, 98, 99, 101, 102 and 103.

6. The isolated monoclonal antibody of claim 5, wherein the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74, 75, 77, 78, 79, 81, 82 and 83, (b) a CDR2 comprising an amino acid

sequence selected from the group consisting of SEQ ID NOs: 84, 85, 87, 88, 89, 91, 92 and 93, or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74, 75, 77, 78, 79, 81, 82 and 83 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 85, 87, 88, 89, 91, 92 and 93.

7. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 67, 68, 69, 71, 72 and 73.

8. The isolated monoclonal antibody of claim 7, wherein the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 45, 47, 48, 49, 51, 52 and 53, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 57, 58, 59, 61, 62 and 63, or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 45, 47, 48, 49, 51, 52 and 53 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 57, 58, 59, 61, 62 and 63.

9. The isolated monoclonal antibody of claim 5, wherein the antibody further comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 64, 65, 67, 68, 69, 71, 72 and 73.

10. The isolated monoclonal antibody of claim 9, wherein the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 74, 75, 77, 78, 79, 81, 82 and 83, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 84, 85, 87, 88, 89, 91, 92 and 93, (c) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 45, 47, 48, 49, 51, 52 and 53, and (d) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 57, 58, 59, 61, 62 and 63.

11. The antibody of claim 4, wherein the antibody comprises heavy and light chain variable regions comprising:

a) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 44, 54, and 64 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 74, 84, and 94;

b) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 45, 55, and 65 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 75, 85, and 95;

c) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 47, 57, and 67 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 77, 87, and 97;

d) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 48, 58, and 68 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 78, 88, and 98;

e) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 49, 59, and 69 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 79, 89, and 99;

f) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 51, 61, and 71 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 81, 91, and 101;

g) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 52, 62, and 72 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 82, 92, and 102; and

h) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 53, 63, and 73 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOs: 83, 93, and 103.

12. The isolated monoclonal antibody of claim 4 further comprising one or more amino acid modifications.

13. The isolated monoclonal antibody of claim 11 further comprising one or more amino acid modifications.

14. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a light chain variable region comprising an amino acid sequence

of SEQ ID NO:8, wherein said amino acid sequence comprises one or more amino acid modifications.

15. The isolated monoclonal antibody of claim 13, wherein the modification is a substitution.

16. The isolated monoclonal antibody of claim 14, wherein the substitution is a position selected from the group consisting of A10, T13, G52, N53, N54, R56, P57, S58, S78, R81, S82, Q91, Y93, S95, S96, L97, S98, G99, S100 and V101.

17. The isolated monoclonal antibody of claim 15, wherein the substitution is selected from the group consisting of A10V, T13A, G52S, G52Y, G52H, G52F, N53G, N54K, N54R, R56K, P57G, P57W, P57N, S58V, S58F, S58R, S78T, R81Q, S82A, Q91R, Q91G, Y93W, S95F, S95Y, S95G, S95W, S95E, S96G, S96A, S96Y, S96W, S96R, L97M, L97G, L97R, L97V, S98L, S98W, S98V, S98R, G99A, G99E, S100A, S100V, V101Y, V101L and V101E.

18. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:18, wherein said amino acid sequence comprises one or more amino acid modifications.

19. The isolated monoclonal antibody of claim 18, wherein the modification is a substitution.

20. The isolated monoclonal antibody of claim 19, wherein the substitution is a position selected from the group consisting of N54 and S56.

21. The isolated monoclonal antibody of claim 20, wherein the substitution is selected from the group consisting of N54G, N54Q, N54A, S56A and S56G.

22. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:12, wherein said amino acid sequence comprises one or more amino acid modifications.

23. The isolated monoclonal antibody of claim 22, wherein the modification is a substitution.

24. The isolated monoclonal antibody of claim 23, wherein the substitution is a position selected from the group consisting of T25, D52, N53, N54, N55, D95, N98 and G99.

25. The isolated monoclonal antibody of claim 24, wherein the substitution is selected from the group consisting of T25S, D52Y, D52F, D52L, D52G, N53C, N53K, N53G, N54S, N55K, D95G, N98S, G99H, G99L and G99F.

26. An isolated monoclonal antibody that binds to an epitope of human activated Protein C (human aPC, SEQ ID NO:3), wherein said epitope comprises residues from a heavy chain of human aPC.

27. An isolated monoclonal antibody that binds to an epitope of human activated Protein C (human aPC, SEQ ID NO:3), wherein said epitope comprises S195 of SEQ ID NO:3.

28. An isolated monoclonal antibody that binds to an epitope of human activated Protein C, wherein said epitope comprises one or more residues selected from the group consisting of D60, K96, S97, T98, T99, E170, V171, M172, S173, M175, A190, S195, W215, G216, E217, G218, and G218 of SEQ ID NO:3.

29. An isolated monoclonal antibody that binds to the active site of activated Protein C.

30. An isolated monoclonal antibody, wherein said antibody binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein said antibody is a fully human antibody.

31. The isolated monoclonal antibody of claims 1-30, wherein the antibody is selected from the group consisting of an IgG1, an IgG2, an IgG3, an IgG4, an IgM, an IgA1, an IgA2, a secretory IgA, an IgD, an IgE antibody, and antibody fragment .

32. The isolated monoclonal antibody of claims 1-30, wherein the antibody binds to human activated Protein C.

33. The isolated monoclonal antibody of claim 32, wherein the antibody further binds to a non-human species of activated Protein C.

34. The antibody of claims 1-30, wherein blood clotting time in the presence of the antibody is shortened.

35. An antibody which would compete with the antibody of claim 1-30.

36. A pharmaceutical composition comprising a therapeutically effective amount of the monoclonal antibody of any of claims 1-30 and a pharmaceutically acceptable carrier.

37. A method for treating genetic or acquired deficiencies or defects in coagulation comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 36 to a patient.

38. A method for treating coagulopathy comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 36 to a patient.

39. The method of claim 38, wherein the coagulopathy is hemophilia A, B or C.

40. The method of claim 38, wherein the coagulopathy is selected from the group consisting of trauma-induced coagulopathy or severe bleeding patients.

41. The method of claim 38, further comprising administering a clotting factor.

42. The method of claim 41, wherein the clotting factor is selected from the group consisting of Factor VIIa, Factor VIII or Factor IX.

43. A method for shortening bleeding time comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 36 to a patient.

44. An isolated nucleic acid molecule encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 14, 15, 17, 18, 19, 21, 22 and 23.

45. An isolated nucleic acid molecule encoding an antibody that binds to activated Protein C and inhibits anticoagulant activity but has minimal binding to unactivated Protein C, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 4, 5, 7, 8, 9, 11, 12 and 13.

Figure 1

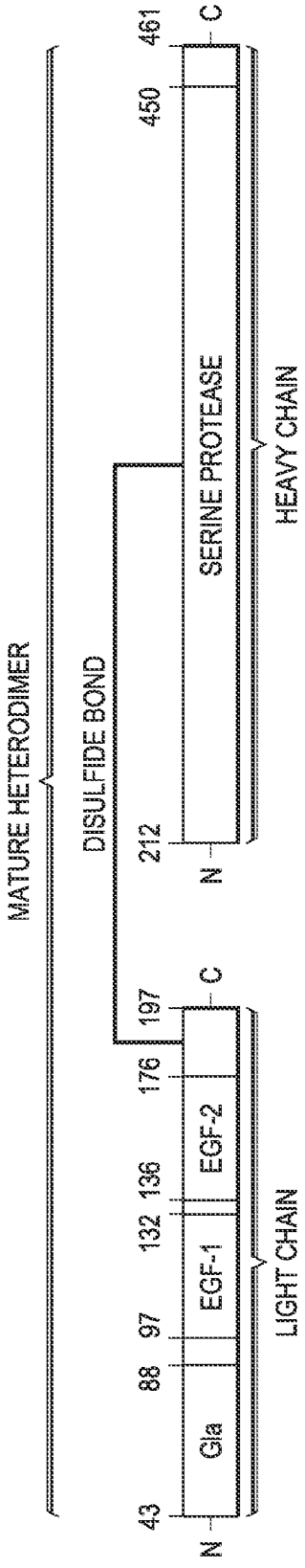

MATURE HETERODIMER
DISULFIDE BOND
N
43
Gla
88
97
EGF-1
132
136
EGF-2
176
197
C
LIGHT CHAIN
N
212
SERINE PROTEASE
450
461
C
HEAVY CHAIN

Figure 2

HEAVY CHAIN		HCDR1	HCDR2	HCDR3
C717 HEAVY CHAIN	46	ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
C7A23 HEAVY CHAIN	46	ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
T46J23 HEAVY CHAIN	46	ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
C22J13 HEAVY CHAIN	46	ASGFTFSSNYMSWVRQAPGKGLEWVAVISYDGSNKYYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCALTGRSGWMRFPNWFDPWGQO	
C25K23 HEAVY CHAIN	46	ASGFTFSSYWMSWVRQAPGKGLEWVSGVSWNGSRTHYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
C26B9 HEAVY CHAIN	46	ASGFTFSSYGMHWVRQAPGKGLEWVSVIYS-GGSTYYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
03E7 HEAVY CHAIN	46	ASGFTFSSYSMNWVRQAPGKGLEWVSAISGSGGSTYYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCALTGRSGWMRFPNWFDPWGQO	
T46P19 HEAVY CHAIN	46	ASGFTFSGYGMHWVRQAPGKGLEWVSGINWNGGSTGYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
R41C17 HEAVY CHAIN	46	ASGFTFSNYAMSWVRQSPGKGLEWVAVISYDGREKYYSDSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQO	
R41E3 HEAVY CHAIN	46	ASGFTFNNYAMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK	GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARMG-------RAFDIWGQO	
				GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRRV--RGIYDAFDMWGQO
				GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNRAT--RSGYYYFDSWGQO
				GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRG-------RTFDYWGQO
				GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARADSSSAGRWAGSLDYWGQO

Figure 2

LIGHT CHAIN		LCDR1	LCDR2	LCDR3
C717 LIGHT CHAIN	17	TISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPD		RFSGSKSGTSASLAISGLRSEDEAAYYCSSYVGSDL---VVFGGGTKI
C7A23 LIGHT CHAIN	17	TISCSGSSSNIGN-NYVSWYQQLPGTAPKLLIYRNNQRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSDLSGPYVLFGGGTKI
T46J23 LIGHT CHAIN	16	TISCTGTSSNIGAGYDVHWYQQLPGTAPKLLIYDNNNRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLNG--VVFGGGTKI
C22J13 LIGHT CHAIN	17	TISCSGSDSNIGS-NAVNWYQQLPGTAPKLLIYDNNKRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCSSYTSSNT---VVFGGGTKI
C25K23 LIGHT CHAIN	17	TISCTGSSSNIGAAYDVHWYQQLPGTAPKLLIYGNNKRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSSLSG--SVFGGGTKI
C26B9 LIGHT CHAIN	17	TISCSGSSSNIRS-NTVNWYQQLPGTAPKLLIYGNSNRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSSLSGSG-DVVFGGGTKI
03E7 LIGHT CHAIN	17	TISCTGSSSNIGAGFDVHWYQQLPGTAPKLLIYGNSNRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCATWQDTLTG--WMFGGGTKI
T46P19 LIGHT CHAIN	16	TISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNINRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCSSYTRSAT---LVFGGGTKI
R41C17 LIGHT CHAIN	16	TISCTGSSSNIGAGYVVHWYQQLPGTAPKLLIYRNNHRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLNG--RVFGGGTKI
R41E3 LIGHT CHAIN	16	TISCSGSSSNIGN-NAVNWYQQLPGTAPKLLIYSNNQRPSGVPD		RFSGSKSGTSASLAISGLRSEDEADYYCSSYTSSST H--VVFGGGTKI

Figure 3

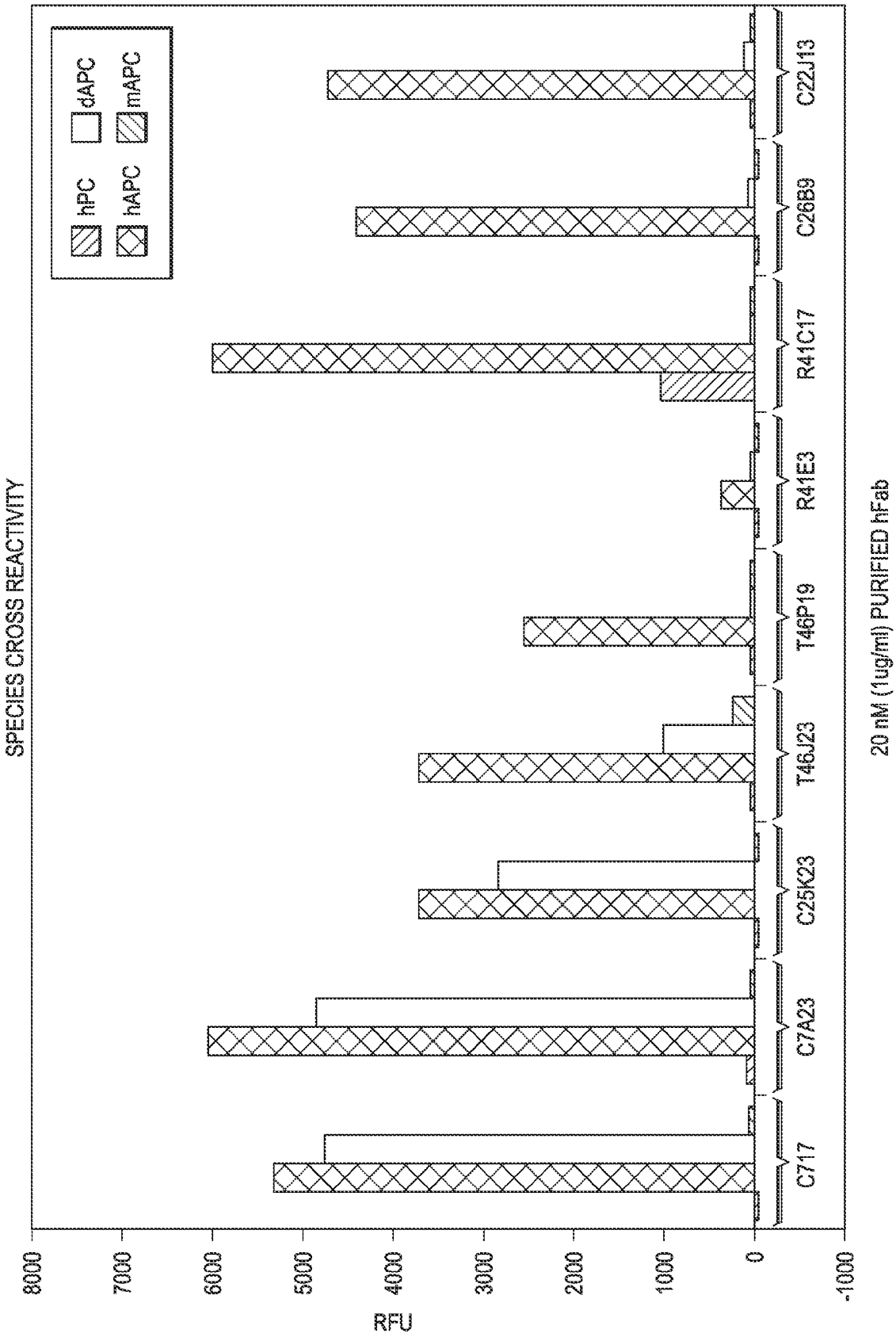

SPECIES CROSS REACTIVITY
hPC
dAPC
hAPC
mAPC
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
RFU
C717
C7A23
C25K23
T46J23
T46P19
R41E3
R41C17
C26B9
C22J13
20 nM (1ug/ml) PURIFIED hFab

Figure 4

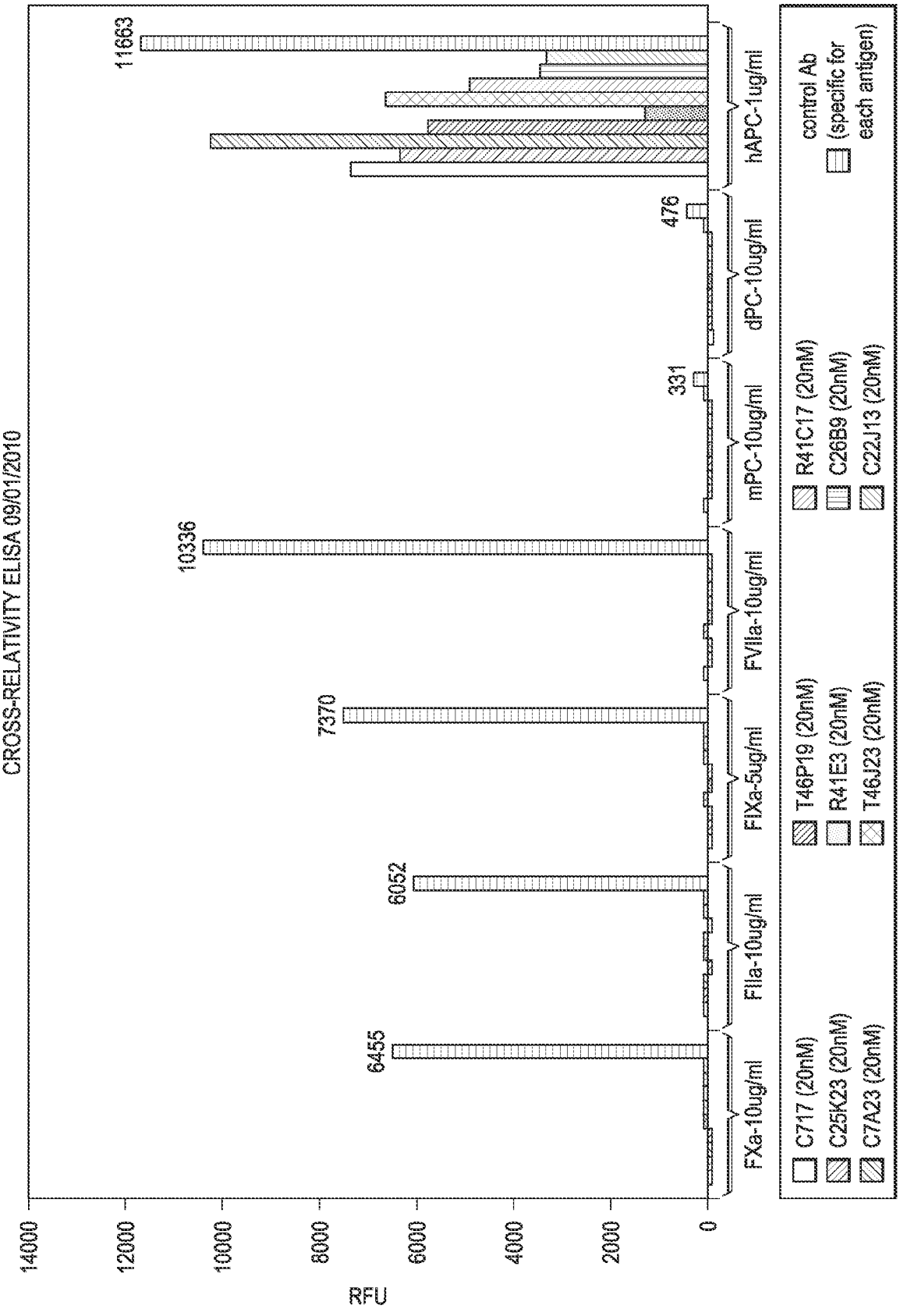

CROSS-RELATIVITY ELISA 09/01/2010
RFU
14000
12000
10000
8000
6000
4000
2000
0
6455
6052
7370
10336
331
476
11663
FXa-10ug/ml
FIIa-10ug/ml
FIXa-5ug/ml
FVIIa-10ug/ml
mPC-10ug/ml
dPC-10ug/ml
hAPC-1ug/ml
C717 (20nM)
C25K23 (20nM)
C7A23 (20nM)
T46P19 (20nM)
R41E3 (20nM)
T46J23 (20nM)
R41C17 (20nM)
C26B9 (20nM)
C22J13 (20nM)
control Ab
(specific for
each antigen)

Figure 5

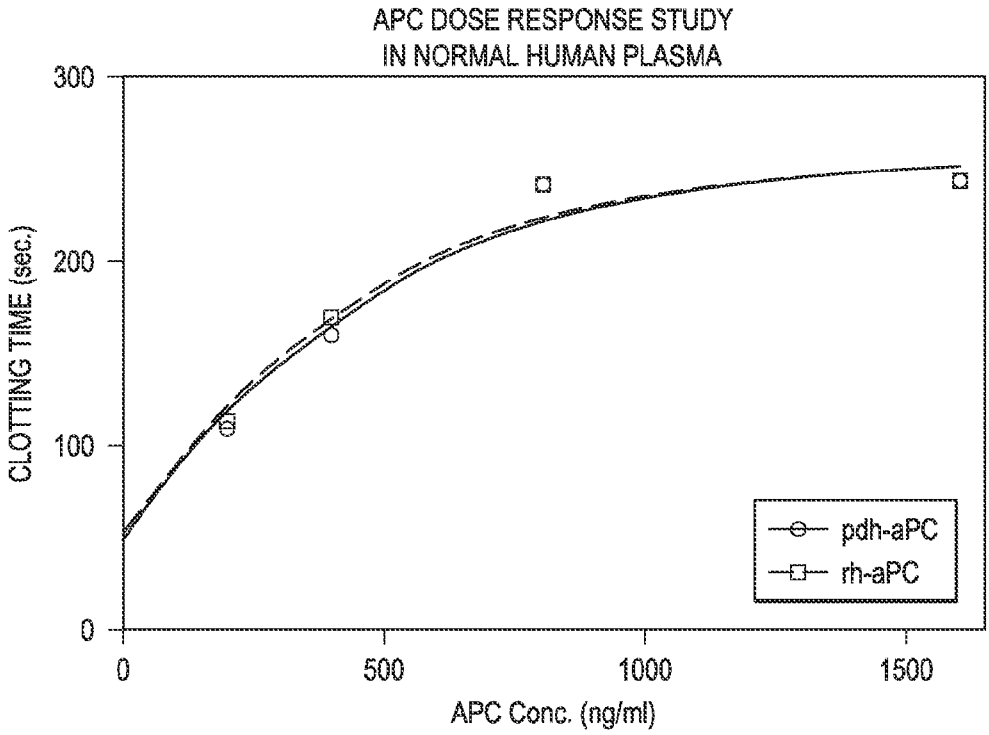


Figure 6

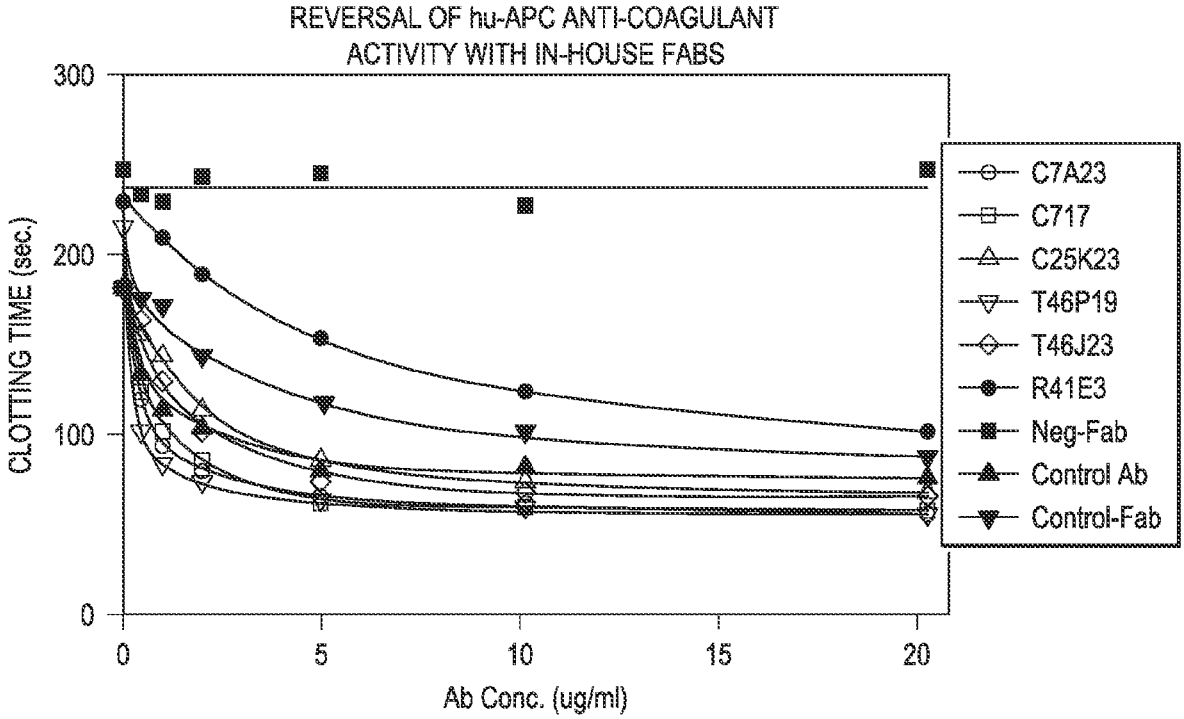


Figure 6

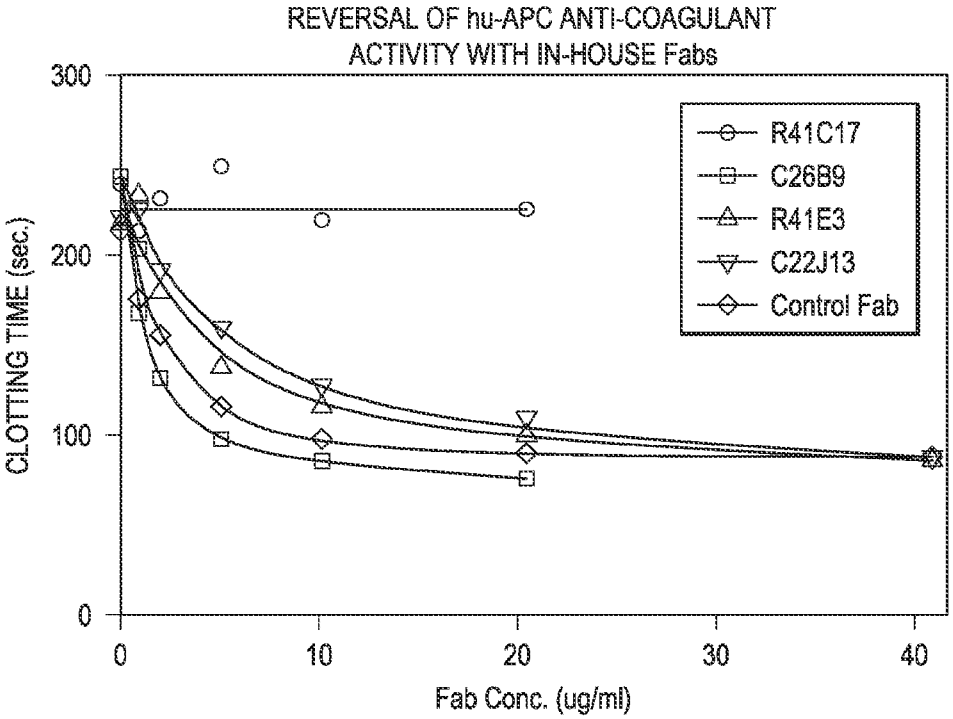


Figure 7

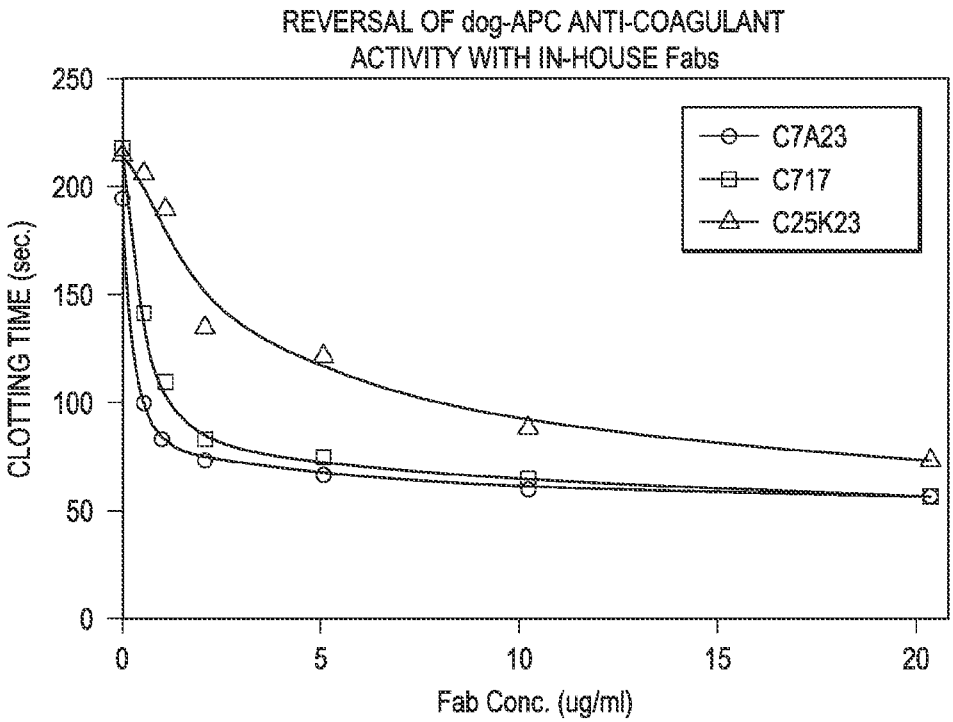
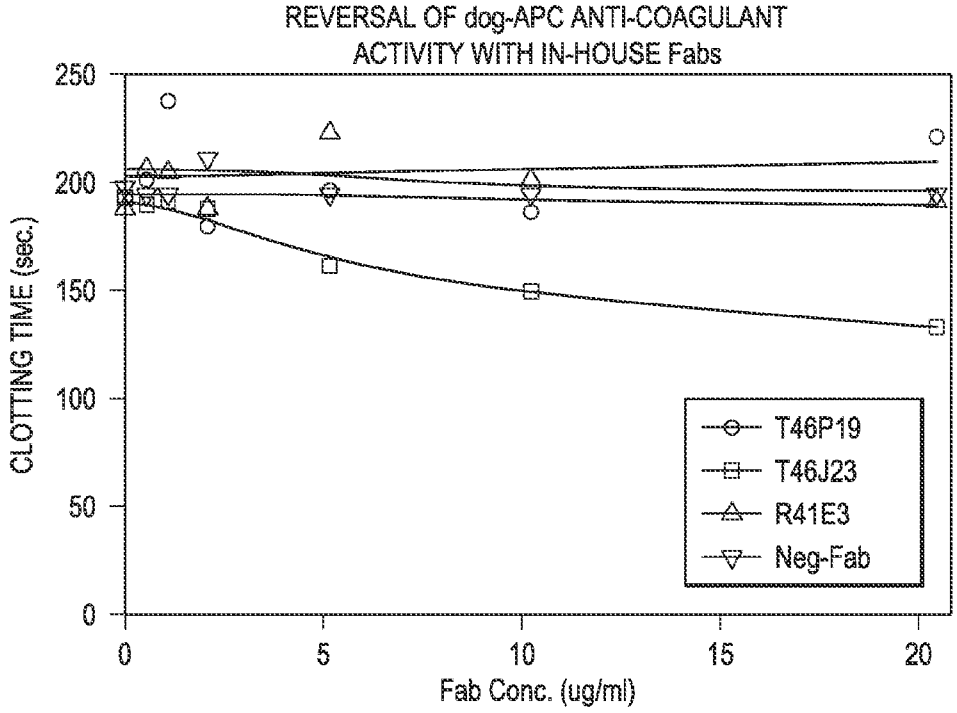


Figure 7



dog-APC (400 ng/ml)

Fab Conc. (ug/ml) (Fab:APC)	% INHIBITION					
	C7A23	C717	C25K23	T46P19	T46J23	R41E3
20.4 (60:1)	95.56	95.16	84.61	0.00	41.48	0.00
10.2 (30:1)	91.59	92.14	75.90	0.00	30.50	0.00
5.08 (15:1)	**88.11**	**48.35**	**55.77**	**0.00**	**21.12**	**0.00**
2.04 (6:1)	82.50	80.27	47.25	0.00	3.45	0.00
1.02 (3:1)	75.32	64.44	15.22	0.00	0.62	0.00
0.51 (1.5:1)	64.59	45.19	5.68	0.00	2.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00

Figure 8

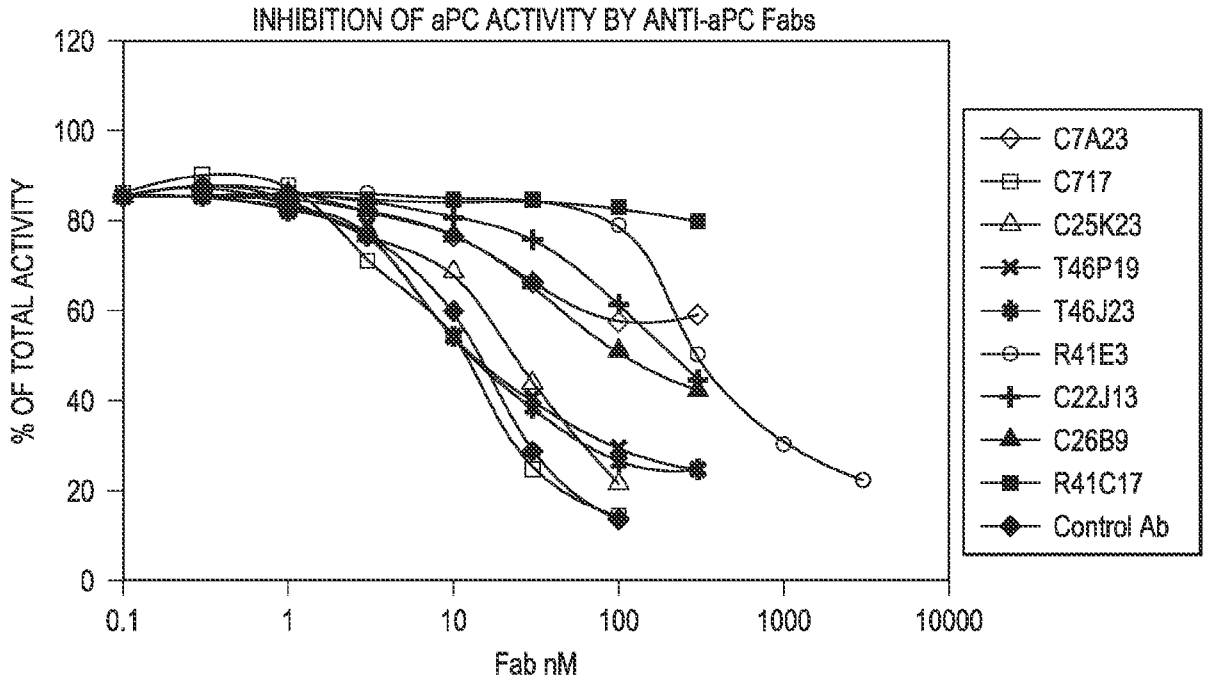


Figure 9

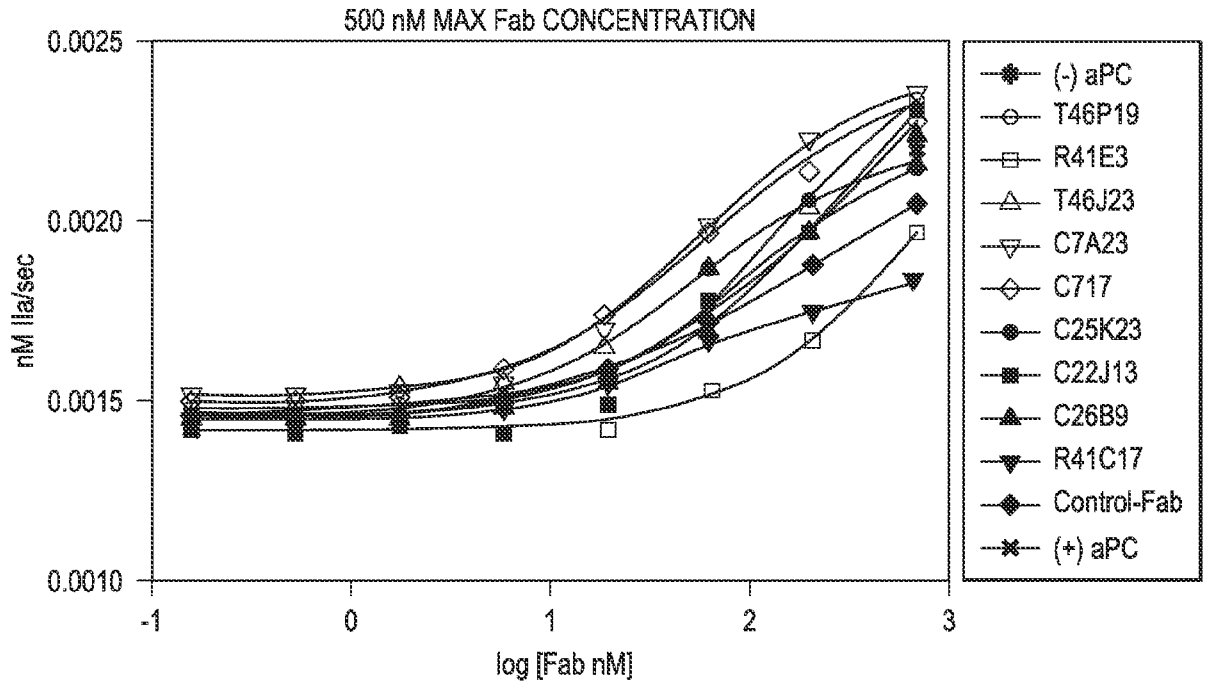
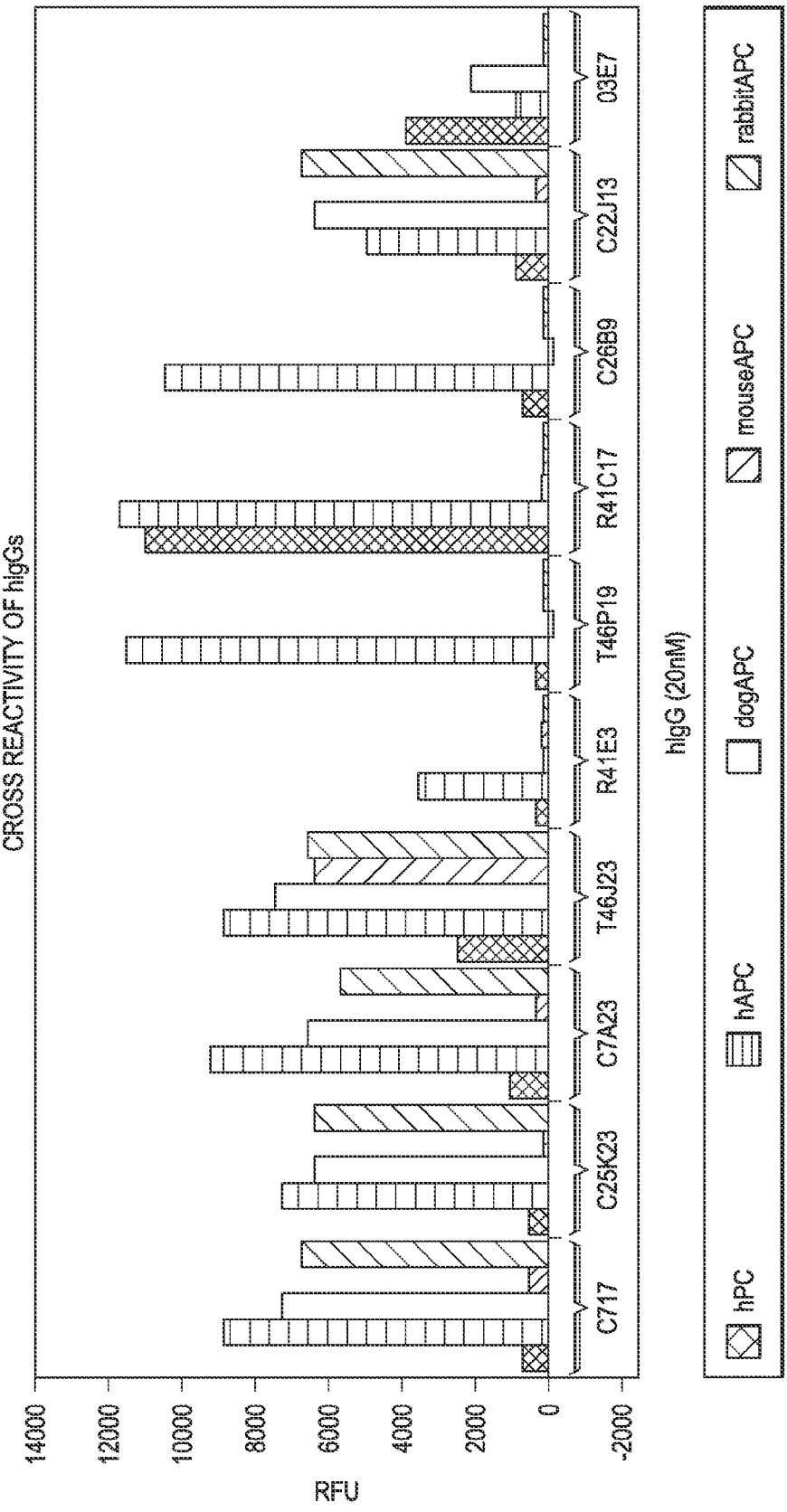
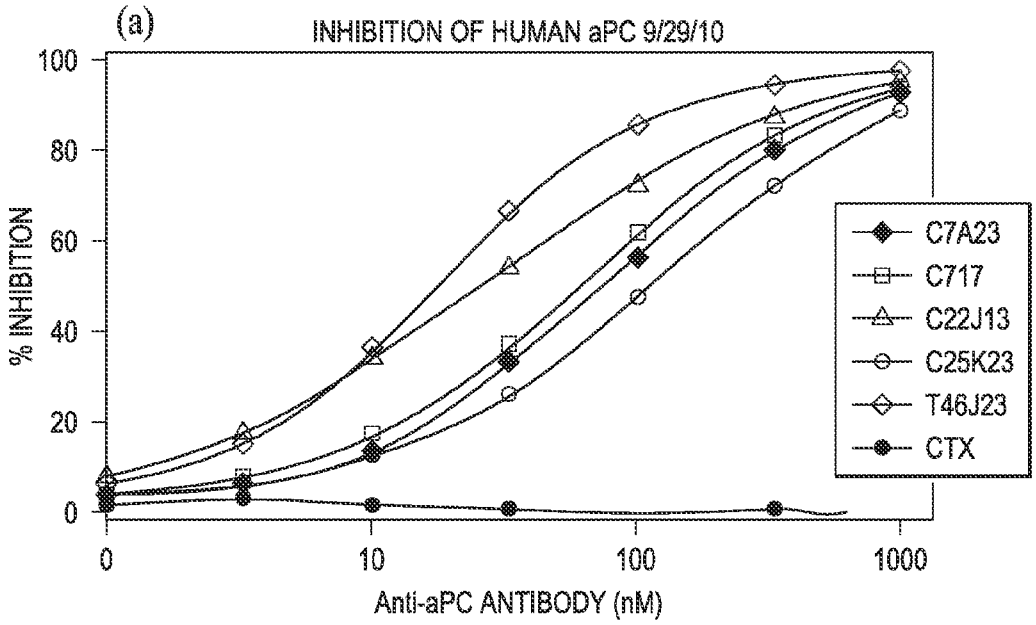


Figure 10



10/20

Figure 11


(a)
INHIBITION OF HUMAN aPC 9/29/10
% INHIBITION
100
80
60
40
20
0
0
10
100
1000
Anti-aPC ANTIBODY (nM)
C7A23
C717
C22J13
C25K23
T46J23
CTX

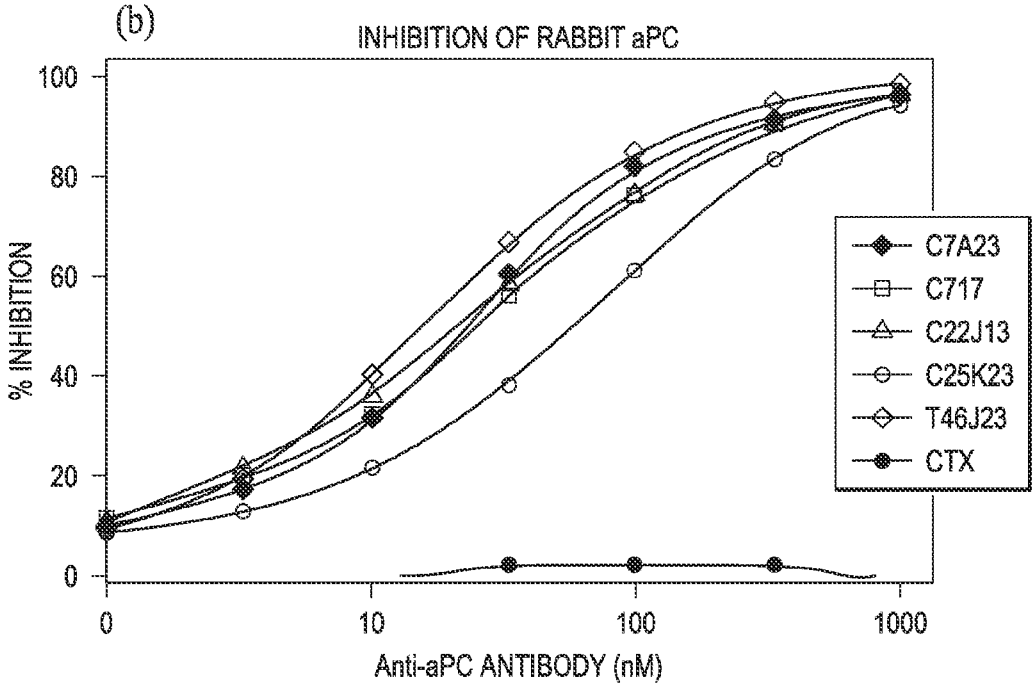
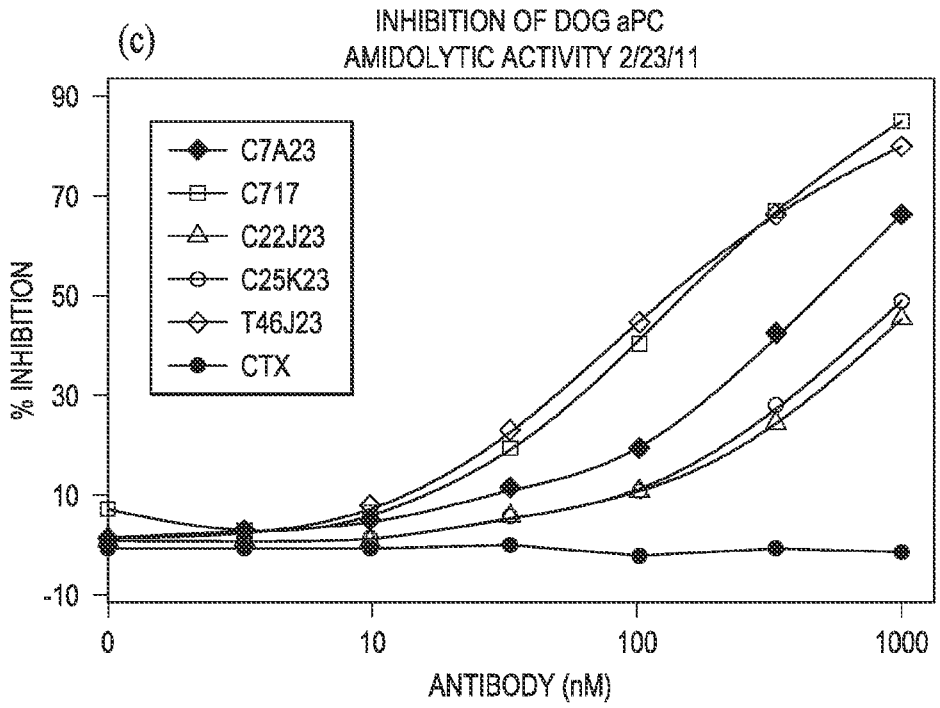

(b)
INHIBITION OF RABBIT aPC
% INHIBITION
100
80
60
40
20
0
0
10
100
1000
Anti-aPC ANTIBODY (nM)
C7A23
C717
C22J13
C25K23
T46J23
CTX

Figure 11


(c)
INHIBITION OF DOG aPC
AMIDOLYTIC ACTIVITY 2/23/11
% INHIBITION
90
70
50
30
10
-10
0
10
100
1000
ANTIBODY (nM)
C7A23
C717
C22J23
C25K23
T46J23
CTX

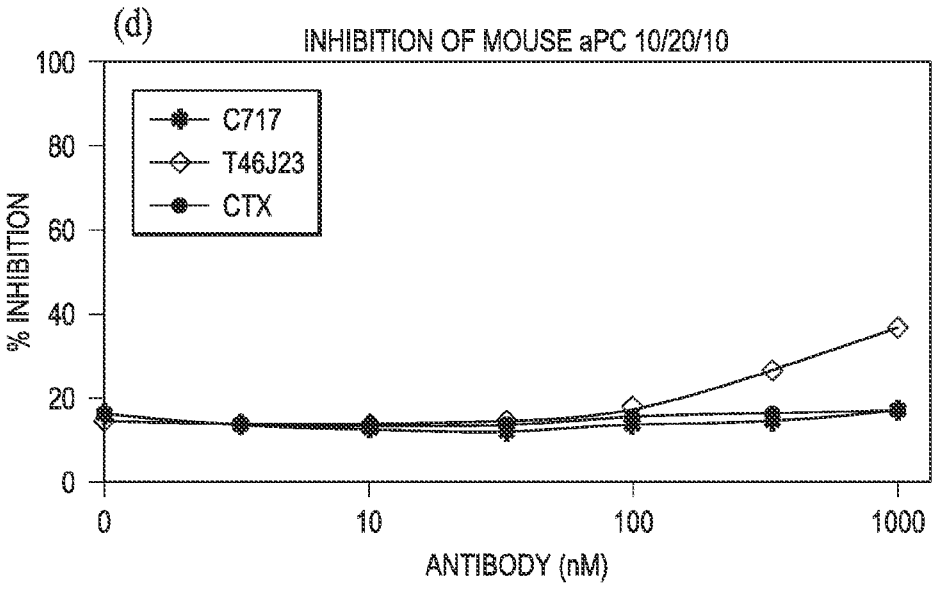

(d)
INHIBITION OF MOUSE aPC 10/20/10
% INHIBITION
100
80
60
40
20
0
0
10
100
1000
ANTIBODY (nM)
C717
T46J23
CTX

Figure 12

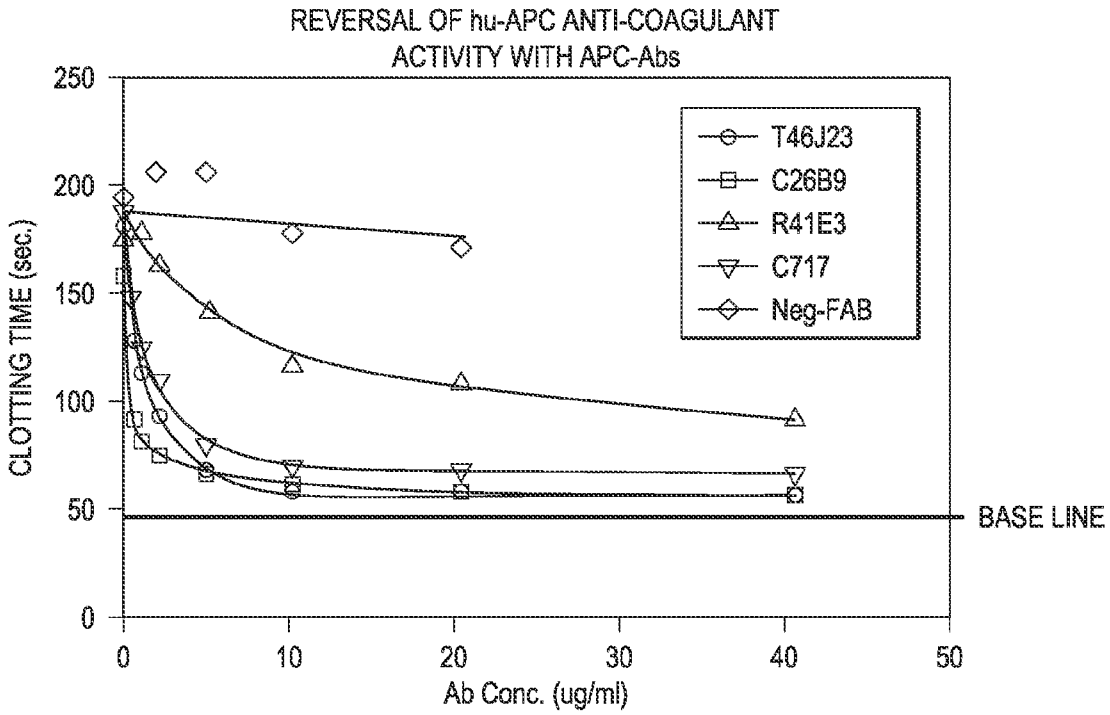

REVERSAL OF hu-APC ANTI-COAGULANT ACTIVITY WITH APC-Abs
CLOTTING TIME (sec.)
250
200
150
100
50
0
0
10
20
30
40
50
Ab Conc. (ug/ml)
T46J23
C26B9
R41E3
C717
Neg-FAB
BASE LINE

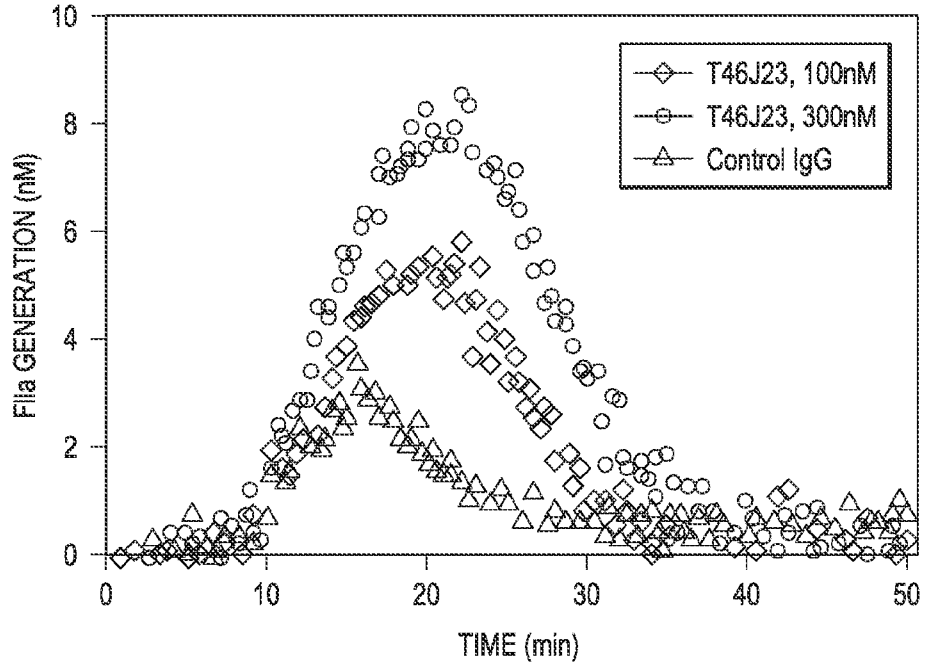
Figure 12

hu-APC (400 ng/ml)

Fab Conc. (ug/ml) (Ab:APC)	CLOTTING TIME					% INHIBITION				
	T46J23	C26B9	R41E3	C717	Neg-IgG	T46J23	C26B9	R41E3	C717	Neg-IgG
163.2 (163:1)			75					80.02		
81.6 (81:1)			80.8					75.43		
40.8 (40:1)	53	56	91.5	62.6		98.45	96.64	66.98	92.89	
20.4 (20:1)	57.9	58.2	109.2	67.4	170.5	94.67	94.53	53.00	89.33	16.07
10.2 (10:1)	59.1	62.0	117	73.2	177.8	93.74	90.89	46.84	85.04	10.88
5.08 (5:1)	68.8	66.3	142.4	79.6	205.5	86.24	86.77	26.78	80.30	-8.82
2.04 (2:1)	93.3	73.5	165.1	109	204.9	67.31	79.87	8.85	58.52	-8.39
1.02 (1:1)	114.1	81.5	177.5	124.8	169.8	51.24	72.20	-0.95	46.81	16.57
0.51 (0.5:1)	129.0	91.5	174.4	147.1	158.1	39.72	62.61	1.50	30.30	24.89
0	180.4	156.8	176.3	188	193.1	0.00	0.00	0.00	0.00	0.00
50% FACT	51.0	52.5	49.7	53.0	52.5					

* R41E3 IS LESS POTENT THAN OTHER IgGs IN INHIBITING THE hu-APC IN APTT ASSAY

Figure 13


T46J23, 100nM
T46J23, 300nM
Control IgG
FIIa GENERATION (nM)
TIME (min)
0
2
4
6
8
10
0
10
20
30
40
50

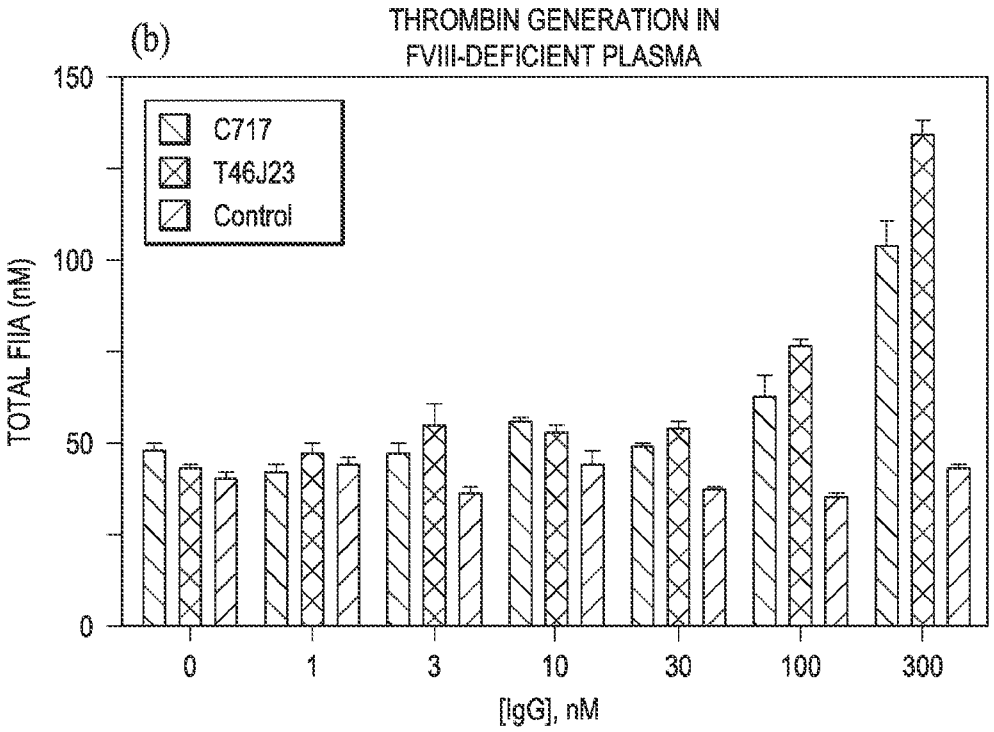
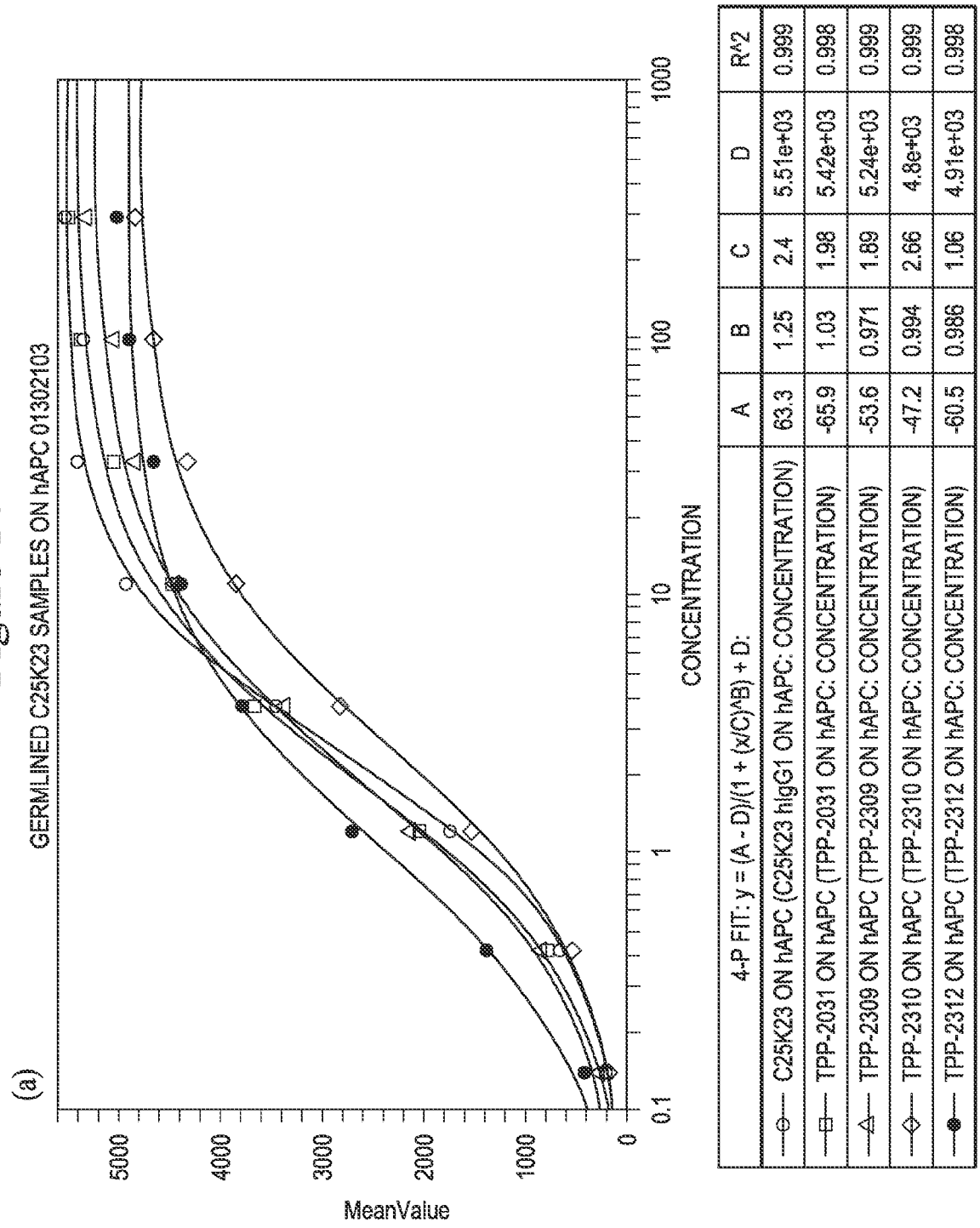
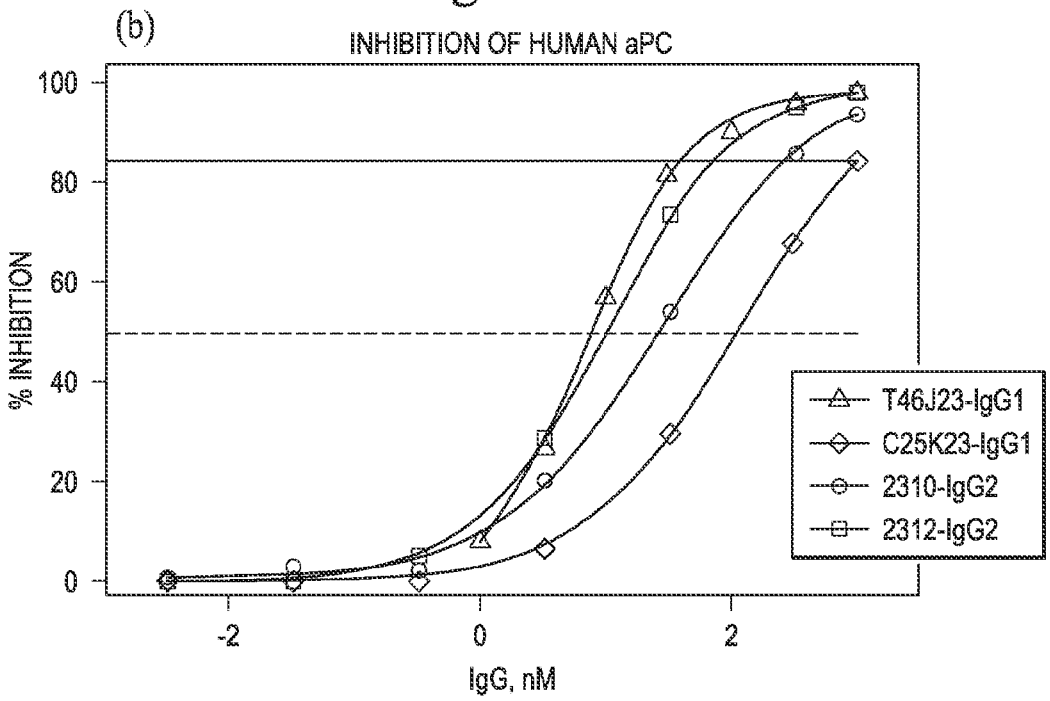

(b)
THROMBIN GENERATION IN
FVIII-DEFICIENT PLASMA
C717
T46J23
Control
TOTAL FIIA (nM)
[IgG], nM
0
50
100
150
0
1
3
10
30
100
300

Figure 14



4-P FIT: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
C25K23 ON hAPC (C25K23 hIgG1 ON hAPC: CONCENTRATION)	63.3	1.25	2.4	5.51e+03	0.999
TPP-2031 ON hAPC (TPP-2031 ON hAPC: CONCENTRATION)	-65.9	1.03	1.98	5.42e+03	0.998
TPP-2309 ON hAPC (TPP-2309 ON hAPC: CONCENTRATION)	-53.6	0.971	1.89	5.24e+03	0.999
TPP-2310 ON hAPC (TPP-2310 ON hAPC: CONCENTRATION)	-47.2	0.994	2.66	4.8e+03	0.999
TPP-2312 ON hAPC (TPP-2312 ON hAPC: CONCENTRATION)	-60.5	0.986	1.06	4.91e+03	0.998

Figure 14


(b)
INHIBITION OF HUMAN aPC
% INHIBITION
100
80
60
40
20
0
-2
0
2
IgG, nM
T46J23-IgG1
C25K23-IgG1
2310-IgG2
2312-IgG2

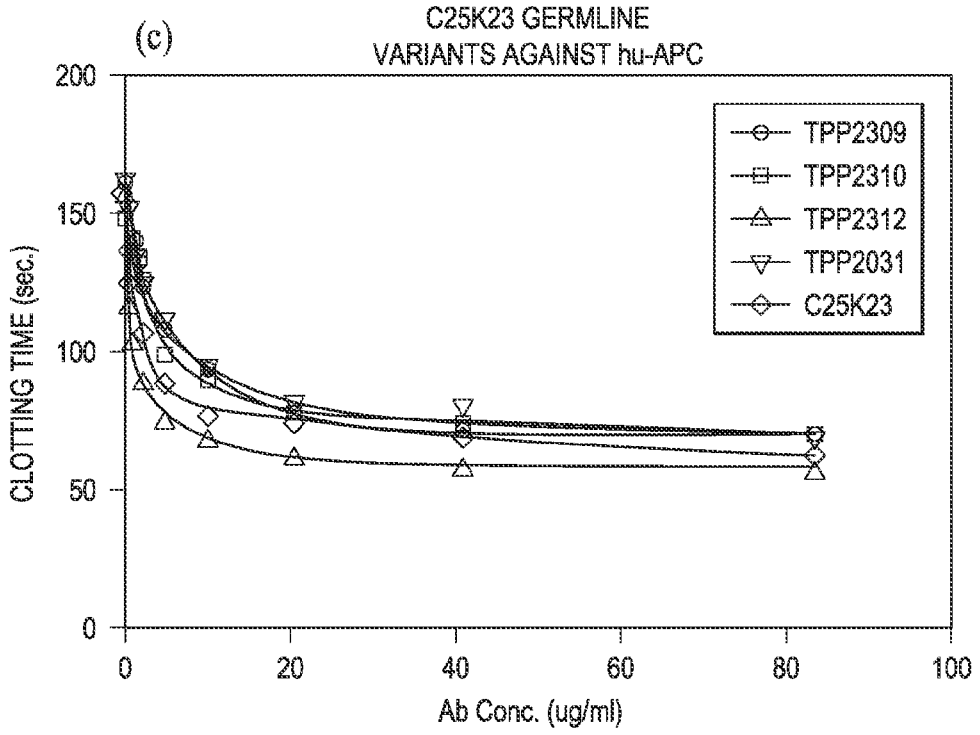

(c)
C25K23 GERMLINE
VARIANTS AGAINST hu-APC
CLOTTING TIME (sec.)
200
150
100
50
0
0
20
40
60
80
100
Ab Conc. (ug/ml)
TPP2309
TPP2310
TPP2312
TPP2031
C25K23

Figure 15

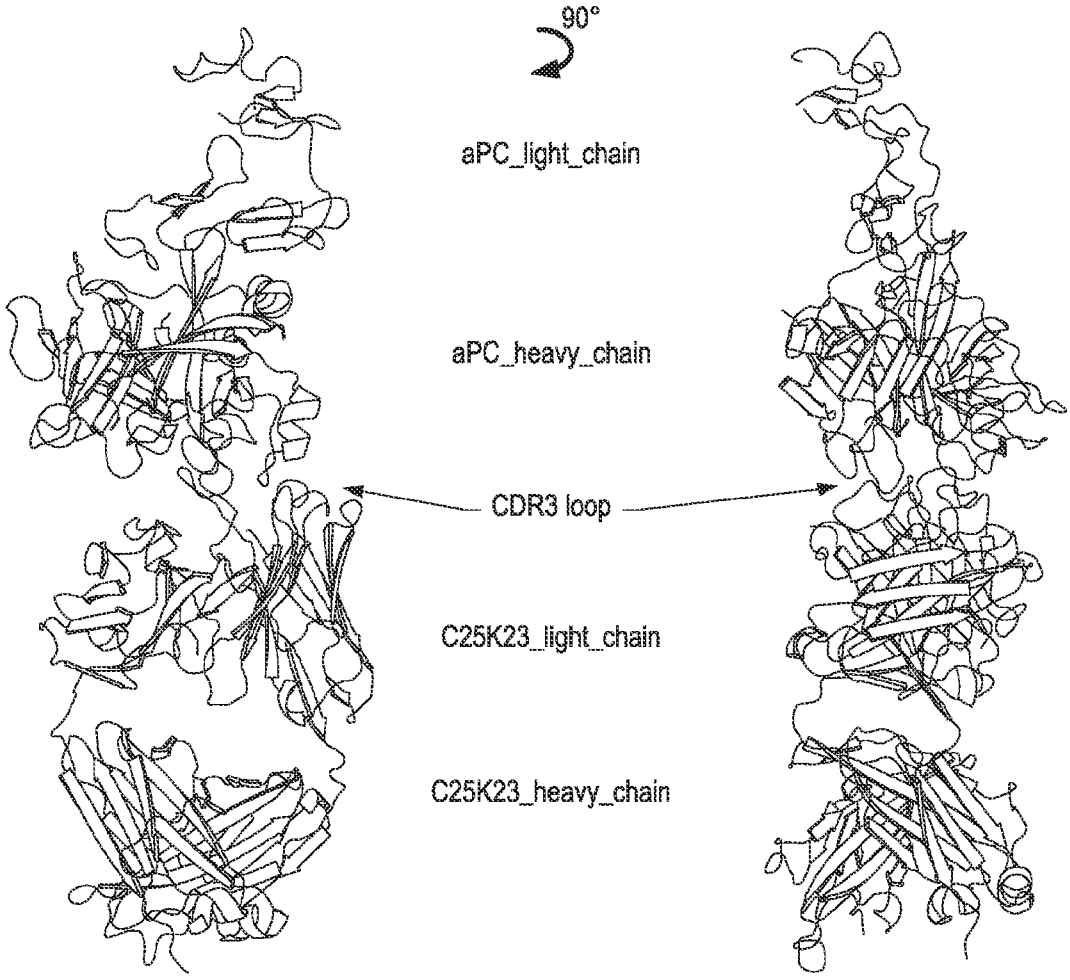

90°
aPC_light_chain
aPC_heavy_chain
CDR3 loop
C25K23_light_chain
C25K23_heavy_chain

Figure 16

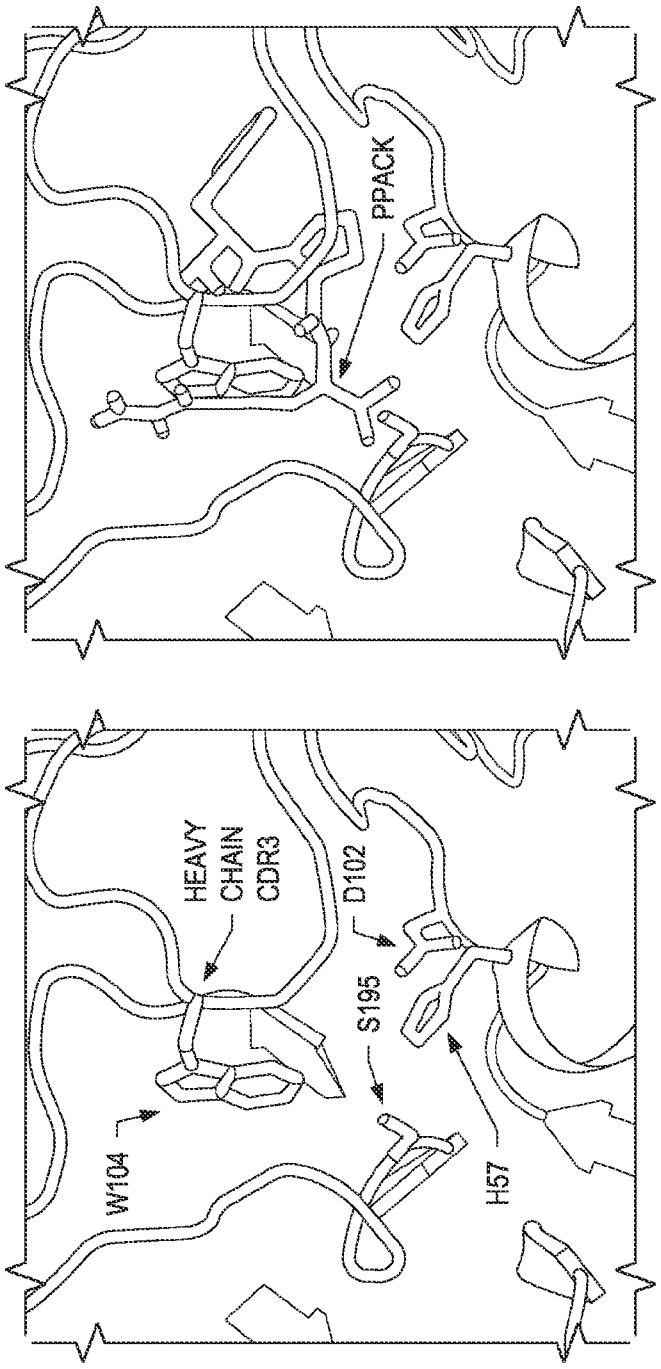
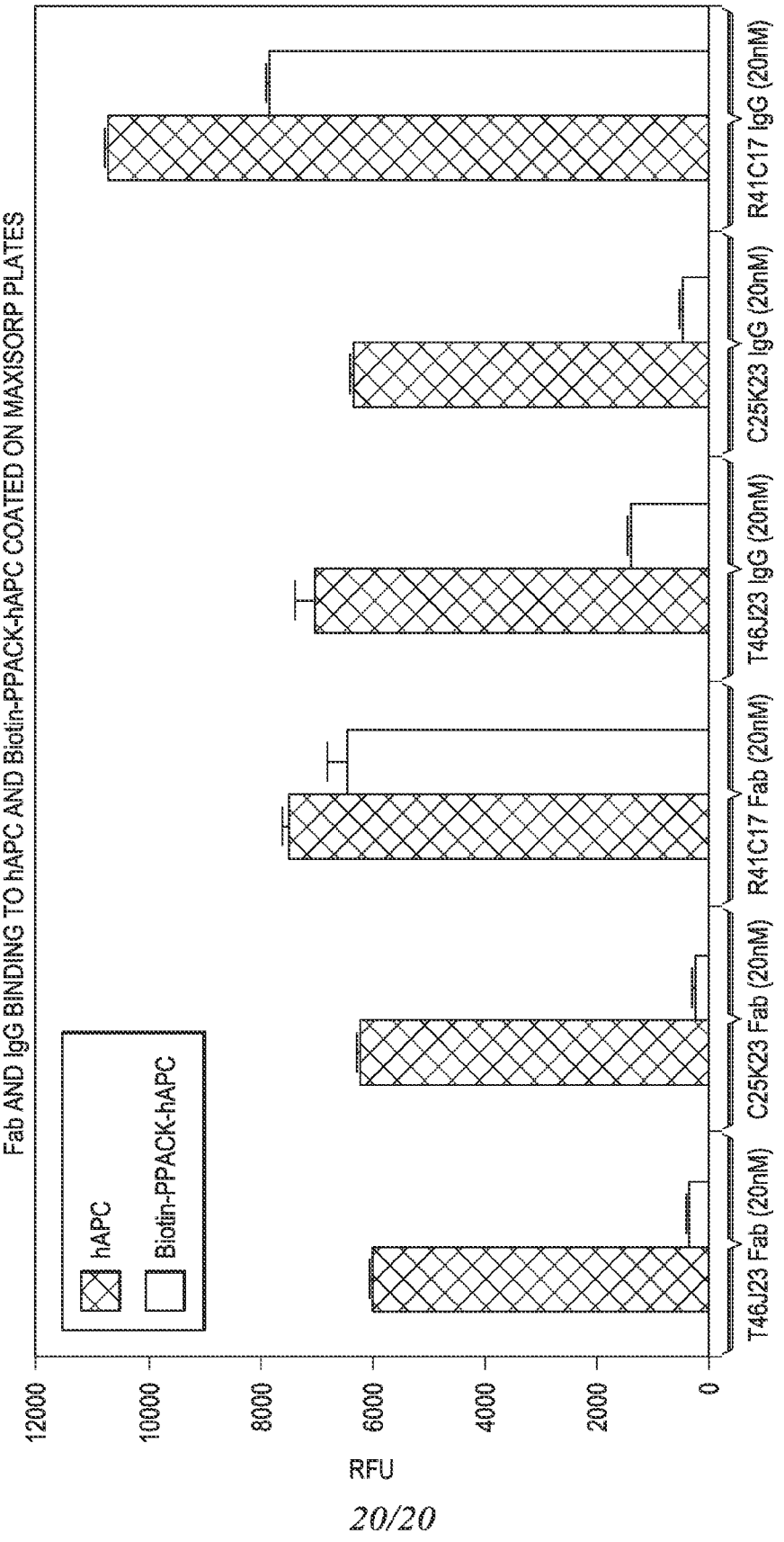


Figure 17


Fab AND IgG BINDING TO hAPC AND Biotin-PPACK-hAPC COATED ON MAXISORP PLATES
hAPC
Biotin-PPACK-hAPC
RFU
12000
10000
8000
6000
4000
2000
0
T46J23 Fab (20nM)
C25K23 Fab (20nM)
R41C17 Fab (20nM)
T46J23 IgG (20nM)
C25K23 IgG (20nM)
R41C17 IgG (20nM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/72243

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/00, 39/395; C07K 16/00, 16/24 (2014.01)
USPC - 424/139.1, 158.1; 530/387.1; 514/1.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) Classification(s): A61K 39/00, 39/395; C07K 16/00, 16/24, 16/28 (2014.01)
USPC Classification(s): 424/139.1, 158.1, 130.1; 530/387.1; 514/1.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; ProQuest; ScienceDirect; monoclonal, antibody, mAb, 'activated protein c', apc, substitution, administer, 'factor VIIa', 'factor VIIIi', 'factor IX', 'autoprothrombin IIA'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	PRESTON, RJ et al. Multifunctional Specificity of the Protein C/Activated Protein C Gla Domain. 25 July 2006. The Journal Of Biological Chemistry, Vol. 281, pp. 28850-28857; DOI: 10.1074/jbc.M604966200; page 28852, right column, second paragraph to page 28853, left column, first paragraph; page 28853, left column, first paragraph	26, 32/26 --- 31(in-part), 32(in-part), 33 (in-part), 34(in-part), 35 (in-part), 36(in-part), 37 (in-part), 38(in-part), 39 (in-part), 40(in-part), 41 (in-part), 42(in-part), and 43(in-part)
X --- Y	US 2009/0110683 A1 (XU, J et al.) April 30, 2009; abstract; paragraphs [0008], [0009], [0014], [0016], [0021], [0024], [0053], [0101], [0103]	29, 30, 32/29, 32/30, 33/32/29, 33/32/30, 34/29, 34/30, 35/29, 35/30, 36/29, 36/30, 37/36/29, 37/36/30, 38/36/29, 38/36/30, 39/38/36/29, 39/38/36/30, 40/38/36/29, 40/38/36/30, 43/36/29, 43/36/30 --- 5, 14, 16, 22-25, 27, 28, 31(in-part), 32(in-part), 33 (in-part), 34(in-part), 35 (in-part), 36(in-part), 37 (in-part), 38(in-part), 39 (in-part), 40 (in-part) ..continued...

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2014 (22.02.2014)	10 MAR 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/72243

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	US 2009/0110683 A1 (XU, J et al.) April 30, 2009; abstract; paragraphs [0008], [0009], [0014], [0016], [0021], [0024], [0053], [0101], [0103]	...from previous.. 41 (in-part), 42(in-part), and 43(in-part) ---------- 1-4, 6-13, 15, 17-21, 31 (in-part), 32(in-part), 33 (in-part), 34(in-part), 35 (in-part), 36(in-part), 37 (in-part), 38(in-part), 39 (in-part), 40(in-part), 41 (in-part), 42(in-part), and 43(in-part), 44, 45
Y	WO 2003/091415 A2 (BRADBURY, A et al.) November 6, 2003; page 62, lines 9-31, table 9	5, 31(in-part), 32(in-part), 33(in-part), 34(in-part), 35(in-part), 36(in-part), 37(in-part), 38(in-part), 39(in-part), 40(in-part), 41(in-part), 42(in-part), and 43(in-part),
Y	WO 2012/007516 A1 (HANSSON, M, et al) January 19, 2012; page 19, line 19-page 20, lines 6-11; page 25, lines 8-12; figure 15	14, 16, 31(in-part), 32 (in-part), 33(in-part), 34 (in-part), 35(in-part), 36 (in-part), 37(in-part), 38 (in-part), 39(in-part), 40 (in-part), 41(in-part), 42 (in-part), and 43(in-part),
Y	US 8,039,597 B2 (RAITANO, AB et al) October 18, 2011; figure 2AR; column 105, lines 11-18; column 106, lines 7-10)	22-25, 31(in-part), 32 (in-part), 33(in-part), 34 (in-part), 35(in-part), 36 (in-part), 37(in-part), 38 (in-part), 39(in-part), 40 (in-part), 41(in-part), 42 (in-part), and 43(in-part),
Y	GALE, AJ et al. Nonenzymatic Anticoagulant Activity Of The Mutant Serine Protease Ser360Ala-Activated Protein C Mediated By Factor Va. 1997. Protein Science, Vol. 6, pp. 132-140; DOI: 10.1002/pro.5560060115; page 132, left column, first paragraph to right column, paragraph 1; page 133, left column, second paragraph	27, 31(in-part), 32 (in-part), 33(in-part), 34 (in-part), 35(in-part), 36 (in-part), 37(in-part), 38 (in-part), 39(in-part), 40 (in-part), 41(in-part), 42 (in-part), and 43(in-part),
Y	US 6,838,437 B2 (KAUFMAN, RJ et al.) January 4, 2005; column 7, lines 54-64	41, 42
Y --- A	US 6,989,241 B2 (ESMON, CT et al.) January 24, 2006; column 4, lines 11-24	31 --- 1-4, 6-13, 15, 17-21, 31 (in-part), 32(in-part), 33 (in-part), 34(in-part), 35 (in-part), 36(in-part), 37 (in-part), 38(in-part), 39 (in-part), 40(in-part), 41 (in-part), 42(in-part), and 43(in-part), 44, 45
A	US 7,879,322 B2 (KNEISSEL, M et al.) February 1, 2011; column 16, lines 41-65; column 121, line 16-column 123, line 30	1, 3, 4, 11-13, 15, 17, 31 (in-part), 32(in-part), 33 (in-part), 34(in-part), 35 (in-part), 36(in-part), 37 (in-part), 38(in-part), 39 (in-part), 40(in-part), 41 (in-part), 42(in-part), and 43(in-part), 44
A	US 7,244,430 B2 (THROSBY, M et al.) July 17, 2007; column 251, line 30-column 252, line 2; Claim 4	2, 45

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(51)Int.Cl.
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A61K 39/395(2006.01)
C07K 16/00(2006.01)
C07K 16/24(2006.01)

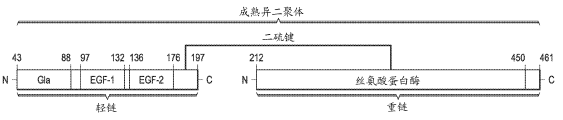
权利要求书4页 说明书29页
序列表45页 附图22页

(54)发明名称

针对活化蛋白C(aPC)的单克隆抗体

(57)摘要

本文提供对其酶原蛋白C(PC)有最低结合的针对人活化蛋白C(aPC)的抗体、抗原-结合抗体片段(Fab)以及其它蛋白质支架。此外,这些aPC结合蛋白可潜在地阻断aPC的抗凝活性以诱导凝血。这些结合体的治疗用途及淘选以及筛选特异性抗体的方法描述于本文。



CN 104812402 A

1.分离的单克隆抗体，其中所述抗体结合至活化蛋白C并抑制抗凝活性、但对未活化蛋白C具有最低结合，其中所述抗体包含含有选自SEQ ID NOs：14、15、17、18、19、21、22、23、109、111、113、115、117以及119的氨基酸序列的重链可变区。

2.分离的单克隆抗体，其中所述抗体结合至活化蛋白C并抑制抗凝活性、但对未活化蛋白C具有最低结合，其中所述抗体包含含有选自SEQ ID NOs：4、5、7、8、9、11、12、13、108、110、112、114、116以及118的氨基酸序列的轻链可变区。

3.如权利要求1的分离的单克隆抗体，其进一步包含含有选自SEQ ID NOs：4、5、7、8、9、11、12、13、108、110、112、114、116以及118的氨基酸序列的轻链可变区。

4.如权利要求3的分离的单克隆抗体，其中所述抗体包含含有下列的重链与轻链可变区：

a)重链可变区，其含有SEQ ID NO：14的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：4的氨基酸序列；

b)重链可变区，其含有SEQ ID NO：15的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：5的氨基酸序列；

c)重链可变区，其含有SEQ ID NO：17的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：7的氨基酸序列；

d)重链可变区，其含有SEQ ID NO：18的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：8的氨基酸序列；

e)重链可变区，其含有SEQ ID NO：19的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：9的氨基酸序列；

f)重链可变区，其含有SEQ ID NO：21的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：11的氨基酸序列；

g)重链可变区，其含有SEQ ID NO：22的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：12的氨基酸序列；

h)重链可变区，其含有SEQ ID NO：23的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：13的氨基酸序列；

i)重链可变区，其含有SEQ ID NO：109的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：108的氨基酸序列；

j)重链可变区，其含有SEQ ID NO：111的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：110的氨基酸序列；

k)重链可变区，其含有SEQ ID NO：113的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：112的氨基酸序列；

l)重链可变区，其含有SEQ ID NO：115的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：114的氨基酸序列；

m)重链可变区，其含有SEQ ID NO：117的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：116的氨基酸序列；以及

n)重链可变区，其含有SEQ ID NO：119的氨基酸序列；以及轻链可变区，其含有SEQ ID NO：118的氨基酸序列。

5.分离的单克隆抗体，其中所述抗体结合至活化蛋白C并抑制抗凝活性、但对未活化

蛋白 C 具有最低结合，其中所述抗体包含含有选自 SEQ ID NOs :94、95、97、98、99、101、102 以及 103 的氨基酸序列的 CDR3。

6. 如权利要求 5 的分离的单克隆抗体，其中所述抗体进一步包含 :(a) 含有选自 SEQ ID NOs :74、75、77、78、79、81、82 以及 83 的氨基酸序列的 CDR1，(b) 含有选自 SEQ ID NOs :84、85、87、88、89、91、92 以及 93 的氨基酸序列的 CDR2，或 (c) 含有选自 SEQ ID NOs :74、75、77、78、79、81、82 以及 83 的氨基酸序列的 CDR1 以及含有选自 SEQ ID NOs :84、85、87、88、89、91、92 以及 93 的氨基酸序列的 CDR2 两者。

7. 分离的单克隆抗体，其中所述抗体结合至活化蛋白 C 并抑制抗凝活性、但对未活化蛋白 C 具有最低结合，其中所述抗体包含含有选自 SEQ ID NOs :64、65、67、68、69、71、72 以及 73 的氨基酸序列的 CDR3。

8. 如权利要求 7 的分离的单克隆抗体，其中所述抗体进一步包含 :(a) 含有选自 SEQ ID NOs :44、45、47、48、49、51、52 以及 53 的氨基酸序列的 CDR1，(b) 含有选自 SEQ ID NOs :54、55、57、58、59、61、62 以及 63 的氨基酸序列的 CDR2，或 (c) 含有选自 SEQ ID NOs :44、45、47、48、49、51、52 以及 53 的氨基酸序列的 CDR1 以及含有选自 SEQ ID NOs :54、55、57、58、59、61、62 以及 63 的氨基酸序列的 CDR2 两者。

9. 如权利要求 5 的分离的单克隆抗体，其中所述抗体进一步包含含有选自 SEQ ID NOs :64、65、67、68、69、71、72 以及 73 的氨基酸序列的 CDR3。

10. 如权利要求 9 的分离的单克隆抗体，其中所述抗体进一步包含 :(a) 含有选自 SEQ ID NOs :74、75、77、78、79、81、82 以及 83 的氨基酸序列的 CDR1，(b) 含有选自 SEQ ID NOs :84、85、87、88、89、91、92 以及 93 的氨基酸序列的 CDR2，(c) 含有选自 SEQ ID NOs :44、45、47、48、49、51、52 以及 53 的氨基酸序列的 CDR1，以及 (d) 含有选自 SEQ ID NOs :54、55、57、58、59、61、62 以及 63 的氨基酸序列的 CDR2。

11. 如权利要求 4 的抗体，其中所述抗体包含含有下列的重链与轻链可变区 :

a) 轻链可变区，其包含含有 SEQ ID NOs :44、54 与 64 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :74、84 与 94 的氨基酸序列 ;

b) 轻链可变区，其包含含有 SEQ ID NOs :45、55 与 65 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :75、85 与 95 的氨基酸序列 ;

c) 轻链可变区，其包含含有 SEQ ID NOs :47、57 与 67 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :77、87 与 97 的氨基酸序列 ;

d) 轻链可变区，其包含含有 SEQ ID NOs :48、58 与 68 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :78、88 与 98 的氨基酸序列 ;

e) 轻链可变区，其包含含有 SEQ ID NOs :49、59 与 69 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :79、89 与 99 的氨基酸序列 ;

f) 轻链可变区，其包含含有 SEQ ID NOs :51、61 与 71 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :81、91 与 101 的氨基酸序列 ;

g) 轻链可变区，其包含含有 SEQ ID NOs :52、62 与 72 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :82、92 与 102 的氨基酸序列 ;以及

h) 轻链可变区，其包含含有 SEQ ID NOs :53、63 与 73 的氨基酸序列 ;以及重链可变区，其包含含有 SEQ ID NOs :83、93 与 103 的氨基酸序列。

12. 如权利要求 4 的分离的单克隆抗体，其进一步包含一个或多个氨基酸修饰。

13. 如权利要求 11 的分离的单克隆抗体，其进一步包含一个或多个氨基酸修饰。

14. 分离的单克隆抗体，其中所述抗体结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合，其中所述抗体包含含有 SEQ ID NO :8 的氨基酸序列的轻链可变区，其中所述氨基酸序列包含一个或多个氨基酸修饰。

15. 如权利要求 13 的分离的单克隆抗体，其中所述修饰为置换。

16. 如权利要求 14 的分离的单克隆抗体，其中所述置换选自下列的位置 :A10、T13、G52、N53、N54、R56、P57、S58、S78、R81、S82、Q91、Y93、S95、S96、L97、S98、G99、S100 以及 V101。

17. 如权利要求 15 的分离的单克隆抗体，其中所述置换选自下列 :A10V、T13A、G52S、G52Y、G52H、G52F、N53G、N54K、N54R、R56K、P57G、P57W、P57N、S58V、S58F、S58R、S78T、R81Q、S82A、Q91R、Q91G、Y93W、S95F、S95Y、S95G、S95W、S95E、S96G、S96A、S96Y、S96W、S96R、L97M、L97G、L97R、L97V、S98L、S98W、S98V、S98R、G99A、G99E、S100A、S100V、V101Y、V101L 以及 V101E。

18. 分离的单克隆抗体，其中所述抗体结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合，其中所述抗体包含含有 SEQ ID NO :18 的氨基酸序列的重链可变区，其中所述氨基酸序列包含一个或多个氨基酸修饰。

19. 如权利要求 18 的分离的单克隆抗体，其中所述修饰为置换。

20. 如权利要求 19 的分离的单克隆抗体，其中所述置换选自 N54 以及 S56 的位置。

21. 如权利要求 20 的分离的单克隆抗体，其中所述置换选自下列 :N54G、N54Q、N54A、S56A 以及 S56G。

22. 分离的单克隆抗体，其中所述抗体结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合，其中所述抗体包含含有 SEQ ID NO :12 的氨基酸序列的轻链可变区，其中所述氨基酸序列包含一个或多个氨基酸修饰。

23. 如权利要求 22 的分离的单克隆抗体，其中所述修饰为置换。

24. 如权利要求 23 的分离的单克隆抗体，其中所述置换选自下列的位置 :T25、D52、N53、N54、N55、D95、N98 以及 G99。

25. 如权利要求 24 的分离的单克隆抗体，其中所述置换选自下列 :T25S、D52Y、D52F、D52L、D52G、N53C、N53K、N53G、N54S、N55K、D95G、N98S、G99H、G99L 以及 G99F。

26. 结合至人活化蛋白 C(人 aPC，SEQ ID NO :3) 的表位的分离的单克隆抗体，其中所述表位包含来自人 aPC 重链的残基。

27. 结合至人活化蛋白 C(人 aPC，SEQ ID NO :3) 的表位的分离的单克隆抗体，其中所述表位包含 SEQ ID NO :3 的 S195。

28. 结合至人活化蛋白 C 的表位的分离的单克隆抗体，其中所述表位包含选自下列的一个或多个残基 :SEQ ID NO :3 的 D60、K96、S97、T98、T99、E170、V171、M172、S173、M175、A190、S195、W215、G216、E217、G218 以及 G218。

29. 结合至活化蛋白 C 的活性位点的分离的单克隆抗体。

30. 分离的单克隆抗体，其中所述抗体结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合，其中所述抗体为完全人抗体。

31. 如权利要求 1-30 的分离的单克隆抗体，其中所述抗体选自下列：IgG1、IgG2、IgG3、IgG4、IgM、IgA1、IgA2、分泌型 IgA、IgD、IgE 抗体以及抗体片段。

32. 如权利要求 1-30 的分离的单克隆抗体，其中所述抗体结合至人活化蛋白 C。

33. 如权利要求 32 的分离的单克隆抗体，其中所述抗体进一步结合至非人物种的活化蛋白 C。

34. 如权利要求 1-30 的抗体，其中凝血时间在所述抗体存在下被缩短。

35. 与如权利要求 1-30 的抗体竞争的抗体。

36. 药物组合物，其包含治疗有效量的如权利要求 1-30 中任一项的单克隆抗体以及药学上可接受的载体。

37. 治疗凝血方面的遗传性或后天缺乏或缺陷的方法，其包含向患者施用治疗有效量的如权利要求 36 的药物组合物。

38. 治疗凝血紊乱的方法，其包含向患者施用治疗有效量的如权利要求 36 的药物组合物。

39. 如权利要求 38 的方法，其中所述凝血紊乱为 A 型血友病、B 型血友病或 C 型血友病。

40. 如权利要求 38 的方法，其中所述凝血紊乱选自创伤引起的凝血紊乱或重度出血患者。

41. 如权利要求 38 的方法，其进一步包含施用凝血因子。

42. 如权利要求 41 的方法，其中所述凝血因子选自因子 VIIa、因子 VIII 或因子 IX。

43. 缩短出血时间的方法，其包含向患者施用治疗有效量的如权利要求 36 的药物组合物。

44. 编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中所述抗体包含含有选自 SEQ ID NOs：14、15、17、18、19、21、22 以及 23 的氨基酸序列的重链可变区。

45. 编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中所述抗体包含含有选自 SEQ ID NOs：4、5、7、8、9、11、12 以及 13 的氨基酸序列的轻链可变区。

针对活化蛋白 C (aPC)的单克隆抗体

[0001] 本申请要求 2012 年 11 月 29 日提交的美国临时专利申请号 61/731,294 和 2013 年 3 月 15 日提交的美国临时专利申请号 61/786,472 的优先权,它们的公开内容因此以其整体作为参考并入本文。

序列表提交

与本申请相关的序列表以电子形式通过 EFS-Web 提交,并因此以其整体作为参考并入说明书。

[0002] 实施方式领域

提供了分离的单克隆抗体和其片段,其优先结合人蛋白 C 的活化形式 (aPC)。

[0003] 背景

人蛋白 C (PC) 酶原在肝中作为 461 个氨基酸残基前体合成并分泌到血液中 (如 SEQ ID NO: 1 中所示)。在分泌之前,单链多肽前体通过除去二肽 (Lys156-Arg157) 和 42 个氨基酸残基前原前导序列(preproleader)转化成异二聚体。异二聚体形式 (417 个残基) 由通过二硫桥连接的轻链 (155aa, 21 kDa) 和重链 (262aa, 41 kDa) 组成 (如 SEQ ID NO: 2 中所示)。PC 酶原包含凝血酶切割位点,导致“活化肽”的去除和 PC 活化成活化的 PC(aPC) 形式 (405 个残基) ,其显示于 SEQ ID NO: 3 中。图 1 提供人 PC 及其活化形式 aPC 的卡通描绘。人 PC 包含 9 个 Gla- 残基和 4 个用于 N- 联糖基化的潜在的位点。轻链包含 Gla 结构域和 2 个 EGF- 样结构域。重链带有活性丝氨酸蛋白酶结构域。

[0004] PC 通常以 3-5ug/ml(~65nM) 在健康人血液中循环且其半衰期为 6-8 小时。循环型 PC 酶原的主要形式是异二聚体形式。PC 的轻链含有一个富含 γ- 羧基谷氨酸 (Gla) 的结构域 (45aa) 、两个 EGF 样结构域 (46aa) 和接头序列。PC 的重链带有 12-aa 的高度极性“活化肽”以及具有典型的丝氨酸蛋白酶催化三联体的催化结构域。

[0005] 人 PC 经历广泛的翻译后修饰,包括糖基化,维生素 K 依赖性 γ- 羧基化和 γ- 羟基化 (1-2)。它含有 23%的碳水化合物(以重量计)和 4 个潜在的 N- 联糖基化位点(一个在轻链 Asn97 和三个在重链 Asn248/313/329)。 其 Gla 结构域含有 9 个 Gla 残基并负责 PC 钙依赖性结合至带负电磷脂膜。Gla 结构域也可结合内皮蛋白 C 受体 (EPCR) ,其在 PC 活化期间与内皮膜上的凝血酶以及凝血调节蛋白密切合作。

[0006] 蛋白 C 酶原通常转化为它的活性酶 - 活化蛋白 C(aPC) 以具有生物效力。PC 途径的活性是由 PC 活化以及 aPC 失活的比率所控制。PC 活化以两步骤过程发生在内皮细胞表面上。其需要 PC 结合 (经由 Gla 结构域) 至内皮细胞上的 EPCR,然后是 PC 通过凝血酶 / 凝血调节蛋白复合物的蛋白水解活化。由内皮细胞表面上的凝血酶 / 凝血调节蛋白催化的在人 PC 重链 Arg12 处的单一切割释放 12-aa 的 AP 并且将酶原 PC 转化成活性丝氨酸蛋白酶 aPC。因此,PC 和的 aPC 的氨基酸序列之间的主要区别是 PC 中存在 12-aa 活化肽,而在 APC 中不存在。PC 活化成 aPC 也诱导构象变化 ;因此只有 aPC 而不是 PC 可在其酶活性位点中被苯甲脒或以氯甲基酮 (CMK) 肽抑制剂标记。近来已解析无 Gla- 结构域 aPC 在与 CMK- 抑制剂的复合物中的晶体结构。人血浆中的主要 aPC 灭活剂为以 100nM 存在于人血浆中的蛋白 C 抑制剂 (PCI),其为丝氨酸蛋白酶抑制蛋白超家族的成员。在生理条件下,aPC

以极低浓度 (1-2ng/ml 或 40pM) 循环于人血液中，半衰期为 20-30min。

[0007] 蛋白 C 途径充当对抗血栓的天然防御机制。其不同于其它抗凝剂，因为它是一种按需系统 (on-demand system)，当凝血反应增强时其能够放大抗凝反应。在受伤之后，产生凝血酶用于凝血。同时，凝血酶也通过结合至排列在血管表面上的凝血调节蛋白而触发抗凝反应，这促使蛋白 C 活化。因此，aPC 生成大体上与凝血酶浓度以及 PC 水平成比例。

[0008] 蛋白 C 途径作为凝血过程的主要调解者的生理学重要性通过三个临床发现显示 :(a) 与蛋白 C 缺乏相关的严重血栓并发症以及通过蛋白 C 补充纠正该缺陷的能力 ; (b) 与蛋白 C 辅因子 (蛋白 S) 缺乏相关的家族性血栓形成倾向 ;以及 (c) 与在其底物 (因子 V Leidei R506Q) 中的遗传性突变有关的血栓风险，使其对于被 aPC 切割具有抗性 (Bernard,GR et.al.N Engl J Med 2001,344 :699-709 综述)。

[0009] 相对于其它维生素 K 依赖性凝血因子，aPC 作为抗凝剂通过两种凝血辅因子 - 因子 Va 以及 VIIIa 的蛋白水解失活来发挥作用，从而抑制凝血酶生成。作为降低的凝血酶水平的结果，由凝血酶诱导的炎症、促凝血以及抗纤维蛋白溶解反应降低。aPC 也通过与纤溶酶原活化因子抑制剂 (PAI) 形成复合物而直接促成增强的纤维蛋白溶解反应。

[0010] 除了其抗凝功能以外，aPC 也引起细胞保护效应，包括抗炎症以及抗凋亡活性，以及内皮屏障功能的保护。aPC 对细胞的这些直接细胞保护效应需要 EPCR 以及 G 蛋白偶联受体，蛋白酶活化受体 -1(PAR-1)。因此，aPC 促进纤维蛋白溶解作用并抑制血栓与炎症。aPC 的抗凝以及细胞保护功能似乎是可分开的。大多数的细胞保护效应主要与 aPC 的抗凝活性无关，且已生成带有最小抗凝活性以及正常细胞保护活性的 aPC 突变体。同样地，也已报导高抗凝但非细胞保护性 aPC 突变体。

[0011] aPC 轻链的 C 端也是高度带电区域，其在蛋白酶结构域中的活性位点的相反侧上含有残基 Gly142-Leu155。E149A-aPC 具有与野生型 aPC 不可区别的酰胺分解活性，但于活化部分凝血活酶时间 (aPTT) 凝血分析中因为对蛋白 S 辅因子活性的敏感性增加而在抗凝活性方面增加超过 3 倍。E149A-aPC 显示在血浆凝血分析中有高活性的抗凝活性以及在体内有高活性的抗血栓效力。该突变体在 LPS 诱发的致死内毒素血症鼠模型中也具有降低的细胞保护以及死亡率降低活性。这暗示，需要 aPC 的细胞保护活性来降低鼠模型中的死亡率。与之相比，aPC 的抗凝活性对于死亡率降低既非必要也非足够。aPC 已用于治疗败血症，一种危及生命的与高凝血性以及综合性炎症反应相关的状况。在败血症中，aPC 疗法的严重副作用为在 2% 患者中所发生的大出血。这一严重的副作用限制其临床使用。

[0012] 概述

提供针对人活化蛋白 C(aPC) 的单克隆抗体。在至少一个实施方式中，该抗 -aPC 单克隆抗体对 aPC 的酶原蛋白 C 表现最小结合。

[0013] 在一些实施方式中，所提供针对 aPC 的单克隆抗体已被最佳化，例如增加亲和力、增加功能活性或降低来自种系序列的差异。

[0014] 也提供分离的单克隆抗体所结合的人 aPC 上的特异性表位。进一步提供编码该特异性表位的分离的核酸分子。

[0015] 也提供包含抗 -aPC 单克隆抗体的药物组合物以及治疗遗传性与后天性凝血缺乏或缺陷诸如 A 型及 B 型血友病的方法。也提供通过将抗 -aPC 单克隆抗体施用给有需要的患者而缩短出血时间的方法。也提供生产结合人 aPC 的单克隆抗体的方法。

[0016] 附图简述

技术人员将理解，下述图仅供说明目的。附图不意欲以任何方式限制本教导的范围。

[0017] 图 1 显示人活化蛋白 C 以其成熟异二聚体形式的卡通图。

[0018] 图 2 显示，重链以及轻链 CDR 的氨基酸序列比对在由人 Fab 抗体库所鉴定的 10 个抗 -aPC Fab 中显示。

[0019] 图 3 描绘通过直接 ELISA 表征抗 -APC Fab 的图。ELISA 板以每孔 100ng 包被人 PC(hPC)、人 aPC(hAPC)、犬 aPC(dAPC)、小鼠 aPC(mAPC)。在 X 轴上标出的经纯化 Fab 以 20nM(1ug/ml) 被添加至板。通过二次抗体 (抗 - 人 Fab-HRP)、继而 HRP 底物 AmplexRed 来检测结合的 Fab。经纯化 Fab 优先结合至人 aPC，并且除了 Fab R41C17 以外显示较少至不结合人 PC。一个 Fab T46J23 也显示与小鼠 aPC 的一些结合。

[0020] 图 4 显示利用 ELISA 的抗 -PC Fab 的结合选择性。

[0021] 图 5 描绘显示利用 aPTT 通过在人 aPC 中跟踪标定 (spiking) 而以剂量依赖性方式抑制正常人血浆血块形成的图。50% 混合的人正常血浆在 52 秒内形成血块。100、200、400、800 或 1600ng/ml 的人 aPC 与血浆的预温育以剂量依赖性方式延长凝血时间。观察到重组人 aPC(rh-APC) 以及血浆衍生人 aPC(pdh-APC) 有近乎相同的效力。

[0022] 图 6 描绘显示在人正常血浆中抗 -aPC Fab 抑制人 aPC 并且引起血块形成的图。400ng/ml 的人 aPC 将血浆凝血时间从 52 秒延长至 180 秒。0、0.5、1、2、5、10 或 20ug/ml 的对照抗体 (对照) 或其 Fab(对照 -Fab) 或选定 Fab 与 aPC 的温育以剂量依赖性方式降低凝血时间 (上图)。也在 40ug/ml 测试三种 Fab(R41E3、C22J13、对照 -Fab) 寻求更高效应 (下图)。

[0023] 图 7 显示在 aPTT 中抗 -aPC Fab 抑制犬 aPC 并且引起血块形成。

[0024] 图 8 显示抗 -aPC Fab 对于 aPC 的酰胺分解活性的影响。人 aPC 蛋白 (20nM) 首先与等体积抗 -aPC Fab(1-3000nM) 在室温预温育 20 分钟，然后将最多 1mM 的显色底物 SPECTROZYME PCa 添加至反应混合物。在 Fab 存在下测量最终浓度为 10nM 的人 aPC 的酰胺分解活性。水解率在 Fab 存在下受到抑制，达到 80% 的最大降低。

[0025] 图 9 显示抗 -aPC Fab 对于 aPC 的因子 Va(FVa) 失活活性的影响。

[0026] 图 10 显示利用 ELISA 抗 -aPC 人 IG1 的结合特异性并显示抗 -aPC 人 IgG1 的物种交叉反应性。ELISA 板以 1ug/ml 包被人 PC(hPC)、人 aPC(hAPC)、犬 aPC、小鼠 aPC、兔 aPC。将纯化 IgG(20nM) 添加至板。通过二次抗体 (抗 - 人 IgG-HRP)、继而为 HRP 底物 AmplexRed 来检测结合的 IgG。五个抗 -aPC 人 IgG1 与犬和兔 aPC 交叉反应而一个 IgG1 也结合小鼠 aPC。

[0027] 图 11 显示抗 -aPC IgG 对于物种 aPC 的酰胺分解活性的影响 -(a) 人、(b) 兔、(c) 犬与 (d) 小鼠。aPC 蛋白 (20nM) 首先与等体积抗 -aPC-hIgG1(1-1000nM) 在室温预温育 20 分钟，然后将最多 1mM 的显色底物 SPECTROZYME PCa 添加至反应混合物。在 Fab 存在下测量最终浓度为 10nM 的 aPC 的酰胺分解活性。水解速率在 IgG 存在下受到抑制。使用阴性对照抗体 (抗 -CTX-hIgG1)。

[0028] 图 12 显示在人血浆凝血分析 (aPTT) 中抗 -aPC-hIgG1 缩短凝血时间并且引起凝血。

[0029] 图 13 显示抗 -aPC-IgG1 对于重度血友病患者血浆的影响。在内皮细胞以及凝血

调节蛋白存在下，PC 被活化成 aPC 并减少凝血酶生成。不同于对照 Ab，抗 -aPC- 抗体快速地抑制该新生成的 aPC 并增加凝血酶生成达 5-10x。增加的凝血酶生成将在患有凝血紊乱的患者中导致改进的凝血。

[0030] 图 14 显示抗 -aPC- 抗体变体的活性概况。类似于亲代抗体 C25K23，该变体 (a) 以高亲和力结合至 aPC、(b) 在经纯化系统中有力抑制 aPC 活性、以及 (c) 在人血浆凝血分析中缩短凝血时间导致凝血。

[0031] 图 15 显示卡通图，描绘复合物结构精修为最终 R 工作 (R_{work}) =0.201，R 游离 (R_{free}) =0.241。左图与右图显示具有 90° 旋转改变的相同复合物结构。来自 Fab C25K23 的 HCDR3 环与 aPC 重链具有广泛的相互作用。

[0032] 图 16 显示，在左图中显示 Fab C25K23 重链的 CDR3 环中残基 Trp104 附近的相互作用的推近视图。其阻断 aPC 的活性位点 (在催化上重要的残基 His57、Asp102，以及 Ser195) 的可接近性。右图显示 Fab C25K23 以类似于 PPACK 抑制剂的方式抑制 aPC 活性，因为 Trp104 以及 PPACK 在活性位点处占据相同区域。

[0033] 图 17 显示描绘利用 ELISA 在 Fab 以及 IgG 二种形式中抗 -aPC 抗体的图，其结合或不结合至活性位点阻断的 aPC。

[0034] 详细描述

如上述，本公开内容提供抗体，包括特异地结合至人蛋白 C 活化形式 (aPC)、但对人蛋白 C 的酶原形式 (PC) 表现比较少反应性或无反应性的单克隆抗体以及其它结合蛋白。

[0035] 为了本专利文件的目的，下列术语将以下文所列定义使用。

[0036] *定义*

在适当时，以单数使用的术语也将包括复数，反之亦然。在下面列出的任何定义与任何其它文件、包括并入本文做为参考资料的任何文件中的该词的用法相冲突的情况下，除非明确意指为相反含义 (例如在术语最初使用的文件中)，否则对于解释本说明书及其相关权利要求的目的，应当总是以下面列出的定义为准。除非另有说明，否则使用“或”表示“和 / 或”。除非另有说明或使用“一或多”为明确不恰当的，否则使用“一”在此表示“一或多”。“包含 (comprise、comprises、comprising)”以及“包括 (include、includes、including)”的应用可交换且不具限制性。例如，术语“包括”应意味着“包括但不限于”。

[0037] 术语“蛋白 C”或“PC”如本文所用意指呈其酶原形式的蛋白 C 的任一变体、同种型和 / 或物种同系物，其被细胞天然地表达并存在于血浆中，且与蛋白 C 的活化形式不同。

[0038] 术语“活化蛋白 C”或“aPC”如本文所用意指蛋白 C 的活化形式，其通过没有存在于蛋白 C 中的 12 个氨基酸活化肽而表征。

[0039] 如本文所用，“抗体”意指完整抗体及其任何抗原结合片段 (也即“抗原 - 结合部分”) 或单链。该术语包括天然发生的或由正常免疫球蛋白基因片段重组过程所形成的全长免疫球蛋白分子 (例如，IgG 抗体)，或是免疫球蛋白分子的免疫活性部分，诸如抗体片段，其保留特异结合活性。不论结构为何，抗体片段与全长抗体所识别的相同抗原结合。例如，抗 -aPC 单克隆抗体片段结合至 aPC 的表位。抗体的抗原结合功能可以由全长抗体的片段来执行。术语抗体的“抗原 - 结合部分”所涵盖的结合片段实例包括 ：(i)Fab 片段，由 VL、VH、CL 与 CH1 结构域组成的单价片段 ；(ii)$F(ab')_2$ 片段，包含在铰链区由二硫桥连接的两个 Fab 片段的二价片段 ；(iii) 由 VH 与 CH1 结构域组成的 Fd 片段 ；(iv) 由抗体单臂的 VL

与 VH 结构域组成的 Fv 片段 ;(v) dAb 片段 (Ward et al.,(1989) Nature 341 :544-546),其由 VH 结构域组成 ;(vi) 分离的的互补决定区 (CDR) ;(vii) 微抗体、双抗体、三抗体、四抗体及 κ 抗体 (参见,例如 Ill et al.,Protein Eng 1997 ;10 :949-57) ;(viii) 骆驼 IgG ;以及 (ix) IgNAR。此外,尽管 Fv 片段的两个结构域 VL 与 VH 由个别基因所编码,但它们可以使用重组方法、通过合成接头连接,使它们制成单一蛋白质链,其中 VL 与 VH 区配对形成单价分子 (已知为单链 Fv(scFv) ;参见,例如 Bird et al.(1988)Science 242 :423-426 ;和 Huston et al(1988)Proc.Natl.Acad.Sci.USA 85 :5879-5883)。该单链抗体也意欲含括在术语抗体的“抗原 - 结合部分”中。这些抗体片段使用本领域技术人员已知的常规技术获得,且以与完整抗体相同的方式来分析片段的效用。

[0040] 另外,预期抗原结合片段可含括在抗体模拟物中。术语“抗体模拟物”或“模拟物”如本文所用意指表现与抗体类似的结合、却是较小的可选抗体或非抗体蛋白的蛋白质。这种抗体模拟物可包含于支架中。术语“支架”指用于将带有订制功能与特性的新产物工程化的多肽平台。

[0041] 如本文所用,术语“抗 -aPC 抗体”意指特异地结合至 aPC 的表位的抗体。当在体内结合至 aPC 的表位时,本文所公开的抗 -aPC 抗体放大凝血级联的一或多个方面。

[0042] 如本文所用,术语“抑制结合”及“阻断结合”(例如,参见抑制 / 阻断 aPC 底物结合至 aPC) 可互换使用并包括部分与完全抑制或阻断蛋白质与其底物,诸如抑制或阻断至少约 10%、约 20%、约 30%、约 40%、约 50%、约 60%、约 70%、约 80%、约 90%、约 95%、约 96%、约 97%、约 98%、约 99% 或约 100%。如本文所用,“约”表示指定数值的 +/- 10%。

[0043] 提及抑制和 / 或阻断 aPC 底物结合至 aPC 时,术语抑制与阻断也包括,当与抗 -aPC 抗体接触时,aPC 对生理底物的结合亲和力与 aPC 不与抗 -aPC 抗体接触时相比可测量的降低,例如,阻断 aPC 与其底物包括因子 Va 或与因子 VIIIa 相互作用至少约 10%、约 20%、约 30%、约 40%、约 50%、约 60%、约 70%、约 80%、约 90%、约 95%、约 96%、约 97%、约 98%、约 99% 或约 100%。

[0044] 术语“单克隆抗体”或“单克隆抗体组分”如本文所用意指单一分子组分的抗体分子制剂。单克隆抗体组分对特定表位表现单一结合特异性以及亲和力。因此,术语“人单克隆抗体”意指表现单一结合特异性的抗体,其具有衍生自人种系免疫球蛋白序列的可变区与恒定区。人抗体可包括不被人种系免疫球蛋白序列编码的氨基酸序列 (例如,通过在体外随机或定点诱变、或通过体内体细胞突变而引入的突变)。

[0045] “分离的抗体”如本文所用,意欲指基本上不含其它生物分子的抗体,包括具有不同抗原特异性的抗体 (例如,结合至 aPC 的分离的抗体基本上不含结合 aPC 以外的抗原的抗体)。在一些实施方式中,分离的抗体以干重计为至少约 75%、约 80%、约 90%、约 95%、约 97%、约 99%、约 99.9% 或约 100% 纯。在一些实施方式中,纯度可通过诸如柱层析、聚丙烯酰胺凝胶电泳或 HPLC 分析的方法来测量。但是,结合至人 aPC 的表位、同种型或变体的分离的抗体对其它相关抗原例如来自其它物种 (例如 aPC 物种同系物) 可具有交叉反应性。此外,分离的抗体可基本上不含其它细胞物质和 / 或化学品。如本文所用,“特异性结合”意指结合至预定抗原的抗体。典型地,表现“特异性结合”的抗体以至少约 105 M-1 的亲和力结合至抗原且以比不相干抗原 (例如 BSA、酪蛋白) 的结合亲和力高的亲和力结合至该抗原,例如至少两倍高。短语“识别抗原的抗体”以及“对抗原具有特异性的抗体”在本文可与术语

“特异地结合至抗原的抗体”互换使用。

[0046] 如本文所用，术语“最低结合”意指不结合至指定抗原和 / 或对特定抗原表现低亲和力的抗体。典型地，对抗原具有最低结合的抗体以低于约 102 M-1 的亲和力结合至该抗原，且不以比其结合至不相干抗原高的亲和力结合至预定抗原。

[0047] 如本文所用，术语“高亲和力”对抗体诸如 IgG 抗体而言意指至少约 107 M-1 的结合亲和力，在至少一个实施方式中至少约 108 M-1、在一些实施方式中至少约 109 M-1、1010 M-1、1011 M-1 或更高，例如最多 1013 M-1 或更高。但是，“高亲和力”结合对其它抗体同种型可改变。例如，对 IgM 同种型的“高亲和力”结合意指至少约 107 M-1 的结合亲和力。如本文所用，“同种型”意指由重链恒定区基因编码的抗体类型（例如 IgM 或 IgG1）。

[0048] “互补决定区”或“CDR”意指抗体分子的重链可变区或轻链可变区内三个高变区中的一个，其形成与被结合抗原的三维结构互补的 N 端抗原 - 结合面。从重链或轻链的 N 端开始，这些互补决定区分别表示为“CDR1”、“CDR2”以及“CDR3”[Wu TT, Kabat EA, Bilofsky H, Proc Natl Acad Sci U S A. 1975 Dec ;72(12) :5107 和 Wu TT, Kabat EA, J Exp Med. 1970 Aug 1 ;132(2) :211]。CDR 涉及抗原 - 抗体结合，且 CDR3 包含对抗原 - 抗体结合具有特异性的独特区域。因此，抗原 - 结合位点可包括六个 CDR，其包含来自重链与轻链 V 区的每一个的 CDR 区。

[0049] 术语“表位”意指抗体特异地结合或相互作用的抗原的范围或区域，其在一些实施方式中指示抗原在物理上与抗体接触之处。相反地，术语“抗原互补位 (paratope)”意指抗原特异地结合于其上的抗体的范围或区域。如果对应抗体结合是互相排他性的，也即一个抗体的结合排除另一个抗体的同时结合，通过竞争结合表征的表位被称为是重叠的。如果抗原能够容纳两个对应抗体同时结合，则表位被称为是个别的（独特的）。

[0050] 术语“竞争抗体”如本文所用，意指结合至与对抗如本文所述 aPC 的抗体大概、实质上或基本上相同、或甚至相同表位的抗体。“竞争抗体”包括具有重叠表位特异性的抗体。因此，竞争抗体能够有效与如本文所述抗体竞争结合至 aPC。在一些实施方式中，竞争抗体可结合至与如本文所述抗体相同的表位。换个角度来看，竞争抗体具有与如本文所述抗体相同的表位特异性。

[0051] 如本文所述，“保守性置换”意指多肽修饰，其涉及将一或多个氨基酸置换成具有相似生物化学特性但不造成多肽的生物或生物化学功能丧失的氨基酸。“保守性氨基酸置换”是其中将氨基酸残基以具有相似侧链的氨基酸残基取代。具有相似侧链的氨基酸残基家族在本领域中已经定义。该家族包括下列 :具有碱性侧链（例如赖氨酸、精氨酸、组氨酸）、酸性侧链（例如天冬氨酸、谷氨酸）、不带电极性侧链（例如甘氨酸、天冬酰胺、谷氨酰胺、丝氨酸、苏氨酸、酪氨酸、半胱氨酸）、非极性侧链（例如丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、甲硫氨酸、色氨酸）、β 分支侧链（例如苏氨酸、缬氨酸、异亮氨酸）以及芳香族侧链（例如酪氨酸、苯丙氨酸、色氨酸、组氨酸）的氨基酸。本公开内容的抗体可具有一或多个仍保留抗原结合活性的保守性氨基酸置换。

[0052] 关于核酸以及多肽，术语“实质同源性”表示两个核酸或两个多肽或其指定序列在应用适当核苷酸或氨基酸插入或缺失最佳比对并比较时，在至少约 80% 的核苷酸或氨基酸，通常至少约 85%，在一些实施方式中约 90%、91%、92%、93%、94% 或 95%，在至少一个实施方式中至少约 96%、97%、98%、99%、99.1%、99.2%、99.3%、99.4% 或 99.5% 的核苷酸或氨基酸中是

相同的。可选地，当区段在选择杂交条件下会与该链的互补体杂交时，存在核酸的实质同源性。与本文陈述的特定核酸序列以及氨基酸序列具有实质同源性的核酸序列以及多肽序列也包括在内。

[0053] 两个序列之间的同一性百分比是序列所共有的相同位置的数目的函数（也即，% 同源性 = 相同位置数 / 位置总数 x 100），考虑空位数以及各个空位长度，其需要被引入用于两个序列的最佳比对。序列比较以及两个序列之间的同一性百分比测定可使用数学计算法来完成，例如而非限制，为 VectorNTI ™ 的 AlignX ™ 模块 (Invitrogen Corp.，Carlsbad，CA)。对于 AlignX ™，多重比对的缺省参数为：空位开放罚分：10；空位延伸罚分：0.05；空位分离罚分范围：8；比对延迟的同一性 %：40（更多详细内容见于 http：//www.invitrogen.com/site/us/en/home/LINNEA-Online-Guides/LINNEA-Communities/Vector-NTI-Community/Sequence-analysis-and-data-management-software-for-PCs/AlignX-Module-for-Vector-NTI-Advance.reg.us.html）。

[0054] 测定查询序列（本公开内容的序列）以及目标序列之间的最佳整体匹配的另一个方法也称为全局序列比对 (global sequence alignment)，其可使用 CLUSTALW 计算机程序 (Thompson et al.，Nucleic Acids Research，1994，2(22)：4673-4680) 来测定，该计算机程序是以 Higgins 等人的算法 (Computer Applications in the Biosciences(CABIOS)，1992，8(2)：189-191) 为基础。在序列比对时，查询序列以及目标序列均为 DNA 序列。该全局序列比对的结果是以同一性百分比来表示。可用于 DNA 序列的 CLUSTALW 比对中以通过成对比对来计算同一性百分比的参数为：矩阵 =IUB、k-tuple=1、顶对角数目（Number of Top Diagonals）=5、空位罚分 =3、空位开放罚分 =10、空位延伸罚分 =0.1。关于多重比对，可使用下列 CLUSTALW 参数：空位开放罚分 =10、空位延伸罚分 =0.05；空位分离罚分范围 =8；比对延迟的同一性 %=40。

[0055] 核酸可存在于整个细胞中、在细胞裂解物中、或呈部分纯化或实质上纯的形式。当从在天然环境中与核酸通常缔合的其它细胞组分纯化出来时，核酸是“分离的”或“使其实质上纯的”。为了分离核酸，可使用诸如下列的标准技术：碱 /SDS 处理、CsCl 成带、柱层析、琼脂糖凝胶电泳以及本领域熟知的其它技术。

[0056] *针对活化蛋白 C的单克隆抗体*

已知 aPC 的抗凝特性。在血友病中或在伤口导致止血暂时丧失的创伤患者中稳态失调的出血性病症可通过 aPC 抑制剂治疗。抗体、其抗原 - 结合片段以及其它 aPC 特异性蛋白质支架可用于提供靶向特异性来抑制 aPC 蛋白质的子集发挥作用，同时保留余者。假定血浆中 aPC 浓度 (<4ng/ml) 对 PC(4ug/ml) 差异为至少 1000 倍，任一种潜在 aPC 抑制剂疗法的增加的特异性有助于阻断 aPC 在高循环过量 PC 存在下发挥作用。

[0057] 阻断 aPC 的抗凝功能的 aPC 特异性抗体可作为治疗剂用于患有出血性病症的患者，出血性病症包括，例如血友病、带有抑制剂的血友病患者、创伤引起的凝血紊乱、在通过 aPC 治疗败血症期间严重出血的患者、择期手术引起的出血诸如移植、心脏手术、整形外科手术、或月经过多引起的过度出血。

[0058] 具有长循环半衰期的抗 -aPC 抗体可用于治疗慢性疾病如血友病。具有较短半衰期的 aPC 抗体片段或 aPC- 结合蛋白质支架对于急性用途（例如在创伤中的治疗用途）可更为有效。因为 aPC 是多功能蛋白质，包括抗体、抗原 - 结合抗体片段、aPC- 特异性蛋白质

支架的亲和性与靶向特异性增加的选择性 aPC 功能阻断剂 (SAFB) 可选择性地仅只阻断一个 aPC 功能而不影响其它 aPC 功能。

[0059] 通过淘选以及筛选对抗人 aPC 的人抗体库来鉴定 aPC- 结合抗体。经鉴定的抗体表现不结合或最低结合至人 PC。对分离的每一单克隆抗体的重链可变区以及轻链可变区进行测序并鉴定其 CDR 区。对应于 aPC- 特异性单克隆抗体每一个的重链区与轻链区的序列标识号 (“SEQ ID NO：”) 归纳于表 1 中。

[0060] 表 1. 人抗 aPC 抗体

克隆	轻链可变区	SEQ ID	重链可变区	SEQ ID
C7A23	QSVLTQPPSASGTPGQRVT ISCSGSSSNIGNNYVSWYQ QLPGTAPKLLIYRNNQRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCQSYDSD LSGPYVLFGGGTKLTVLG	SEQ ID NO: 4	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTS	SEQ ID NO: 14
C7I7	QSVLTQPPSASGTPGQRVT ISCTGSSSNIGAGYDVHWY QQLPGTAPKLLIYGNSNRP SGVPDRFSGSKSGTSASLAI SGLRSEDEAAYYCSSYVGS DLVVFGGGTKLTVLG	SEQ ID NO: 5	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTS	SEQ ID NO: 15
O3E7	QSVLTQPPSTSGTPGQRVTI SCTGSSSNIGAGFDVHWY QQLPGTAPKLLIYGNSNRP SGVPDRFSGSKSGTSASLAI SGLRSEDEADYYCATWQD TLTGWMFGGGTKLTVLG	SEQ ID NO: 6	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYSMNWVRQ APGKGLEWVSAISGSGGSTY YADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCAR DRRVRGIYDAFDMWGQGTL VTVTS	SEQ ID NO: 16
C22J13	QSVLTQPPSASGTPGQRVT ISCSGSDSNIGSNAVNWYQ QLPGTAPKLLIYDNNKRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCSSYTSSN TVVFGGGTKLTVLG	SEQ ID NO: 7	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSNYMSWVRQ APGKGLEWVAVISYDGSNK YYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAMYYCA LTGRSGWMRFPNWFDPWG QGTLVTVTS	SEQ ID NO: 17
C25K23	QSVLTQPPSASGTPGQRVT ISCTGSSSNIGAAYDVHWY QQLPGTAPKLLIYGNNKRP SGVPDRFSGSKSGTSASLAI SGLRSEDEADYYCQSYDSS LSGSVFGGGTKLTVLG	SEQ ID NO: 8	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYWMSWVRQ APGKGLEWVSGVSWNGSRT HYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA LTGRSGWMRFPNWFDPWG QGTLVTVTS	SEQ ID NO: 18
C26B9	QSVLTQPPSASGTPGQRVT ISCSGSSSNIRSNTVNWYQ QLPGTAPKLLIYGNSNRPS GVPDRFSGSKSGTSASLAIS GLRSEDEADYYCQSYDSSL SGDVVFGGGTKLTVLG	SEQ ID NO: 9	EVQLLESGGGLVQPGGSLRL SCAASGFTFSSYGMHWVRQ APGKGLEWVSVIYSGGSTY YADSVKGRFTISRDNSKNTL YLQMNSLRAEDTAVYYCAR MGRAFDIWGQGTLVTVTS	SEQ ID NO: 19
R41C17	LTQPPSASGTPGQRVTISCT GSSSNIGAGYVVHWYQQL PGTAPKLLIYRNNHRPSGV PDRFSGSKSGTSASLAISGL RSEDEADYYCAAWDDSLN GRVFGGGTKLTVLGQPKA APSVTLFP	SEQ ID NO: 10	EVQLLESGGGLVQPGGSLRL SCAASGFTFSNYAMSWVRQ SPGKGLEWVAVISYDGREK YYSDSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA RDRGRTFDYWGQGTLVTVT SASTKGPSVF	SEQ ID NO: 20
R41E3	LTQPPSASGTPGQRVTISCS GSSSNIGNNAVNWYQQLP GTAPKLLIYSNNQRPSGVP DRFSGSKSGTSASLAISGLR	SEQ ID NO: 11	EVQLLESGGGLVQPGGSLRL SCAASGFTFNNYAMTWVRQ APGKGLEWVSGVSWNGSRT HYADSVKGRFTISRDNSKNT	SEQ ID NO: 21

克隆	轻链可变区	SEQ ID	重链可变区	SEQ ID
	SEDEADYYCSSYTSSSTHV VFGGGTKLTVLGQPKAAP SVTL		LYLQMNSLRAEDTAVYYCA RADSSSAGRWAGSLDYWG QGTLVTVTSASTKGPSVF	
T46J23	LTQPPSASGTPGQRVTISCT GTSSNIGAGYDVHWYQQL PGTAPKLLIYDNNNRPSGV PDRFSGSKSGTSASLAISGL RSEDEADYYCAAWDDSLN GVVFGGGTKLTVLGQPKA APSVTLFP	SEQ ID NO: 12	EVQLLESGGGLVQPGGSLRL SCAASGFTFGNHWMTWVR QAPGKGLEWVSGVSWNGSR THYADSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYC ALTGRSGWMRFPNWFDPW GQGTLVTVTSASTKGPSVF	SEQ ID NO: 22
T46P19	LTQPPSASGTPGQRVTISCT GSSSNIGAGYDVHWYQQL PGTAPKLLIYGNINRPSGVP DRFSGSKSGTSASLAISGLR SEDEADYYCSSYTRSATLV FGGGTKLTVLGQPKAAPS VTLFP	SEQ ID NO: 13	EVQLLESGGGLVQPGGSLRL SCAASGFTFSGYGMHWVRQ APGKGLEWVSGINWNGGST GYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCA RNRATRSGYYYFDSWGQGT LVTVTSASTKGPSVF	SEQ ID NO: 23

[0061] 在一个实施方式中，提供结合至人活化蛋白C(aPC)并抑制抗凝活性、但对未活化蛋白C具有最低结合的分离的单克隆抗体，其中该抗体包含含有选自SEQ ID NO:14-23的氨基酸序列的重链可变区。

[0062] 在另一个实施方式中，提供结合至人活化蛋白C(aPC)并抑制抗凝活性、但对未活化蛋白C具有最低结合的分离的单克隆抗体，其中该抗体包含含有选自SEQ ID NO:4-13的氨基酸序列的轻链可变区。

[0063] 在另一个实施方式中，提供结合至人活化蛋白C(aPC)并抑制抗凝活性、但对未活化蛋白C具有最低结合的分离的单克隆抗体，其中该抗体包含含有选自SEQ ID NO:14-23的氨基酸序列的重链可变区、以及含有选自SEQ ID NO:4-13的氨基酸序列的轻链可变区。

[0064] 在其它实施方式中，抗体包含重链可变区以及轻链可变区，该重链可变区以及轻链可变区包含：

重链可变区，其含有SEQ ID NO:14的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:4的氨基酸序列；

重链可变区，其含有SEQ ID NO:15的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:5的氨基酸序列；

重链可变区，其含有SEQ ID NO:16的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:6的氨基酸序列；

重链可变区，其含有SEQ ID NO:17的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:7的氨基酸序列；

重链可变区，其含有SEQ ID NO:18的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:8的氨基酸序列；

重链可变区，其含有SEQ ID NO:19的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:9的氨基酸序列；

重链可变区，其含有SEQ ID NO:20的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:10的氨基酸序列；

重链可变区，其含有SEQ ID NO:21的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:11的氨基酸序列；

重链可变区，其含有SEQ ID NO:22的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:12的氨基酸序列；以及

重链可变区，其含有SEQ ID NO:23的氨基酸序列；以及轻链可变区，其含有SEQ ID NO:13的氨基酸序列。

[0065] 表2中所示为结合至人aPC的单克隆抗体的每一重链与轻链的CDR区（“CDR1”、“CDR2”以及“CDR3”）的SEQ ID NOs归纳。

[0066] 表2. 人抗-aPC抗体的CDR区的序列标识符

克隆	轻链可变区			重链可变区		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
C7A23	44	54	64	74	84	94
C7I7	45	55	65	75	85	95
O3E7	46	56	66	76	86	96
C22J13	47	57	67	77	87	97
C25K23	48	58	68	78	88	98
C26B9	49	59	69	79	89	99
R41C17	50	60	70	80	90	100
R41E3	51	61	71	81	91	101
T46J23	52	62	72	82	92	102
T46P19	53	63	73	83	93	103

[0067] 在一个实施方式中，提供结合至人活化蛋白C(aPC)的分离的单克隆抗体，其中该抗体包含含有选自SEQ ID NO:94-103的氨基酸序列的CDR3。这些CDR3由淘选以及筛选期间鉴定的抗体的重链鉴定。在又一个实施方式中，此抗体进一步包含(a)含有选自SEQ ID NO:74-83的氨基酸序列的CDR1、(b)含有选自SEQ ID NO:84-93的氨基酸序列的CDR2，或(c)含有选自SEQ ID NQ:74-83的氨基酸序列的CDR1以及含有选自SEQ ID NO:84-93的氨基酸序列的CDR2两者。

[0068] 在另一个实施方式中，提供共有来自淘选以及筛选期间鉴定的抗体的轻链之一的CDR3的抗体。因此，也提供分离的单克隆抗体，其中该抗体结合至活化蛋白C且抑制抗凝活性、但对未活化蛋白C具有最低结合，其中该抗体包含含有选自SEQ ID NO:64-73的氨基酸序列的CDR3。在又一个实施方式中，此抗体进一步包含(a)含有选自SEQ ID NO:44-53的氨基酸序列的CDR1、(b)含有选自SEQ ID NO:54-63的氨基酸序列的CDR2、或(c)含有选自SEQ ID NO:44-53的氨基酸序列的CDR1以及含有选自SEQ ID NO:54-63的氨基酸序列的CDR2两者。

[0069] 在另一个实施方式中，抗体含有来自从筛选以及淘选鉴定的抗体的重链与轻链的CDR3。提供分离的单克隆抗体，其中该抗体结合至活化蛋白C且抑制抗凝活性、但对未活化蛋白C具有最低结合，其中该抗体包含含有选自SEQ ID NO:94-103的氨基酸序列的CDR3，以及含有选自SEQ ID NO:64-73的氨基酸序列的CDR3。在又一个实施方式中，此抗体进一步包含(a)含有选自SEQ ID NO:74-83的氨基酸序列的CDR1、(b)含有选自SEQ ID NO:

84-93 的氨基酸序列的 CDR2、(c) 含有选自 SEQ ID NO :44-53 的氨基酸序列的 CDR1，和 / 或 (d) 含有选自 SEQ ID NO :54-63 的氨基酸序列的 CDR2。

[0070] 在一些实施方式中，该抗体包含重链可变区以及轻链可变区，该重链可变区以及轻链可变区包含 ：

轻链可变区，其包含含有 SEQ ID NO :44、54 与 64 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :74、84 与 94 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :45、55 与 65 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :75、85 与 95 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :46、56 与 66 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :76、86 与 96 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :47、57 与 67 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :77、87 与 97 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :48、58 与 68 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :78、88 与 98 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :49、59 与 69 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :79、89 与 99 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :50、60 与 70 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :80、90 与 100 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :51、61 与 71 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :81、91 与 101 的氨基酸序列 ；

轻链可变区，其包含含有 SEQ ID NO :52、62 与 72 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :82、92 与 102 的氨基酸序列 ；以及

轻链可变区，其包含含有 SEQ ID NO :53、63 与 73 的氨基酸序列 ；以及重链可变区，其包含含有 SEQ ID NO :83、93 与 103 的氨基酸序列。

[0071] 也提供结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的分离的单克隆抗体，其中该抗体包含与选自 SEQ ID NO :4-13 中所示氨基酸序列的氨基酸序列具有至少 89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 99.5% 同一性的氨基酸序列。

[0072] 也提供结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的分离的单克隆抗体，其中该抗体包含与选自 SEQ ID NO :14-23 中所示氨基酸序列的氨基酸序列具有至少 89%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99% 或 99.5% 同一性的氨基酸序列。

[0073] 抗体可具有物种特异性或与多种物种交叉反应。在一些实施方式中，抗体可与人、小鼠、大鼠、兔、豚鼠、猴、猪、犬、猫或其它哺乳动物物种的 aPC 特异地反应或交叉反应。

[0074] 抗体可为抗体不同类型的任一种，诸如但不限于 IgG1、IgG2、IgG3、IgG4、IgM、IgA1、IgA2、分泌型 IgA、IgD、以及 IgE 抗体。

[0075] 在一个实施方式中，提供针对人活化蛋白 C 的分离的完全人单克隆抗体。

[0076] *抗 -aPC抗体的最佳化变体*

在一些实施方式中，经淘选以及筛选的抗体可最佳化，例如增加对 aPC 的亲和力、进一

步降低对 PC 的任何亲和力、改进对不同物种的交叉反应性或改进 aPC 的阻断活性。这样的最佳化可以例如通过使用抗体的 CDR 或与 CDR 紧密相近的氨基酸残基、也即与 CDR 相邻约 3 或 4 个残基的定点饱和诱变来进行。

[0077] 也提供具有对 aPC 增加的亲和力或高亲和力的单克隆抗体。在一些实施方式中，抗 -aPC 抗体具有至少约 107 M-1 的结合亲和力，在一些实施方式中至少约 108 M-1 、在一些实施方式中至少约 109 M-1 、1010 M-1 、1011 M-1 或更高，例如最多 1013 M-1 或更高。

[0078] 在一些实施方式中，可引入其它氨基酸修饰以降低来自种系序列的差异。在其它实施方式中，可引入氨基酸修饰以促使供大规模生产过程的抗体生产。

[0079] 在一些实施方式中，提供特异地结合至人活化蛋白 C 的分离的抗 -aPC 单克隆抗体，该抗体包含一或多个氨基酸修饰。在一些实施方式中，该抗体包含 1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或 20 或更多个修饰。

[0080] 因此，在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含含有 SEQ ID NO :8 中所示氨基酸序列的轻链，其中该氨基酸序列含有一或多个氨基酸修饰。在一些实施方式中，轻链的修饰为置换、插入或缺失。在一些实施方式中，修饰位于轻链的 CDR 中。在其它实施方式中，修饰位于轻链的 CDR 以外。

[0081] 在一些实施方式中，SEQ ID NO :8 的轻链修饰是在选自以下位置处 :G52、N53、N54、R56、P57、S58、Q91、Y93、S95、S96、L97、S98、G99、S100 以及 V101。修饰可以是例如下列置换的一个 :G52S、G52Y、G52H、G52F、N53G、N54K、N54R、R56K、P57G、P57W、P57N、S58V、S58F、S58R、Q91R、Q91G、Y93W、S95F、S95Y、S95G、S95W、S95E、S96G、S96A、S96Y、S96W、S96R、L97M、L97G、L97R、L97V、S98L、S98W、S98V、S98R、G99A、G99E、S100A、S100V、V101Y、V101L 或 V101E。另外，在一些实施方式中，抗体可包含两个或更多个来自下列的置换 :G52S、G52Y、G52H、G52F、N53G、N54K、N54R、R56K、P57G、P57W、P57N、S58V、S58F、S58R、Q91R、Q91G、Y93W、S95F、S95Y、S95G、S95W、S95E、S96G、S96A、S96Y、S96W、S96R、L97M、L97G、L97R、L97V、S98L、S98W、S98V、S98R、G99A、G99E、S100A、S100V、V101Y、V101L 或 V101E。

[0082] 在一些实施方式中，SEQ ID NO :8 的轻链进一步包含在选自下列位置的一或多个处的修饰 :A10、T13、S78、R81 以及 S82。在一些实施方式中，在轻链位置 A10 处的修饰为 A10V。在一些实施方式中，在轻链位置 T13 处的修饰为 T13A。在一些实施方式中，在轻链位置 S78 处的修饰为 S78T。在一些实施方式中，在轻链位置 R81 处的修饰为 R81Q。在一些实施方式中，在轻链位置 S82 处的修饰为 S82A。在一些实施方式中，SEQ ID NO :8 的轻链包含下列修饰的两个或更多 :A10V、T13A、S78T、R81Q 以及 S82A。在一些实施方式中，SEQ ID NO :8 的轻链包含所有修饰 A10V、T13A、S78T、R81Q 以及 S82A。

[0083] 在其它实施方式中，提供特异地结合至人活化形式的蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :18 中所示氨基酸序列的重链，其中该氨基酸序列含有一个或多个氨基酸修饰。在一些实施方式中，轻链的修饰为置换、插入或缺失。

[0084] 在一些实施方式中，SEQ ID NO :18 的重链进一步包含在位置 N54 或 S56 处的修饰。在一些实施方式中，在重链位置 N54 处的修饰为 N54G、N54Q 或 N54A。在一些实施方式中，在重链位置 S56 处的修饰为 S56A 或 S56G。

[0085] 在一些实施方式中，可进行氨基酸修饰以促使供大规模生产过程的抗体生产。例如，在一些实施方式中，可进行修饰以降低抗体的疏水性表面区域，用于改进生物物理特性

（例如最低聚集 / 粘性）。在一些实施方式中，在 SEQ ID NO :8 的轻链中进行额外修饰。在一些实施方式中，SEQ ID NO :8 的轻链的修饰在位置 Y33 处。在一些实施方式中，轻链中的修饰与 Y33 为 Y33A、Y33K 或 Y33D。在一些实施方式中，在 SEQ ID NO :18 的重链中进行额外修饰。在一些实施方式中，SEQ ID NO :18 的重链的修饰在位置 Y32、W33、W53 或 W110 的一个或多个处。在一些实施方式中，SEQ ID NO :18 的重链中的修饰选自 Y32A、Y32K、Y32D、W33A、W33K、W33D、W53A、W53K、W53D、W110A、W110K 或 W110D。

[0086] 在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :108 中所示氨基酸序列的轻链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :110 中所示氨基酸序列的轻链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :112 中所示氨基酸序列的轻链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :114 中所示氨基酸序列的轻链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :116 中所示氨基酸序列的轻链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :118 中所示氨基酸序列的轻链。

[0087] 在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :109 中所示氨基酸序列的重链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :111 中所示氨基酸序列的重链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :113 中所示氨基酸序列的重链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :115 中所示氨基酸序列的重链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :117 中所示氨基酸序列的重链。在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有 SEQ ID NO :119 中所示氨基酸序列的重链。

[0088] 在一些实施方式中，提供结合至人活化蛋白 C 的分离的单克隆抗体，其中该抗体包含具有SEQ ID NO :12 中所示氨基酸序列的轻链，其中该氨基酸序列包含一或多个氨基酸修饰。在一些实施方式中，轻链的修饰为置换、插入或缺失。在一些实施方式中，修饰位于轻链的 CDR 中。在其它实施方式中，修饰位于轻链的 CDR 以外。

[0089] 在一些实施方式中，SEQ ID NO :12 的轻链的修饰在选自以下位置处 :T25、D52、N53、N54、N55、D95、N98 或 G99。修饰可以是例如下列置换中的一个 :T25S、D52Y、D52F、D52L、D52G、N53C、N53K、N53G、N54S、N55K、D95G、N98S、G99H、G99L 或 G99F。另外，在一些实施方式中，抗体可含有两个或更多个来自以下的置换 :T25S、D52Y、D52F、D52L、D52G、N53C、N53K、N53G、N54S、N55K、D95G、N98S、G99H、G99L 或 G99F。

[0090] 在又一个实施方式中，提供结合至人活化形式的蛋白 C 的分离的抗 -aPC 单克隆抗体，其中该抗体包含具有 SEQ ID NO :22 中所示的氨基酸序列的重链，其中该氨基酸序列包含一或多个氨基酸修饰。在一些实施方式中，轻链的修饰为置换、插入或缺失。

[0091] *表位*

也提供结合至人活化蛋白 C 的表位的分离的单克隆抗体，其中该表位包含来自 SEQ ID NO :3 中所示人 aPC 的重链的一个或多个残基。

[0092] 在一些实施方式中，表位可包括人 aPC 的活性位点。在一些实施方式中，活性位点可包含人 aPC 的氨基酸残基 S195。

[0093] 在一些实施方式中，表位可包含 SEQ ID NO :3 中所示人活化蛋白 C 的选自以下的一或多个残基 :D60、K96、S97、T98、T99、E170、V171、M172、S173、M175、A190、S195、W215、G216、E217、G218 以及 G218。

[0094] 也提供可与本文所述抗体中任一竞争结合人活化蛋白 C 的抗体。例如，这样的竞争抗体可结合至上述的一或多个表位。

[0095] *核酸、载体以及宿主细胞*

也提供编码上述单克隆抗体中任一的分离的核酸分子。

[0096] 因此，提供编码结合至人活化蛋白 C 的抗体的分离的核酸分子。

[0097] 在一些实施方式中，提供编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中该抗体包含含有选自 SEQ ID NO :34-43 的核酸序列的重链可变区。

[0098] 在一些实施方式中，提供编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中该抗体包含含有选自 SEQ ID NO :24-33 的核酸序列的轻链可变区。

[0099] 在一些实施方式中，提供编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中该抗体包含含有选自 SEQ ID NO :14-23 的氨基酸序列的重链可变区。

[0100] 在一些实施方式中，提供编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中该抗体包含含有选自 SEQ ID NO :4-13 的氨基酸序列的轻链可变区。

[0101] 在另一个实施方式中，提供编码结合至活化蛋白 C 且抑制抗凝活性、但对未活化蛋白 C 具有最低结合的抗体的分离的核酸分子，其中该抗体包含含有选自 SEQ ID NO : 14-23 的氨基酸序列的重链可变区或含有选自 SEQ ID NO :4-13 的氨基酸序列的轻链可变区，以及一或多个在重链可变区或轻链可变区中的氨基酸修饰。

[0102] 另外，也提供包含编码上述单克隆抗体中任一的分离的核酸分子的载体以及包含该载体的宿主细胞。

[0103] *制备针对 aPC的抗体的方法*

可通过在宿主细胞中表达编码本发明实施方式中的一个的单克隆抗体的可变区的核苷酸序列重组制备单克隆抗体。借助表达载体，含有该核苷酸序列的核酸可在适于生产的宿主细胞中转染并表达。因此，也提供用于生产与人 aPC 结合的单克隆抗体的方法，其包含 :

(a) 将编码单克隆抗体的核酸分子转染至宿主细胞中，

(b) 培养宿主细胞以在宿主细胞中表达该单克隆抗体，并任选地分离且纯化所生产的单克隆抗体，其中核酸分子包含编码该单克隆抗体的核苷酸序列。

[0104] 在一个实例中，为了表达抗体或其抗体片段，将通过标准分子生物学技术所得的编码部分或全长轻链与重链的 DNA 插入表达载体中，使得基因操作性地连接至转录与翻译控制序列。在本文中，术语“操作性地连接”意欲表示抗体基因连接至载体中，使得载体中

的转录与翻译控制序列发挥其调节抗体基因的转录与翻译的意图功能。选择与所用表达宿主细胞相容的表达载体以及表达控制序列。抗体轻链基因以及抗体重链基因可插入个别载体中，或更典型地，两个基因被插入相同表达载体中。通过标准方法（例如抗体基因片段以及载体上的互补限制位点的连接，或如果不存在限制位点为平端连接）将抗体基因插入表达载体中。本文所述抗体的轻链与重链可变区可通过将其插入已编码期望同种型的重链恒定区与轻链恒定区的表达载体中而用于产生任一种抗体同种型的全长抗体基因，使得 VH 区段在载体中操作性地连接至 CH 区段，且 VL 区段在载体中操作性地连接至 CL 区段。附加地或可选地，重组型表达载体可编码有助于抗体链从宿主细胞分泌的信号肽。抗体链基因可被克隆至载体中，使得信号肽符合读框地连接至抗体链基因的氨基端。信号肽可以是免疫球蛋白信号肽或异源信号肽（也即，来自非免疫球蛋白蛋白质的信号肽）。

[0105] 除了编码抗体链的基因以外，重组型表达载体带有在宿主细胞中控制抗体链基因表达的调节序列。术语“调节序列”意欲包括启动子、增强子以及控制抗体链基因转录或翻译的其它表达控制元件（例如，聚腺苷酸化信号）。该调节序列描述于例如 Goeddel；Gene Expression Technology.Methods in Enzymology 185,Academic Press,San Diego,Calif.(1990) 中。本领域技术人员将理解，表达载体的设计，包括调节序列的选择，可取决于这些因素如要转化的宿主细胞、期望蛋白质的表达水平等。用于哺乳动物宿主细胞表达的调节序列的实例包括在哺乳动物细胞中指导高水平蛋白质表达的病毒元件，诸如衍生自巨细胞病毒 (CMV)、猿猴病毒 40(SV40)、腺病毒（例如腺病毒主要晚期启动子 (AdMLP)）以及多瘤病毒的启动子和 / 或增强子。可选地，可使用非病毒调节序列，诸如泛素启动子或 β - 球蛋白启动子。

[0106] 除了抗体链基因以及调节序列以外，重组型表达载体还可带有其它序列，诸如调节载体在宿主细胞中复制的序列（例如复制起点）以及可选择标记基因。可选择标记基因有助于选择载体已被引入的宿主细胞（参见，例如美国专利第 4,399,216 号、第 4,634,665 号与第 5,179,017 号，全部是 Axel 等人的）。例如，可选择标记基因通常赋予已被引入载体的宿主细胞对药物诸如 G418、潮霉素或甲氨蝶呤的抗性。可选择标记基因的实例包括二氢叶酸还原酶 (DHFR) 基因（用于带有甲氨蝶呤选择 / 扩增的 dhfr- 宿主细胞）以及 neo 基因（用于 G418 选择）。

[0107] 关于轻链以及重链的表达，将编码重链与轻链的表达载体通过标准技术转染至宿主细胞中。术语“转染”的各种形式意欲涵盖广泛不同的将外源性 DNA 引入原核或真核宿主细胞的常用技术，例如电穿孔、磷酸钙沉淀、DEAE- 葡聚糖转染以及类似技术。尽管在理论上可在原核或真核宿主细胞中表达抗体，在真核细胞包括哺乳动物宿主细胞中抗体的表达是典型的，因为该真核细胞且尤其是哺乳动物细胞比原核细胞更有可能组装并分泌正确折叠及在免疫上具有活性的抗体。

[0108] 用于表达重组型抗体的哺乳动物宿主细胞的实例包括中国仓鼠卵巢 (CHO 细胞）（包括 dhfr-CHO 细胞，在 Urlaub and Chasin,(1980)Proc.Natl.Acad.Sci.USA 77：4216-4220 中所述，使用 DHFR 可选择标记，例如 R.J.Kaufman and P.A.Sharp(1982)Mol.Biol.159：601-621 中所述）、NSO 骨髓瘤细胞、COS 细胞、HKB11 细胞以及 SP2 细胞。当编码抗体基因的重组型表达载体被引入哺乳动物宿主细胞中时，通过将宿主细胞培养足以允许抗体在宿主细胞中表达或将抗体分泌至宿主细胞所生长的培养基中的时间段来生产抗体。

可使用标准蛋白质纯化方法由培养基回收抗体，诸如超滤、尺寸排阻层析、离子交换层析以及离心。

[0109] *部分抗体序列表达完整抗体的用途*

抗体主要通过位于六个重链与轻链 CDR 中的氨基酸残基与目标抗原相互作用。由于该原因，CDR 中的氨基酸序列在个别抗体之间比在 CDR 以外序列更为多样。因为 CDR 序列负责大多数抗体 - 抗原相互作用，其可表达模拟特定天然发生抗体的特性的重组型抗体，这是通过构建包括嫁接至具有不同特性的不同抗体的框架序列上的特定天然发生抗体的 CDR 序列的表达载体而实施（参见，例如 Riechmann, L. et al., 1998, Nature 332 :323-327 ; Jones, P. et al., 1986, Nature 321 :522-525 ;和 Queen, C. et al., 1989, Proc. Natl. Acad. Sci. U. S. A. 86 :10029-10033）。该框架序列可以由包括种系抗体基因序列的公共 DNA 数据库获得。这些种系序列将不同于成熟抗体基因序列，因为它们将不包括完全组装的可变基因，所述基因通过 V(D)J 接合在 B 细胞成熟期间形成。不需要获得特定抗体的完整 DNA 序列以便重新产生具有与原始抗体相似的结合特性的完整重组型抗体（参见 WO 99/45962）。跨越 CDR 区的部分重链与轻链抗体就这个目的来说通常是足够的。部分序列用于确定促成重组抗体可变基因的种系可变以及接合基因区段。然后，种系序列用于填充可变区的缺失部分。重链与轻链前导序列在蛋白质成熟期间被切割且不促成最终抗体的特性。为此，需要使用对应种系前导序列用于表达构建体。为了添加缺失序列，可将克隆的 cDNA 序列与合成寡核苷酸通过连接或 PCR 扩增而组合。可选地，可将整个可变区作为一组短、重叠寡核苷酸般合成并通过 PCR 扩增组合以产生完全合成的可变区克隆。这个过程具有某些优势，诸如排除或纳入或特定限制位点、或者是特定密码子最佳化。

[0110] 重链以及轻链转录子的核苷酸序列被用来设计合成寡核苷酸的重叠组，以产生带有与天然序列相同的氨基酸编码能力的合成 V 序列。合成重链与轻链序列可不同于天然序列。例如，重复核苷酸碱基串被打断以促使寡核苷酸合成以及 PCR 扩增 ;最佳化翻译起始位点依据 Kozak 规则并入 (Kozak, 1991, J. Biol. Chem. 266 :19867-19870) ;且限制位点在翻译起始位点的上游或下游被工程化。

[0111] 就重链以及轻链可变区而言，最佳化编码以及对应非编码链序列在对应非编码寡核苷酸的约中点分解成 30-50 个核苷酸节段。因此，就每一链而言，寡核苷酸可以组装成重叠的双链组，其跨越 150-400 个核苷酸的区段。然后，将该库用作模板产生 150-400 个核苷酸的 PCR 扩增产物。通常，单一可变区寡核苷酸组会分解成个别扩增以产生两种重叠 PCR 产物的两个库。然后，这些重叠产物通过 PCR 扩增被组合，以形成完整的可变区。在 PCR 扩增中包含重链或轻链恒定区的重叠片段以产生可容易克隆至表达载体构建体中的片段也是期望的。

[0112] 然后，经再构建的重链与轻链可变区与克隆的启动子、翻译起始、恒定区、3' 未翻译、聚腺苷酸化和转录终止序列组合形成表达载体构建体。重链与轻链表达构建体可组合进单一载体内、共转染、连续转染或个别转染至宿主细胞中，然后被融合以形成表达两种链的宿主细胞。

[0113] 因此，在另一方面，人抗 -aPC 抗体的结构特征用于产生结构上相关的人抗 -aPC 抗体，其保留结合至 aPC 的功能。更具体地，单克隆抗体的具体鉴定的重链与轻链区的一或多个 CDR 可以重组方式与已知人框架区以及 CDR 组合以生成其它经重组工程化的人抗 -aPC

抗体。

[0114] *药物组合物*

也提供药物组合物，其包含治疗有效量的抗 -aPC 单克隆抗体以及药学上可接受的载体。“药学上可接受的载体”是可被添加至活性成分中以协助配制或稳定制品且不引起对患者明显不良的毒性效应的物质。该载体的实例为本领域技术人员所熟知且包括水、糖诸如麦芽糖或蔗糖、白蛋白、盐诸如氯化钠等。其它载体描述于例如 E.W.Martin 的 Remington's Pharmaceutical Sciences 中。该组合物将包含治疗有效量的至少一种抗 -TFPI 单克隆抗体。

[0115] 药学上可接受的载体包括无菌水溶液或分散液以及无菌粉末用于无菌注射溶液或分散液的临时制备。该介质以及试剂对于药物活性物质的应用是本领域已知的。组合物在一些实施方式中配制成胃肠外注射。组合物可配制成溶液、微乳液、脂质体或其它适于高药物浓度的有序结构。载体可以是溶剂或分散介质，其含有例如水、乙醇、多元醇（例如甘油、丙二醇、以及液体聚乙二醇、与类似物），及其适当的混合物。在一些情况下，其在组合物中将包括等渗剂，例如糖、多元醇诸如甘露醇、山梨醇、或氯化钠。

[0116] 无菌注射溶液可通过将活性化合物在适当溶剂中以需要量与上述一种成分或成分组合按需要掺入、然后灭菌微量过滤而制备。一般而言，分散液是通过将活性化合物掺入含有碱性分散介质以及上述所需其它成分的无菌媒介中制备。在用于制备无菌注射溶液的无菌粉末的情况下，一些制备方法为真空干燥以及冷冻干燥（冻干），其生成活性成分加上来自其先前无菌过滤的其溶液的任一种其它所需成分的粉末。

[0117] *药物用途*

单克隆抗体可用于治疗凝血方面的遗传性以及后天性缺乏或缺陷的治疗目的。例如，在上述实施方式中的单克隆抗体可用于阻断 aPC 与其底物的相互作用，所述底物可包括因子 Va 或因子 VIIIa。

[0118] 单克隆抗体在治疗止血病症诸如血小板减少症、血小板病症以及出血性病症（例如 A 型血友病、B 型血友病以及 C 型血友病）方面具有治疗用途。该病症可通过对有需要的患者施用治疗有效量的抗 -aPC 单克隆抗体而治疗。单克隆抗体在诸如创伤与出血性中风的适应症中治疗不受控制的出血方面具有治疗用途。因此，也提供用于缩短出血时间的方法，其包含向有需要的患者施用治疗有有效量的抗 -aPC 单克隆抗体。

[0119] 在另一个实施方式中，抗 -aPC 抗体可用作为经 aPC 治疗的患者的解毒剂，经 aPC 治疗的患者包括例如，其中 aPC 被用于治疗败血症或出血性病症。

[0120] 抗体可用作为单药疗法或与其它疗法组合以解决止血病症。例如，一或多种抗体与凝血因子诸如因子 VIIa、因子 VIII 或因子 IX 的共施用认为可用于治疗血友病。在一个实施方式中，提供用于治疗凝血方面的遗传性与后天性缺乏或缺陷的方法，其包含施用 (a) 第一数量的结合至人组织因子途径抑制剂的单克隆抗体，以及 (b) 第二数量的因子 VIII 或因子 IX，其中该第一数量以及第二数量共同有效治疗所述缺乏或缺陷。在另一个实施方式中，提供用于治疗凝血方面的遗传性与后天性缺乏或缺陷的方法，其包含施用 (a) 第一数量的结合至人组织因子途径抑制剂的单克隆抗体，以及 (b) 第二数量的因子 VIII 或因子 IX，其中该第一数量以及第二数量共同有效治疗该缺乏或缺陷，且另外其中因子 VII 是不共施用的。也包括药物组合物，其包含治疗有效量的单克隆抗体以及因子 VIII 或因子 IX 的

组合，其中该组合物不含有因子 VII。“因子 VII”包括因子 VII 以及因子 VIIa。该组合疗法有可能降低凝血因子的必须输注频率。共施用或组合疗法表示施用两种各自分别配制或共同配制在一个组合物中的治疗药物，且当分别配制时，在大致相同时间或在不同时间施用，但是在相同的治疗期间施用。

[0121] 在一些实施方式中，本文所述一或多个抗体可以组合应用以解决止血病症。例如，两种或更多种本文所述抗体的共施用认为可用于治疗血友病或其它止血病症。

[0122] 药物组合物可以胃肠外施用给患 A 型血友病或 B 型血友病的个体，其剂量与频率可随着出血事件的严重性而改变，或在预防性疗法的情况下，可随着患者的凝血缺乏的严重性而改变。

[0123] 组合物可作为推注剂或通过连续输注被施用给患者。例如，作为 Fab 片段的本发明抗体的推注施用可以是 0.0025 至 100mg/kg 体重、0.025 至 0.25mg/kg、0.010 至 0.10mg/kg 或 0.10-0.50mg/kg 的数量。对于连续输注，作为 Fab 片段的本发明抗体可以 0.001 至 100mg/kg 体重 / 分钟、0.0125 至 1.25mg/kg/ 分钟、0.010 至 0.75mg/kg/ 分钟、0.010 至 1.0mg/kg/ 分钟或 0.10-0.50mg/kg/ 分钟施用 1-24 小时、1-12 小时、2-12 小时、6-12 小时、2-8 小时或 1-2 小时期间。对于作为全长抗体（具有完整恒定区）的本发明抗体的施用，剂量数可以是约 1-10mg/kg 体重、2-8mg/kg 或 5-6mg/kg。该全长抗体通常会通过输注而施用，延长三十分钟至三小时的期间。施用频率将取决于状况严重性。频率可以是每周三次到每两周至六个月一次。

[0124] 此外，组合物可经由皮下注射施用给患者。例如，剂量为 10 至 100mg 的抗 -aPC 抗体可经由皮下注射每周、每两周或每月施用给患者。

[0125] 如本文所用，“治疗有效量”表示在体内有效增加凝血时间或在体内以其它方式对有需要的患者产生可测量益处所需的抗 -aPC 单克隆抗体或该抗体以及因子 VIII 或因子 IX 的组合的数量。确切数量将取决于许多因素，包括但不限于治疗组合物的组分以及物理性质、意图的患者群、个别患者考量等，且可由本领域技术人员容易地确定。

实施例

[0126] 本公开内容的各方面可根据下列实施例进一步理解，所述实施例不应以任何方式解释为限制本教导的范围。

[0127] 实例 1. 材料与方法

人 aPC特异性结合体（binder）的筛选

主平板 (Master Plates) 的制备：按照淘选策略，使用 Qpix2(Genetix,Boston,MA USA) 集落挑选器通过将 1880 个克隆挑至含有生长培养基 (2XYT/1% 葡萄糖 /100μg/ml 羧苄青霉素）的 384 孔板 (ThermoFisher Scientific,Weltham, MA USA) 中生成主平板。在 37℃伴随振荡使板生长过夜。

[0128] 表达板的产生：使用 Evolution P3 液体处理器 (Perkin Elmer,Waltham, MA,USA)，将来自主平板的 5μl 培养基转移至含有表达培养基 (2XYT/0.1% 葡萄糖 /100ug/ml 羧苄青霉素）的 384 孔板，并在 30℃温育。当培养物达到 0.5 的 OD 600 时，以 0.5mM 的最终浓度添加 IPTG。然后使板回到 30℃生长过夜。

[0129] 初次 ELISA：将 Maxisorp 384 孔板 (ThermoFisher Scientific,Rochester,NY

USA)包被以1μg/ml的于DPBS(具有Ca/Mg)中的重组型人aPC或人PC(Mol.Innovation),并在4℃温育过夜。以DPBST(PBS+0.05% TWEEN)洗涤经包被ELISA板三次并以MDPBST(PBS+0.05%TWEEN+5%奶)在室温封闭1小时。抽吸封闭的板并将15μl表达培养基以及30μl MDPBST转移至每一孔。在室温温育ELISA板1小时,然后以DPBST洗涤5次。添加抗-hFab-HRP(Jackson ImmunoResearch,1:10,000稀释于DPBST中)至每一孔并在室温温育1小时。然后,以DPBST洗涤板5次。添加Amplex Red(Invitrogen)底物并在485nm的激发与595nm的发射下读板。

[0130] 确认ELISA:使用Qpix2集落挑选器,将推测的阳性克隆从主平板重新排列至含有1ml生长培养基的96深孔板(Qiagen)中并在37℃生长过夜。从主平板接种表达板,且当培养物达到0.5的OD600时以0.5mM最终浓度的IPTG诱导。然后,如上述对表达培养基进行ELISA。

[0131] *使用经生物素化aPC的库选择(溶液中淘选)*

进行两种方法:耗尽PC结合体以及非耗尽总PC与aPC结合体。将Dynabeads M280链霉抗生物素偶联至100nM生物素-TF(组织因子,用于非耗尽)或100nM生物素-PC(耗尽)并由磁性装置捕捉。预先经DPBS/3%BSA/0.05% TWEEN20封闭的1-7.5x1012cfu Fab库噬菌体与用生物素-TF或生物素-PC偶联的链霉抗生物素珠在室温于旋转器上温育2小时。捕获并丢弃生物素-TF(非耗尽)或生物素-PC(耗尽)/链霉抗生物素珠。所得噬菌体上清液与1ml DPBS/3%BSA/0.05% TWEEN20/1mM CaCl 2中的100nM(第一轮)、50nM(第二轮)或10nM(第三轮)生物素-aPC在室温温育2小时或在40℃温育过夜。100ul的链霉抗生物素偶联的磁性珠被添加至噬菌体-aPC溶液并在室温温育30分钟。噬菌体-aPC复合物珠被捕捉在磁性装置上并根据淘选轮数以具有3%BSA或0.05% TWEEN20的DPBS洗涤不同次。以1mg/ml胰蛋白酶洗脱结合的噬菌体并以抑肽酶中和。洗脱的噬菌体随后用来感染10ml以指数生长的大肠杆菌HB101F'并扩增用于下一轮选择。也以CFU滴定(淘选输出)分析噬菌体原液。

[0132] *使用固定化aPC的库选择(固相淘选)*

在4℃,将Maxi-sorp 96孔板的五个孔包被以400ng/孔于DPBS中的重组型aPC过夜。与在溶液中淘选相同,噬菌体库用生物素-TF预处理用于非耗尽或用生物素-PC预处理用于耗尽。所得噬菌体然后被添加至经aPC包被的孔并于室温在振荡器上温育1-2小时。通过根据淘选轮数以具有3%BSA或0.05% TWEEN20的DPBS洗涤不同次,洗掉未结合噬菌体。以1mg/ml胰蛋白酶洗脱结合的噬菌体并以抑肽酶中和。洗脱的噬菌体随后被用来感染10ml以指数生长的大肠杆菌HB101F'并扩增用于下一轮选择。也以CFU滴定(淘选输出)分析噬菌体原液。

[0133] *选择的噬菌体库的扩增:洗脱噬菌体原液使用辅助噬菌体M13K07在HB101F'中扩增用于第2、3和4个选择轮*

使用经每一轮选择洗脱的噬菌体感染体积为10ml的以指数生长的HB101F',并在37℃、50rpm温育45分钟。然后将细菌再悬浮于2xYT培养基中,并铺在两个含有100μg/ml羧苄青霉素、15μg/ml四环素以及1%葡萄糖的15cm琼脂板上,然后在30℃温育过夜。以总计8ml 2xYT/carb/tet来收集来自板的细菌菌苔。

[0134] 将约10μl细胞再悬浮于10ml的2xYT/carb/tet中(OD600为约0.1-0.2)并

在 37℃温育直到 OD600 达到 0.5-0.7。将 5x1010cfu 的 M13K07 辅助噬菌体添加至细胞并在 37℃温育 45 分钟。然后将感染的细胞再悬浮于 15ml 的新鲜 2xYT/carb/ 卡那霉素 (50μg/ml)/tet 并在 30℃振荡过夜以产生噬菌体。通过离心收集噬菌体上清液并过滤通过 0.45μm 过滤器。900μl 的上清液用于下一轮的选择。

[0135] *aPC抗体的 DNA测序分析*

使用标准分子生物学技术制备质粒。使用下列引物进行选定抗体克隆的 DNA 测序。

[0136] a) 引物 A :5’ GAAACAGCTATGAAATACCTATTGC 3’

b) 引物 B :5’ GCCTGAGCAGTGGAAGTCC 3’

c) 引物 C :5’ TAGGTATTTCATTATGACTGTCTC 3’

d) 引物 D :5’ CCCAGTCACGACGTTGTAAAACG 3’

从血浆纯化蛋白 C

以 20x50ml 冷冻原液购买一升的犬或兔血浆,包含肝素作为抗凝剂 (Bioreclamation, Inc.,Westbury,NY)。纯化方法由 Esmon 的实验室所述 (12) 并具有修改。在 4℃解冻血浆,并于加载至 Q-Sepharose 柱用于捕获蛋白 C 与其它维生素 K 依赖性蛋白质之前,在室温使用 0.02M Tris-HCl,pH 7.5、肝素 1U/ml 最终、苯甲脒 HCl 10mM 最终以 1 :1 稀释。以缓冲的 0.15M NaCl 洗涤柱,并使用缓冲的 0.5M NaCl 洗脱蛋白 C。使用 10mM Ca++ 以及 100U/ml 肝素使洗脱液再钙化,并然后加载至 HCP4-Affigel-10 亲和柱上。以含 Ca 缓冲液洗涤柱并以含 EDTA 缓冲液洗脱。经纯化的 PC 经透析过夜至 PBS 缓冲液中,急速冷冻并以 0.5ml 等分试样储存于 -80。纯化产量为来自一升犬血浆的 1.75mg。经纯化 PC 如通过 SDS-PAGE 以及分析型 SEC 测定具有 98% 纯度。

[0137] *Fab表达以及纯化*

对于 Fab 表达,将 5•l sFab 大肠杆菌甘油原液接种至 1ml 生长培养基 (LB,1% 葡萄糖,100 •g/ml 氨苄青霉素) 中,并在 37℃伴随在 250rpm 振荡使培养物生长过夜。然后,将过夜培养物 500 • l 接种至 10ml 预温热 (37℃) 诱导培养基 (LB,0.1% 葡萄糖,100 • g/ml 氨苄青霉素) 中并在 37℃以 250rpm 生长至 OD500 0.6-0.7。将 IPTG 添加至培养物中达 0.5mM 最终浓度用于 Fab 表达,且在 30C 伴随在 250rpm 振荡使培养物生长过夜。次日,在 4℃以 3,000g 离心过夜培养物 15 分钟将培养基与细胞分离。保留上清液以及沉淀 (pellet) 用于 Fab 纯化。在上清液以及沉淀中的 Fab 表达可通过使用抗 -His 抗体的蛋白质印迹分析来确认。

[0138] 对于 Fab 纯化,如 BioInvent 方案所建议使用蛋白 A 柱 (MabSure)。将上清液滤过 0.45um 过滤器以移除碎片并于加载至缓冲液平衡的蛋白 A 柱上之前与一片完全蛋白酶抑制剂 (Roche 11873580001) 混合。以 pH 2-3 缓冲液洗脱 Fab,然后经缓冲液交换至 PBS,pH 7.0。为了从细胞沉淀中释放 Fab,向沉淀添加 1ml 裂解缓冲液。在 4℃于摇晃式平台上温育混合物 1 小时用于裂解,然后在 4℃以 3,000g 离心 30 分钟。澄清的上清液被转移至新的管并加载至蛋白 A 柱上。裂解缓冲液含有新鲜制备的于冷蔗糖溶液 (20% 蔗糖 (w/v)、30mM TRIS-HCL、1mM EDTA,pH 8.0) 中的 1mg/ml 溶菌酶 (Sigma L-6876),2.5U/ml benzonase(Sigma E1014)(25KU/ml,原液 1/10.000) 以及一片完全蛋白酶抑制剂 (Roche 11873580001)。经纯化 Fab 的纯度通过 SDS-PAGE 以及分析型尺寸排阻层析 (SEC) 确认。也监测内毒素水平。

[0139] *PC以及 aPC的蛋白质印迹分析*

在有 DTT(还原)或没有 DTT(非还原)的情况下将经纯化蛋白质 (100ng/ 泳道)与 4x SDS-PAGE 上样染料混合,在 95℃加热 5 分钟,然后加载至 4-12% NuPAGE 凝胶上。通过 i-Blot(Life technologies,Carlsbad,CA) 将蛋白质转移至硝基纤维素膜。探测步骤使用 SNAP-id(Millipore) 进行。在使用 5% 奶 /PBS 封闭 10 分钟后,将膜与不同试剂(例如,用于检测经生物素化 aPC 的链霉抗生物素 -HRP,用于检测犬 aPC 的小鼠抗人 PC 单克隆抗体 HCP-4 以及抗 -PC 山羊多克隆抗体)温育。探测之后为在室温与 HRP 二次抗体温育 10 分钟。在以具有 0.1% TWEEN-20 的 PBS 洗涤印迹之后,使用化学发光底物 (ECL)(Pierce,Rockford,IL) 并暴露于 x 射线胶片而检测来自 HRP 的信号。

[0140] *Fab ELISA*

抗原蛋白(人 PC、人 PC、小鼠 APC、犬 APC) 在 4℃以 100ng/100ul/ 孔于 PBS/Ca 缓冲液 (Life technologies) 中包被在 ELISA 板上过夜。次日,洗涤板 3 次并在室温以 5% PBS/Ca/BSA/Tween20 封闭 1 小时。将可溶性 Fab 添加至每孔并在室温温育 1 小时。在添加抗 - 人 λ - 抗体 -HRP 作为检测抗体之后,在室温温育板 1 小时,充分地洗涤然后使用 Amplex Red 底物依据试剂盒制造商所述来显影。使用荧光板读数仪 (SpectraMax 340pc,Molecular Devices,Sunnyvale,CA) 来测量信号(呈 RFU)。将标准曲线拟合至四参数模型,并从该曲线外推未知的数值。

[0141] 实施例 2.从库淘选 aPC抗体

使用如实施例 1 中所述方法来进行对抗人活化蛋白 C 的完全人 Fab 抗体库的淘选以及筛选。对阳性抗体克隆进行 DNA 测序,得到 10 个独特的抗体序列。抗体的重链以及轻链的比对显示于图 2 中。在 5 个 Fab(C7I7、C7A23、T46J23、C22J13、C25K23) 中发现相同的重链 CDR3 序列。

[0142] 经纯化 Fab 通过一组功能性分析来表征,以评估 :a) 其结合特异性 (aPC 对 PC) ;结合亲和力(通过 ELISA 以及 Biacore) ;以及物种交叉反应性(也即,对不同物种来源的 aPC 的结合,包括人、犬及小鼠)。稍后也使用兔 aPC ;b) 其对其它维生素 K 依赖性凝血因子(例如 FIIa、FVIIa、FIXa、FXa) 的结合选择性 ;c) 其在血浆凝血分析 aPTT 中抑制 aPC 的抗凝活性的效力 ;以及 d) 其在使用酰胺分解活性分析(对小肽底物)以及 FVa 失活分析(对蛋白质底物 FVa) 于缓冲液中对 aPC 的蛋白酶酶活性的影响。

[0143] 实施例 3.aPC特异性抗体的结合亲和力以及交叉物种反应性

这些经纯化抗 -aPC Fab 的抗原 - 结合活性通过如图 3 所示的直接 ELISA 测定。抗原被直接包被在 ELISA 板上。包被抗原包括于 PBS/Ca 缓冲液中 100ng/ 孔的人 PC(血浆衍生)、人 aPC(重组型)、犬 aPC(血浆衍生)以及小鼠 aPC(重组型)。在使用 5% 奶 /PBS 封闭板并以 PBS-Tween20 洗涤板后,将可溶性 Fab(1ug/ml,20nM) 添加至板并在室温伴随着振荡温育 1 小时。使用抗 - 人 Fab(λ) 抗体 -HRP 以及 Aplex red 作为底物来检测 Fab 结合。ELISA 数据显示,所有 Fab 特异地结合至人 aPC 而不是人 PC。一个 Fab,R41C17,对人 PC 显示最低结合。与之相比,R41C17 结合至人 APC 以及人 PC 二者。图 3 中也显示利用 ELISA 的 Fab 的交叉物种反应性。在 8 个 aPC- 特异性结合体中,它们中的 4 个 (C7I7、C7A23、C25K23、T46J23) 也显示与犬 aPC 的交叉反应性,此外,一个 Fab,T46J23,显示一些结合小鼠 aPC。

[0144] 表 3 中显示如通过 ELISA 测得的抗 -aPC 抗体对人 aPC 以及犬 aPC 的 EC_{50}。

[0145] 表 3. 抗 -aPC Fabs 的 ELISA 分析

Fab/ EC_{50} (nM)	C7A23	C7I7	C22J13	C25K23	C26B9	R41C17	R41E3	T46J23	T46P19
人 aPC	2.4	6.3	10.6	4.3	10.3	8.6	10.4	6.7	10.3
犬 aPC	6	3.9		8.2				16.6	

[0146] 通过 Biacore 测定抗 -aPC Fab 的亲和力并且显示于表 4 中。

[0147] 表 4. 抗 -aPC Fabs 的 ELISA 分析

Fab/ KD (nM)	C7A23	C7I7	C22J13	C25K23	C26B9	R41C17	R41E3	T46J23	T46P19
人 aPC	1.9	1.3	16	2	7.9	4.8	11	4.1	7.2
犬 aPC	1.3	1.3	300	5.2				21	

[0148] 实施例 4.抗 -aPC Fab的结合选择性

为了测定这些 fab 的结合选择性，也通过 ELISA 评估其对于酶原人 PC、对凝血酶 (FIIa)，以及对活化因子 II(FIIa，凝血酶)、因子 VII(FVIIa)、因子 IX(FIXa) 与因子 X(FXa) 的结合活性。简言之，ELISA 板包被以人 aPC(1ug/ml)、小鼠 PC(10ug/ml)、犬 PC(10ug/ml)，其它凝血因子 (FIIa、FVIIa、FIXa、FXa)(5-10ug/ml)。将 20nM(1ug/ml) 的抗 -aPC Fab 添加至孔。通过二次抗体 (抗 - 人 Fab-HRP) 然后是 HRP 底物 AmplexRed 检测结合的 Fab。作为阳性对照，对每一抗原具有特异性的对照抗体被用来证明存在包被抗原。

[0149] 如图 4 中所示，最多为浓度 20nM，Fab 没有一个显示结合至因子 IIa、VIIa、IXa 或 Xa。与酶原小鼠 PC 或犬 PC 的结合也是不可检测的。

[0150] 实施例 5.在正常人血浆中抗 -aPC Fab抑制 aPC并诱发血块形成

人 aPC 是有力抗凝剂，且这一功能可以容易地通过如图 5 所示的血浆凝血分析 (aPTT) 证明。在 aPTT 分析中，50% 正常人混合血浆在将 CaCls(引发剂) 添加至血浆与磷脂的混合物之后于 52 秒内形成血块。100、200、400、800 或 1600ng/ml 的人 aPC 与血浆的预温育以剂量依赖性方式延长凝血时间。如图 5 中所示，血浆衍生的 aPC 以及重组型 aPC 得到近乎相同的效力。因为对 Stago 仪器的凝血时间的最大设定为 240 秒，人 aPC 在这个功能分析中的抗凝活性在 aPC 为 800ng/ml 时达到最大。

[0151] 为了评估抗 -aPC Fab 对 aPC 的抗凝活性的潜在抑制性效应，对于良好分析范围在 aPTT 分析中使用 400ng/ml aPC(图 6)。由于施用的 aPC 的抗凝活性，血浆凝血时间从 52 秒延长至 180 秒。将工具小鼠抗 - 人 APC 抗体 (对照) 或其 Fab(对照 Fab) 或 Fab C7A23 在 0、0.5、1、2、5、10 或 20ug/ml 与 aPC(也即 Fab 相对于 aPC 为 1.5x 至 60x 倍过量) 温育以剂量依赖性方式降低凝血时间。Fab C7A23 在倒转人 aPC 的抗凝活性方面比对照 -Fab 有效 4-5 倍。与之相比，阴性对照 Fab(人 Fab λ) 对于凝血时间没有影响。在图 6 中，全长对照抗体 (二价) 在 aPTT 分析中比对照 Fab(单价) 有效 10 倍。这个结果与其在针对 aPC 结合的直接 ELISA 中的 EC 50 值 [对照 (0.56nM) 对对照 Fab(6.56nM)] 相符 (数据未

示出）。因此，暗示当抗 -aPC Fab 转变成 IgG 形式时是更为有力的分子。aPTT 结果暗示，抗 -aPC Fab 明显地抑制 aPC 的抗凝活性并缩短凝血时间。在血浆凝血分析 aPTT 中相比于对照 -Fab 来评估所有的测试 Fab（图 6）。在图 6 的上图中，非特异性人 Fab 被用作为阴性对照，且其如预期地不影响凝血时间。阳性对照（对照以及对照 -Fab）以剂量依赖性方式缩短凝血时间。

[0152] Fab C7A23、C7I7、C25K23、T46J23 以及 T46P19 在 5ug/ml（相对于跟踪标定 aPC(spiked-in aPC) 摩尔过量 15 倍）造成人 aPC 活性的 80-93% 抑制并增强血块形成。它们明确地比对照 -Fab 更为有力。与之相比，Fab R41E3 在相同条件下仅产生 30-40% 的 aPC 活性抑制。R41E3 在 aPTT 中的弱活性可能是因为其 aPC 结合的较弱亲和力，如通过 ELISA 以及 Biacore 所测定。R41E3 Fab 浓度增加至 40ug/ml（相对于 aPC 摩尔过量 100 倍）如在图 6 下图中所示的确造成人 aPC 的 80% 抑制。同样，高剂量 (40ug/ml) 的 C22J13 Fab 产生人 aPC 的 80% 抑制。Fab C26B9 在这个分析中比对照 Fab 更为有力。在下图中，Fab R41C17 对 aPC 活性没有影响，因为其结合 PC 以及 aPC 二者，且在人血浆中比 aPC 丰富超过 1000 倍。这个数据也指出 Fab R41C17 具有与其它 Fab 不同的结合表位。

[0153] 如通过物种 aPC ELISA 数据所指出，4 种 Fab(C7A23、C7I7、C25K23、T46J23) 在纳摩尔亲和力下也结合至犬 aPC，这些 Fab 是通过使用跟踪标定至 50% 混合的人正常血浆中的犬 aPC 的 aPTT 来评价，如图 7 所示。通过 aPTT，犬 aPC 表现与人 aPC 相同的抗凝活性（数据未示出）。犬 aPC 在 300ng/ml 下将凝血时间从 47 秒增加至 117 秒。对照抗体或对照 -Fab 在 0、0.5、1、2、5、10 或 20ug/ml 下与犬 aPC 温育不影响凝血时间，因为通过 ELISA 它们不与犬 aPC 交叉反应。但是，Fab C7A23 明显地以剂量依赖性方式降低凝血时间并且在 5ug/ml 抑制犬 aPC 活性至多 80% 或在 20ug/ml 达 85%。而且，C7A23 在阻断人 aPC 以及犬 aPC 方面在 aPTT 分析中显示相当的效力。Fab C7A23、C717、C25K23 明确地以剂量依赖性方式抑制犬 aPC 活性。在 20ug/ml Fab 浓度，这 3 种 Fab 引起 aPC 的 80-90% 抑制并缩短凝血时间。通过 ELISA 以及 Biacore，Fab T46J23 在高剂量仅提供 40% 抑制，与其对犬 aPC 的结合 (KD=22nM) 比 C7A23、C7I7、C25K23(KD=1-5nM) 更弱相符。与之相比，Fab T46P19 以及 R41E3 如预期地在 APTT 中对犬 aPC 没有影响，因为通过 ELISA 它们不能结合至犬 -aPC。

[0154] 实施例 6. 抗 -aPC Fab对于 aPC的酶活性的影响

活化蛋白 C 为丝氨酸蛋白酶。其催化活性可以通过两种方法来测量 :a) 使用小肽底物的酰胺分解活性分析，以及 b) 使用生理蛋白质底物 FVa 的 FVa 降解分析。

[0155] 人 aPC 的酰胺分解活性通过在缓冲液中使用 aPC 的显色肽底物来研究。10nM 的经纯化 aPC 蛋白与 1mM 的显色底物 SPECTROZYME Pca(Lys-Pro-Arg-pNA，MW 773.9 Da) 温育 30 分钟。底物转换成比色产物（也即 aPC 的酶活性）通过以动力学的方式每 5 分钟读取 OD 450 来监测。使用重组型人 aPC 产生标准曲线。为了测试抗 -aPC Fab 对 aPC 酰胺分解活性的影响（图 8），经纯化 aPC 蛋白 (20nM) 首先与等体积抗 -aPC Fab(1-1000nM) 在室温预温育 20 分钟，然后将显色底物 SPECTROZYME Pca 添加至反应混合物中至多达 1mM。人 aPC 在最终浓度为 10nM 的酰胺分解活性在 Fab 存在下测量。在 Fab 存在下，在最终底物浓度为 1mM 的水解速率受到部分抑制，达到 80% 的最大降低。除了 R41C17 以外，所有 Fab 以剂量依赖性方式抑制 aPC。IC50 与在 ELISA 结合分析中的 EC 50 相关联，因为高亲和力结合体 (C7I7、C7A23、T46P19、T46J23、C25K23) 在这一分析中显示比其余较弱的结合体 (R41E3、

C22J13、C26B9) 快得多的抑制。但是,对于较弱的结合体增加 Fab 的浓度也产生最大抑制。例如,R41E3 在 3,000nM 产生 aPC 活性的约 80% 抑制,而高亲和力结合体在 100nM 达到相同程度的抑制。因此,大多数结合体与 aPC 的活性位点相互作用,导致其酰胺分解活性的抑制。有趣的是,对照抗体产生 aPC 的部分抑制 (40%),并在大于 100nM 的浓度达到平台。当使用增加浓度的 R41C17 Fab 时没有观察到抑制效应。因为其对于人 aPC 的结合亲和力可与利用 Biacore 的具有 KD 值为 4.8nM 的高亲和力结合体相比,该数据指出 R41C17 具有与 aPC 的酶活性位点远离的结合表位。

[0156] 人 aPC 的 FVa 失活活性可通过将人 aPC(180pM) 与生其理蛋白质底物 FVa(1.25nM) 温育,然后添加 FXa 以及凝血酶原至反应混合物以形成凝血酶原酶复合物来测量。添加凝血酶的显色肽底物来检测凝血酶的生成 (图 9)。读出值为凝血酶生成 (FIIa/sec)。经纯化因子 Va(1.25nM) 与 aPC(180pM) 于一定 Fab 浓度范围 (1-500nM) 存在下温育,且在凝血酶原酶 /tenase 分析中评价 FVa 活性。

[0157] 关于生物底物 FVa,Fab 对 aPC 活性的影响通过使用经纯化 Fva 的 FXa- 以及凝血酶生成分析来测量。在这个分析中,0.16U/ml(1.25nM) 的 FVa 在分析缓冲液 (20mM TrisHCl、137nM NaCl、10ug/ml 磷脂、5mM CaCl 2 、1mg/ml BSA) 中于抗体存在或不存在的情况下与 aPC 180pM 温育。在温育 30 分钟后,将 25ul 混合物转移至孔中。然后,在分析缓冲液中将 50ul 人 FXa 以及凝血酶原添加至孔并在 30℃通过使用板读数仪监测凝血酶介导底物水解的动力学。作为 aPC 活性的基线,于不存在添加 Fab 的情况下,aPC 的温育自 0.0022nM FIIa/sec 至 0.0015nM FIIa/sec 改变读出值。

[0158] 向反应混合物添加 Fab 导致 aPC 介导的 FVa 蛋白水解几乎完全抑制并且以剂量依赖性方式凝血酶生成的快速增加。如图 9 中所示,对于通过 aPC 抑制 FVa 蛋白水解的 IC 50 值 在纳摩尔范围内且对所有测试 Fab 是相当的。大多数 Fab 比阳性对照 Fab 更有效。R41E3 因为其对人 aPC 的结合较弱而增加较慢。R41C17 令人惊讶地在这个分析中显示一些活性。当使用小肽底物时这一 Fab 对于 aPC 的抗凝活性利用 aPTT 或对于 aPC 的酰胺分解活性没有影响。这些数据指出,R4117 结合表位明显不同于其它 Fab 的那些。

[0159] 实施例 7. 抗 -aPC IgG的表达以及纯化

通过将 Fv 序列克隆至人 IgG1 表达载体中,所有 10 个抗 -aPC Fab 被转化成人 IgG1。质粒被转染至 HEK293 细胞中用于瞬时表达。抗体被分泌至培养基中并且通过蛋白 A 柱纯化。一个高产量抗体 T47J23-hIgG1 每 200ml 培养物生产 10.3mg。一些抗体每 200ml 仅生产 1mg。也监测内毒素水平 (少于 0.01EU/mg)。

[0160] 类似于经纯化 Fab,所有经纯化 IgG 通过一组功能性分析表征,以评估 :a) 其结合特异性以及结合亲和力 ;b) 其物种交叉反应性 (对不同物种来源的 aPC 的结合,包括兔 aPC) ;c) 使用酰胺分解活性分析,其对物种 aPC 的酶活性的影响 ;以及 d) 其在使用人血浆以及小鼠血浆的血浆凝血分析 aPTT 中抑制 aPC 的抗凝活性的效力。

[0161] 实施例 8. 抗 -aPC IgG的结合特异性以及结合亲和力

如图 10 中所示,ELISA 表明大多数 IgG 抗体如同 Fab 保留其结合特异性,因为它们相对于人 PC 优先结合至人 aPC。另一方面,T41C17 以及 03E7 结合人 aPC 以及人 PC 二者。令人惊讶地,T46J23 在其 Fab 转换成 IgG 之后得到人 PC 结合。通过 ELISA 的滴定实验也表明,一般而言,这些二价 IgG1 的结合亲和力相较于对应单价 Fab 如表 5 中所示增加 2-50 倍。

特别地，低亲和力 Fab R41E3 在 Fab-IgG 转换之后结合亲和力增加几乎 50 倍，EC 50 值为 Fab 的 104nM，对 IgG 的 1.76nM。所有 IgG 显示高亲和力结合至人 APC，EC50 值为亚纳摩尔以及低纳摩尔范围。O3E7-IgG 为最弱的 IgG，EC50 为 16.9nM。

[0162] 表 5. 抗 -aPC IgGs 的 ELISA 分析

EC_{50} (nM)	直接ELISA（aPC包被）			
hIgG1	人 aPC	兔 aPC	犬 aPC	小鼠 aPC
C7A23	3.29	7	10.9	
C7I7	2.66	2	2.3	
C22J13	0.49	0.6	2.0	
C25K23	1.25	2	3.4	
C26B9	0.65			
R41C17	0.55			
R41E3	1.76			
T46J23	2.18	7	1.68	4.6
T46P19	0.37			
O3E7	16.9			

[0163] 也如图 10 中所示，使用 (a) 人、(b) 兔、(c) 犬以及 (d) 小鼠 aPC 与 PC，研究这些 IgG 的物种交叉反应性。在 10 个抗 - 人 aPC IgG 中，5 个 IgG 以高亲和力 (EC_{50} =0.6-7nM) 结合至兔 aPC，没有可检测的至兔 PC 的结合。这 5 个 IgG 也以高亲和力 (EC_{50} =1.7-10nM) 结合至犬 APC，且它们不结合至犬 PC。5 个 IgG 中的一个抗体，T46J23，也以 6nM 的 EC_{50}值结合至小鼠 aPC。T46J23 不结合至小鼠 PC。

[0164] 实施例 9. 抗 -APC IgG在使用酰胺分解活性分析的缓冲液中对于物种 APC酶活性的影响

然后评价 5 个物种交叉反应性 IgG 对于物种 APC 的酰胺分解活性的影响（图 11）。在人 aPC 酰胺分解活性分析中，阴性对照 IgG（抗 -CTX 抗体）不具有抑制效应。5 个 IgG 均以剂量依赖性方式抑制人 aPC。它们的 IC 50 值为 T46J23-IgG 的 18nM；C22J13 的 27nM；C7I7 的 64nM；C7A23 的 78nM，以及 C25K23 的 131nM。

[0165] 在兔 aPC 酰胺分解活性分析中，阴性对照 IgG（抗 -CTX 抗体）不具有抑制效应。5 个 IgG 均以剂量依赖性方式抑制兔 aPC。它们的 IC 50 值为 T46J23-IgG 的 17nM；C22J13 的 24nM；C7I7 的 29nM；C7A23 的 25nM，以及 C25K23 的 74nM。

[0166] 在犬 aPC 酰胺分解活性分析中，阴性对照 IgG（抗 -CTX 抗体）不具有抑制效应。5 个 IgG 以剂量依赖性方式微弱抑制犬 aPC。它们的 IC 50 值为 T46J23-IgG 的 625nM；C22J13 的 1300nM；C7I7 的 147nM；C7A23 的 49nM，以及 C25K23 的 692nM。

[0167] 在小鼠 aPC 酰胺分解活性分析中，仅 T46J23 可抑制小鼠 aPC，尽管其需要高剂量 (1000nM)。C717 以及其它 IgG 对小鼠 aPC 没有效应。这些抗体对于物种 APC 活性的抑制效应归纳于表 6 中。

[0168] 表 6. ELISA 和酰胺分解活性分析

	抗 -aPC EC_{50} (nM)			抗 -aPC IC_{50} (nM)		
	ELISA			酰胺分解活性分析		
hIgG1	人 aPC	兔 aPC	犬 aPC	人 aPC	兔 aPC	犬 aPC
C7A23	3.3	7	10.9	78	25	49
C7I7	2.7	2	2.3	64	29	147
C22J13	0.5	0.6	--	27	24	1300
C25K23	1.2	2	3.4	131	74	692
T46J23	2.2	1.7	1.7	18	17	625

[0169] 图 14(b) 显示，在人 aPC 酰胺分解活性分析中，C25K23 IgG1 的两个变体（称为 2310-IgG2 与 2312-IgG2) 在经纯化系统中显示 aPC 的有力抑制。C25K23 IgG1 具有如 SEQ ID NO :108 中所示的轻链以及如 SEQ ID NO :109 中所示的重链。TPP-2031 为经修饰的 C25K23 IgG，其重链包含修饰 N54G。变体 2310 为经修饰的 C25K23 IgG，其轻链包含如 SEQ ID NO : 112 中所示的修饰 A10V、T13A、S78T、R81Q 以及 S82A，而重链包含如 SEQ ID NO :113 中所示的修饰 N54Q。变体 2312 为经修饰的 C25K23 IgG，其轻链包含如 SEQ ID NO :116 中所示的修饰 A10V、T13A、S78T、R81Q 以及 S82A，而重链包含如 SEQ ID NO :117 中所示的修饰 S56A。该变体对 aPC 也展现如图 14(a) 中所示的高亲和力。TPP-2309 为经修饰的 C25K23 IgG1，其轻链包含如 SEQ ID NO :110 中所示的修饰 A10V、T13A、S78T、R81Q 以及 S82A，而重链包含如 SEQ ID NO :111 中所示的修饰 N54G。

[0170] 实施例 10. 抗 -aPC IgG在正常人血浆中抑制 aPC并诱导血块形成

首先在人血浆凝血分析 (aPTT) 中研究抗 -aPC IgG 对 aPC 抗凝活性的影响并显示于图 12 中。五十百分比 (50%) 人血浆于 aPC 不存在的情况下具有 50-52 sec 的基线凝血时间。向血浆添加人 aPC 如预期地增加凝血时间至 190 sec，因为 aPC 是公知的抗凝剂。aPC 与阴性对照 IgG1（抗 -CTX 抗体）的预温育不改变凝血时间。与之相比，aPC 与抗 -aPC 特异性 IgG 的预温育以剂量依赖性方式明显缩短凝血时间。在 1 :1 摩尔比例，T46J23-IgG 以及 C7I7-IgG 在 1ug/ml 抑制 ~50% 的 aPC 活性（在 400ng/ml）并将凝血时间从 190 sec 缩短至 114 sec。在 20ug/ml，所有三种抗体 (T46J23、C7I7、C26B9) 完全逆转 aPC 的抗凝活性并将凝血回复至正常。R41E3-IgG 在抑制 aPC 方面不如这 3 个 IgG 有效。R41E3 将凝血时间部分回复至 75 sec 并在 163 倍摩尔过量下抑制 ~80% 的 aPC 活性。

[0171] 也在 aPTT 分析中如图 14(c) 中所示研究抗 -aPC IgG 的经修饰变体的效应。同样类似于图 12 中的结果，aPC 与经修饰抗 -aPC 特异性 IgG 的预温育以剂量依赖性方式明显缩短凝血时间。

[0172] 实施例 11. 抗 -aPC IgG在重度血友病患者血浆中抑制 aPC并诱导血块形成

如在图 13 中所示，使用血友病患者血浆在凝血酶生成分析 (TGA) 中进一步研究抗 -APC IgG 对于 aPC 抗凝活性的影响。对沿着血管排列的细胞（内皮细胞）的损伤造成组织因子暴露，导致有限数量的凝血酶生成，称为外源性凝血途径。内皮细胞上的凝血调节蛋白促成 aPC 生成及其抗凝活性。重度血友病血浆仅生成 ~50nM 总凝血酶。向血友病血浆添加抗 -aPC- 抗体以剂量依赖性方式增加凝血酶生成。

[0173] 实施例 12. 共晶体研究

抗体制备与 QC

重组型抗 -aPC 人 Fab(C25K23 以及 T46J23) 在大肠杆菌中被表达并通过蛋白 A 层析纯化成同质性。经纯化 Fab 显示具有 >90% 纯度且通过 SDS-PAGE 以及分析型尺寸排阻层析没有聚集。其功能通过 aPC- 结合分析 (ELISA) 表征。C25K23 以及 T46J23 Fab 如通过 ELISA 所测定以 2-4nM 的相当 EC_{50} 值结合人 aPC 全长以及无 Gla 结构域 aPC。生产 10 毫克的这些 Fab。

[0174] *抗原制备及 QC*

血浆衍生的人 aPC 无 Gla 结构域 (aPC-GD) 购自 Enzyme Research Lab 并且通过 ELISA 表征，以确认其可以被 C25K23 Fab 以及 T46J23 Fab 二者识别。

[0175] *复合物形成*

对于复合物形成，将 0.9mg aPC-GD 与 1.05mg C25K23Fab 混合并于 4℃温育反应混合物 5 小时。将混合物加载至凝胶过滤柱上，以将游离 Fab 或游离 aPC-GD 与 aPC-GD-Fab 复合物分离。收集各级分并通过 SDS-PAGE 在非还原条件下进行分析。重复这个过程三次，并混合含有 aPC-GD-Fab 复合物的级分且浓缩至 10mg/ml。

[0176] 在不同晶体生长条件下进行 aPC-Fab 复合物的结晶以产生适于结构测定的晶体 (最大分辨率 <3Å)。使用高通量晶体筛选试剂盒并鉴定两个命中 (hits)：

a)0.1% 正辛基 - β -D- 葡糖苷、0.1M 柠檬酸三钠盐二水合物 PH 5.5、22% PEG 3350

b)18% 2- 丙醇、0.1M 柠檬酸三钠盐二水合物 PH 5.5、20% PEG 4000。

[0177] *数据收集*

在 2.2Å 分辨率下的结构测定成功地来自 aPC-GD-C25K23Fab 晶体衍射图，通过分子取代，以报导的 aPC 以及 Fab X- 射线结构作为模型 (例如依据 Mather et al.,1996 的 pdb 规则 1 aut)，然后通过模型构建及精修进行。图 15 中显示 aPC 与 C25K23 Fab 结构的卡通表示。如图 15 中所示，C25K23 使用其重链的 CDR3 环来接触 aPC 催化结构域。非常显著地，如图 16 中所示，来自 C25K23 的 W104 侧链插入 aPC 的催化口袋中，与先前报导的 aPC 抑制剂 (三肽抑制剂 PPACK) 具有空间重叠。

[0178] 根据这一结构，确定被抗体结合的 aPC 的表位在 aPC 的重链中。aPC 重链与 Fab 之间的接触残基包括 aPC 残基 D60、K96、S97、T98、T99、E170、V171、M172、S173、M175、A190、S195、W215、G216、E217、G218 以及 G218。

[0179] 尤其对于 Fab C25K23，确定抗原互补位包含 SEQ ID NO :18 中所示重链的残基 S31、Y32、W53、R57、R101、W104、R106、F107、W110，以及 SEQ ID NO :8 中所示轻链的 K55。

[0180] 实施例 13. 活性 -位点结合

使用不可逆的活性 - 位点抑制剂生物素 -PPACK 占据人 aPC 的活性位点，参见图 16。生物素 -PPACK-hAPC 或人 aPC 被包被在 maxisorp96 孔板上。抗 -aPC 抗体 (Fab 以及 IgG) 以 1 :3 从 20nM 连续稀释至 0.007nM 并被添加至经包被的孔，且在室温温育 1 小时。通过 HRP- 缀合抗 - 人或抗 - 小鼠 Fab 抗体、随后与荧光底物 (amplex red 与 H2O2) 温育产生荧光信号 (RFU)，检测结合的抗 -aPC-Fab 或抗 -aPC IgG。通过 Gemini EM 荧光微量板读数仪 (Molecular Devices,Sunnyvale,CA) 来读板。在 20nM 抗体浓度的 RFU 在柱状图中表示为三个重复孔的均值 (+/-SD)。

[0181] 如图 17 中所示，从库鉴定至少两类抗体。第一为受活性位点指引的，包括 T46J23(Fab 与 hIgG) 以及 C25K23(Fab 与 hIgG)，它们不再结合至生物素 -PPACK-hAPC(活

性位点封闭的 hAPC)。第二为非受活性位点指引的，包括 R41C17，认为其为抗 -Gla- 结构域抗体。这些数据提供了可靠证据证明 T46J23 以及 C25K23 对人 aPC 的活性位点结合并解释这些抗体的功能特性，也即完全阻断 hAPC 活性。

[0182] 尽管本发明实施方式已参照特定实施方式以及实施例来说明，应理解可在不偏离随附权利要求范围的忠实精神与范畴的情况下，可做出各种修饰以及变化并且可替换等价物。因此，说明书以及实施例被视为是说明性而非限制的。此外，本文引用的所有论文、书籍、专利申请以及专利的公开内容以其整体并入本文做为参考。

[0001]

序列表

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20 25 30

Ala His Gln Val Leu Arg Ile Arg Lys Arg Ala Asn Ser Phe Leu Glu
35 40 45

Glu Leu Arg His Ser Ser Leu Glu Arg Glu Cys Ile Glu Glu Ile Cys
50 55 60

Asp Phe Glu Glu Ala Lys Glu Ile Phe Gln Asn Val Asp Asp Thr Leu
65 70 75 80

Ala Phe Trp Ser Lys His Val Asp Gly Asp Gln Cys Leu Val Leu Pro
85 90 95

Leu Glu His Pro Cys Ala Ser Leu Cys Cys Gly His Gly Thr Cys Ile
100 105 110

Asp Gly Ile Gly Ser Phe Ser Cys Asp Cys Arg Ser Gly Trp Glu Gly
115 120 125

Arg Phe Cys Gln Arg Glu Val Ser Phe Leu Asn Cys Ser Leu Asp Asn
130 135 140

Gly Gly Cys Thr His Tyr Cys Leu Glu Glu Val Gly Trp Arg Arg Cys
145 150 155 160

Ser Cys Ala Pro Gly Tyr Lys Leu Gly Asp Asp Leu Leu Gln Cys His
165 170 175

Pro Ala Val Lys Phe Pro Cys Gly Arg Pro Trp Lys Arg Met Glu Lys
180 185 190

[0002]

Lys Arg Ser His Leu Lys Arg Asp Thr Glu Asp Gln Glu Asp Gln Val
195 200 205

Asp Pro Arg Leu Ile Asp Gly Lys Met Thr Arg Arg Gly Asp Ser Pro
210 215 220

Trp Gln Val Val Leu Leu Asp Ser Lys Lys Lys Leu Ala Cys Gly Ala
225 230 235 240

Val Leu Ile His Pro Ser Trp Val Leu Thr Ala Ala His Cys Met Asp
245 250 255

Glu Ser Lys Lys Leu Leu Val Arg Leu Gly Glu Tyr Asp Leu Arg Arg
260 265 270

Trp Glu Lys Trp Glu Leu Asp Leu Asp Ile Lys Glu Val Phe Val His
275 280 285

Pro Asn Tyr Ser Lys Ser Thr Thr Asp Asn Asp Ile Ala Leu Leu His
290 295 300

Leu Ala Gln Pro Ala Thr Leu Ser Gln Thr Ile Val Pro Ile Cys Leu
305 310 315 320

Pro Asp Ser Gly Leu Ala Glu Arg Glu Leu Asn Gln Ala Gly Gln Glu
325 330 335

Thr Leu Val Thr Gly Trp Gly Tyr His Ser Ser Arg Glu Lys Glu Ala
340 345 350

Lys Arg Asn Arg Thr Phe Val Leu Asn Phe Ile Lys Ile Pro Val Val
355 360 365

Pro His Asn Glu Cys Ser Glu Val Met Ser Asn Met Val Ser Glu Asn
370 375 380

Met Leu Cys Ala Gly Ile Leu Gly Asp Arg Gln Asp Ala Cys Glu Gly
385 390 395 400

Asp Ser Gly Gly Pro Met Val Ala Ser Phe His Gly Thr Trp Phe Leu
405 410 415

Val Gly Leu Val Ser Trp Gly Glu Gly Cys Gly Leu Leu His Asn Tyr
420 425 430

Gly Val Tyr Thr Lys Val Ser Arg Tyr Leu Asp Trp Ile His Gly His
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[0003]

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Asn Val Asp Asp Thr Leu Ala Phe Trp Ser Lys His Val Asp Gly Asp
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Gln Cys Leu Val Leu Pro Leu Glu His Pro Cys Ala Ser Leu Cys Cys
50 55 60

Gly His Gly Thr Cys Ile Asp Gly Ile Gly Ser Phe Ser Cys Asp Cys
65 70 75 80

Arg Ser Gly Trp Glu Gly Arg Phe Cys Gln Arg Glu Val Ser Phe Leu
85 90 95

Asn Cys Ser Leu Asp Asn Gly Gly Cys Thr His Tyr Cys Leu Glu Glu
100 105 110

Val Gly Trp Arg Arg Cys Ser Cys Ala Pro Gly Tyr Lys Leu Gly Asp
115 120 125

Asp Leu Leu Gln Cys His Pro Ala Val Lys Phe Pro Cys Gly Arg Pro
130 135 140

Trp Lys Arg Met Glu Lys Lys Arg Ser His Leu Asp Thr Glu Asp Gln
145 150 155 160

Glu Asp Gln Val Asp Pro Arg Leu Ile Asp Gly Lys Met Thr Arg Arg
165 170 175

Gly Asp Ser Pro Trp Gln Val Val Leu Leu Asp Ser Lys Lys Lys Leu
180 185 190

Ala Cys Gly Ala Val Leu Ile His Pro Ser Trp Val Leu Thr Ala Ala
195 200 205

His Cys Met Asp Glu Ser Lys Lys Leu Leu Val Arg Leu Gly Glu Tyr
210 215 220

Asp Leu Arg Arg Trp Glu Lys Trp Glu Leu Asp Leu Asp Ile Lys Glu
225 230 235 240

Val Phe Val His Pro Asn Tyr Ser Lys Ser Thr Thr Asp Asn Asp Ile
245 250 255

Ala Leu Leu His Leu Ala Gln Pro Ala Thr Leu Ser Gln Thr Ile Val
260 265 270

Pro Ile Cys Leu Pro Asp Ser Gly Leu Ala Glu Arg Glu Leu Asn Gln
275 280 285

Ala Gly Gln Glu Thr Leu Val Thr Gly Trp Gly Tyr His Ser Ser Arg
290 295 300

Glu Lys Glu Ala Lys Arg Asn Arg Thr Phe Val Leu Asn Phe Ile Lys

[0004]

305 310 315 320

Ile Pro Val Val Pro His Asn Glu Cys Ser Glu Val Met Ser Asn Met
325 330 335

Val Ser Glu Asn Met Leu Cys Ala Gly Ile Leu Gly Asp Arg Gln Asp
340 345 350

Ala Cys Glu Gly Asp Ser Gly Gly Pro Met Val Ala Ser Phe His Gly
355 360 365

Thr Trp Phe Leu Val Gly Leu Val Ser Trp Gly Glu Gly Cys Gly Leu
370 375 380

Leu His Asn Tyr Gly Val Tyr Thr Lys Val Ser Arg Tyr Leu Asp Trp
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Pro

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35 40 45

Gln Cys Leu Val Leu Pro Leu Glu His Pro Cys Ala Ser Leu Cys Cys
50 55 60

Gly His Gly Thr Cys Ile Asp Gly Ile Gly Ser Phe Ser Cys Asp Cys
65 70 75 80

Arg Ser Gly Trp Glu Gly Arg Phe Cys Gln Arg Glu Val Ser Phe Leu
85 90 95

Asn Cys Ser Leu Asp Asn Gly Gly Cys Thr His Tyr Cys Leu Glu Glu
100 105 110

Val Gly Trp Arg Arg Cys Ser Cys Ala Pro Gly Tyr Lys Leu Gly Asp
115 120 125

Asp Leu Leu Gln Cys His Pro Ala Val Lys Phe Pro Cys Gly Arg Pro
130 135 140

Trp Lys Arg Met Glu Lys Lys Arg Ser His Leu Leu Ile Asp Gly Lys
145 150 155 160

[0005]

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165 170 175

Lys Lys Lys Leu Ala Cys Gly Ala Val Leu Ile His Pro Ser Trp Val
180 185 190

Leu Thr Ala Ala His Cys Met Asp Glu Ser Lys Lys Leu Leu Val Arg
195 200 205

Leu Gly Glu Tyr Asp Leu Arg Arg Trp Glu Lys Trp Glu Leu Asp Leu
210 215 220

Asp Ile Lys Glu Val Phe Val His Pro Asn Tyr Ser Lys Ser Thr Thr
225 230 235 240

Asp Asn Asp Ile Ala Leu Leu His Leu Ala Gln Pro Ala Thr Leu Ser
245 250 255

Gln Thr Ile Val Pro Ile Cys Leu Pro Asp Ser Gly Leu Ala Glu Arg
260 265 270

Glu Leu Asn Gln Ala Gly Gln Glu Thr Leu Val Thr Gly Trp Gly Tyr
275 280 285

His Ser Ser Arg Glu Lys Glu Ala Lys Arg Asn Arg Thr Phe Val Leu
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Asn Phe Ile Lys Ile Pro Val Val Pro His Asn Glu Cys Ser Glu Val
305 310 315 320

Met Ser Asn Met Val Ser Glu Asn Met Leu Cys Ala Gly Ile Leu Gly
325 330 335

Asp Arg Gln Asp Ala Cys Glu Gly Asp Ser Gly Gly Pro Met Val Ala
340 345 350

Ser Phe His Gly Thr Trp Phe Leu Val Gly Leu Val Ser Trp Gly Glu
355 360 365

Gly Cys Gly Leu Leu His Asn Tyr Gly Val Tyr Thr Lys Val Ser Arg
370 375 380

Tyr Leu Asp Trp Ile His Gly His Ile Arg Asp Lys Glu Ala Pro Gln
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Lys Ser Trp Ala Pro
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Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
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Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Asp Leu
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Ser Gly Pro Tyr Val Leu Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

Gly

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Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Ala Tyr Tyr Cys Ser Ser Tyr Val Gly Ser
85 90 95

Asp Leu Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

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Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly

[0007]

20 25 30

Phe Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Trp Gln Asp Thr
85 90 95

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100 105 110

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Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser Asn
85 90 95

Thr Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

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20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

[0008]

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

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85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

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20 25 30

Thr Val Asn Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

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100 105 110

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20 25 30

His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Arg Asn Asn His Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
50 55 60

Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu

[0009]

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85 90 95

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115 120

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20 25 30

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35 40 45

Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys
50 55 60

Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu Asp
65 70 75 80

Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser Ser Thr His Val
85 90 95

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100 105 110

Ala Pro Ser Val Thr Leu
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His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Asn Asn Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
50 55 60

[0010]

Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu Asn Gly
85 90 95

Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys
100 105 110

Ala Ala Pro Ser Val Thr Leu Phe Pro
115 120

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Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val
20 25 30

His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Gly Asn Ile Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
50 55 60

Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Arg Ser Ala Thr Leu
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Gln Pro Lys Ala
100 105 110

Ala Pro Ser Val Thr Leu Phe Pro
115 120

<210> 14
<211> 124
<212> PRT
<213> 智人

<400> 14

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Gly Asn His
20 25 30

Trp Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val

[0011]

50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 15
<211> 124
<212> PRT
<213> 智人

<400> 15

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Gly Asn His
20 25 30

Trp Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 16
<211> 122
<212> PRT
<213> 智人

<400> 16

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

[0012]

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Arg Val Arg Gly Ile Tyr Asp Ala Phe Asp Met Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 17
<211> 124
<212> PRT
<213> 智人

<400> 17

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Asn
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 18
<211> 124
<212> PRT
<213> 智人

<400> 18

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

[0013]

35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser
115 120

<210> 19
<211> 115
<212> PRT
<213> 智人

<400> 19

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Val Ile Tyr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Met Gly Arg Ala Phe Asp Ile Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Thr Ser
115

<210> 20
<211> 126
<212> PRT
<213> 智人

<400> 20

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

[0014]

Ala Met Ser Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Arg Glu Lys Tyr Tyr Ser Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Gly Arg Thr Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Thr Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

<210> 21
<211> 133
<212> PRT
<213> 智人

<400> 21

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Asn Tyr
20 25 30

Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Asp Ser Ser Ser Ala Gly Arg Trp Ala Gly Ser Leu Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe
130

<210> 22
<211> 133
<212> PRT
<213> 智人

<400> 22

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0015]

1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Gly Asn His
20 25 30

Trp Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Thr Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe
130

<210> 23
<211> 131
<212> PRT
<213> 智人

<400> 23

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asn Arg Ala Thr Arg Ser Gly Tyr Tyr Tyr Phe Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Thr Ser Ala Ser Thr Lys Gly Pro
115 120 125

Ser Val Phe
130

[0016]

<210> 24
<211> 339
<212> DNA
<213> 智人

<400> 24
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgctctg gaagcagctc caacattggg aataattatg tatcctggta tcagcagctc 120

ccaggaacgg cccccaaact cctcatctat aggaataatc agcggccctc aggggtccct 180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg 240

tccgaggatg aggctgatta ttactgccag tcctatgaca gcgacctgag tggtccctat 300

gtgcttttcg gcggaggaac caagctgacg gtcctaggt 339

<210> 25
<211> 333
<212> DNA
<213> 智人

<400> 25
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacatcggg gcaggttatg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatggtaaca gcaatcggcc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctgc ttattactgc agctcatatg taggcagcga cctcgtggtg 300

ttcggcggag gaaccaagct gacggtccta ggt 333

<210> 26
<211> 336
<212> DNA
<213> 智人

<400> 26
cagtctgtgc tgactcagcc accctcaacg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacatcggg gcaggttttg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatggtaaca gcaatcggcc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgt gctacttggc aagacactct gactggttgg 300

atgttcggcg gaggaaccaa gctgacggtc ctaggt 336

<210> 27
<211> 330
<212> DNA
<213> 智人

<400> 27
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcttgttctg gaagcgactc caacatcgga agtaatgctg ttaattggta tcagcagctc 120

ccaggaacgg cccccaaact cctcatctat gacaataata agcgaccctc aggggtccct 180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg 240

tccgaggatg aggctgatta ttactgcagc tcatatacaa gcagcaacac tgtcgtattc 300

ggcggaggaa ccaagctgac ggtcctaggt 330

[0017]

<210> 28
<211> 336
<212> DNA
<213> 智人

<400> 28
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacatcggg gcagcttatg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatggcaata ataagcgacc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgc cagtcctatg acagcagcct gagtggttcg 300

gtattcggcg gaggaaccaa gctgacggtc ctaggt 336

<210> 29
<211> 336
<212> DNA
<213> 智人

<400> 29
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacatcggg gcagcttatg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatggcaata ataagcgacc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgc cagtcctatg acagcagcct gagtggttcg 300

gtattcggcg gaggaaccaa gctgacggtc ctaggt 336

<210> 30
<211> 336
<212> DNA
<213> 智人

<400> 30
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacattggg gcgggttatg ttgtacattg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tataggaata atcatcggcc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgt gcagcatggg atgacagcct gaatggtcgg 300

gtgttcggcg gaggaaccaa gctgacggtc ctaggt 336

<210> 31
<211> 333
<212> DNA
<213> 智人

<400> 31
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgttctg gaagcagctc caacatcgga aataatgctg taaactggta tcagcagctc 120

ccaggaacgg cccccaaact cctcatctat agtaataatc agcggccctc aggggtccct 180

gaccgattct ctggctccaa gtctggcacc tcagcctccc tggccatcag tgggctccgg 240

tccgaggatg aggctgatta ttactgcagc tcatatacaa gcagcagcac tcatgtggta 300

ttcggcggag gaaccaagct gacggtccta ggt 333

[0018]

<210> 32
<211> 336
<212> DNA
<213> 智人

<400> 32
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggaccagctc caacatcggg gcaggttatg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatgataaca acaatcggcc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgt gcagcatggg atgacagcct gaacggtgtg 300

gtattcggcg gaggaaccaa gctgacggtc ctaggt 336

<210> 33
<211> 333
<212> DNA
<213> 智人

<400> 33
cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60

tcctgcactg ggagcagctc caacatcggg gcaggttatg atgtacactg gtatcagcag 120

ctcccaggaa cggcccccaa actcctcatc tatggtaaca tcaatcggcc ctcaggggtc 180

cctgaccgat tctctggctc caagtctggc acctcagcct ccctggccat cagtgggctc 240

cggtccgagg atgaggctga ttattactgc agctcatata caagaagcgc cactctcgtg 300

ttcggcggag gaaccaagct gacggtccta ggt 333

<210> 34
<211> 372
<212> DNA
<213> 智人

<400> 34
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggGtc cctgagactc 60

tcctgtgcag cctctggatt cacctttggt aatcattgga tgacctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcagtag gacgcactat 180

gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc cctgacaggg 300

agaagtggct ggatgcgctt cccaaactgg ttcgacccct ggggccaagg taccctggtc 360

accgtgacta gt 372

<210> 35
<211> 372
<212> DNA
<213> 智人

<400> 35
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggGtc cctgagactc 60

tcctgtgcag cctctggatt cacctttggt aatcattgga tgacctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcagtag gacgcactat 180

gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc cctgacaggg 300

[0019]

agaagtggct ggatgcgctt cccaaactgg ttcgacccct ggggccaagg taccctggtc 360

accgtgacta gt 372

<210> 36
<211> 366
<212> DNA
<213> 智人

<400> 36
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt cacctttagt agttatagca tgaactgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagagatcga 300

cgggttcggg gaatctatga tgcatttgat atgtggggcc agggtaccct ggtcaccgtg 360

actagt 366

<210> 37
<211> 372
<212> DNA
<213> 智人

<400> 37
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt caccttcagt agcaactaca tgagctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtggcagtt atatcatatg atggaagcaa taaatactac 180

gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccatgt attactgtgc cctgacaggg 300

agaagtggct ggatgcgctt cccaaactgg ttcgacccct ggggccaagg taccctggtc 360

accgtgacta gt 372

<210> 38
<211> 372
<212> DNA
<213> 智人

<400> 38
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt cacctttagt agctattgga tgagctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcagtag gacgcactat 180

gcagactctg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc cctgacaggg 300

agaagtggct ggatgcgctt cccaaactgg ttcgacccct ggggccaggg taccctggtc 360

accgtgacta gt 372

<210> 39
<211> 345
<212> DNA
<213> 智人

<400> 39
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

[0020]

tcctgtgcag cctctggatt caccttcagt agctatggca tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtctcagtt atttatagcg gtggtagcac atactacgca 180

gactccgtga agggcagatt caccatctcc agagacaatt ccaagaacac gctgtatctg 240

caaatgaaca gcctgagagc cgaggacact gccgtgtatt actgtgcgag gatgggaagg 300

gcttttgata tctggggcca aggtaccctg gtcaccgtga ctagt 345

<210> 40
<211> 351
<212> DNA
<213> 智人

<400> 40
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt caccttagc aactatgcga tgagctgggt ccgccagtct 120

ccagggaagg ggctggagtg ggtggctgtt atatcatatg acggaaggga gaagtactac 180

tcagactccg tcaagggacg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagagatcgg 300

ggacgtacct ttgactactg gggccaaggt accctggtca ccgtgactag t 351

<210> 41
<211> 372
<212> DNA
<213> 智人

<400> 41
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt caccttaac aactatgcca tgacctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcagtag gacgcactat 180

gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagagcggat 300

agcagcagcg ccgggaggtg ggccggctca cttgactact ggggccaagg taccctggtc 360

accgtgacta gt 372

<210> 42
<211> 372
<212> DNA
<213> 智人

<400> 42
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt caccttggt aatcattgga tgacctgggt ccgccaggct 120

ccagggaagg ggctggagtg ggtatcgggt gttagttgga atggcagtag gacgcactat 180

gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc cctgacaggg 300

agaagtggct ggatgcgctt cccaaactgg ttcgacccct ggggccaagg taccctggtc 360

accgtgacta gt 372

<210> 43
<211> 366
<212> DNA

[0021]

<213> 智人

<400> 43
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60

tcctgtgcag cctctggatt caccttcagt ggctatggca tgcactgggt ccgccaggct 120

ccaggcaagg ggctggagtg ggtctctggt attaattgga atggtggtag cacaggttat 180

gcagactctg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcaaatga acagcctgag agccgaggac actgccgtgt attactgtgc gagaaatcga 300

gccacccgca gtggctacta ttactttgac tcctggggcc agggtaccct ggtcaccgtg 360

actagt 366

<210> 44
<211> 13
<212> PRT
<213> 智人

<400> 44

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser
1 5 10

<210> 45
<211> 14
<212> PRT
<213> 智人

<400> 45

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val His
1 5 10

<210> 46
<211> 14
<212> PRT
<213> 智人

<400> 46

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Phe Asp Val His
1 5 10

<210> 47
<211> 13
<212> PRT
<213> 智人

<400> 47

Ser Gly Ser Asp Ser Asn Ile Gly Ser Asn Ala Val Asn
1 5 10

<210> 48
<211> 14
<212> PRT
<213> 智人

<400> 48

Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala Tyr Asp Val His
1 5 10

<210> 49
<211> 13
<212> PRT

[0022]

<213> 智人

<400> 49

Ser Gly Ser Ser Ser Asn Ile Arg Ser Asn Thr Val Asn
1 5 10

<210> 50
<211> 14
<212> PRT
<213> 智人

<400> 50

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Val Val His
1 5 10

<210> 51
<211> 13
<212> PRT
<213> 智人

<400> 51

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Ala Val Asn
1 5 10

<210> 52
<211> 14
<212> PRT
<213> 智人

<400> 52

Thr Gly Thr Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val His
1 5 10

<210> 53
<211> 14
<212> PRT
<213> 智人

<400> 53

Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val His
1 5 10

<210> 54
<211> 6
<212> PRT
<213> 智人

<400> 54

Arg Asn Asn Gln Arg Pro
1 5

<210> 55
<211> 6
<212> PRT
<213> 智人

<400> 55

Gly Asn Ser Asn Arg Pro
1 5

<210> 56
<211> 6

[0023]

<212> PRT
<213> 智人

<400> 56

Gly Asn Ser Asn Arg Pro
1 5

<210> 57
<211> 6
<212> PRT
<213> 智人

<400> 57

Asp Asn Asn Lys Arg Pro
1 5

<210> 58
<211> 6
<212> PRT
<213> 智人

<400> 58

Gly Asn Asn Lys Arg Pro
1 5

<210> 59
<211> 6
<212> PRT
<213> 智人

<400> 59

Gly Asn Ser Asn Arg Pro
1 5

<210> 60
<211> 6
<212> PRT
<213> 智人

<400> 60

Arg Asn Asn His Arg Pro
1 5

<210> 61
<211> 6
<212> PRT
<213> 智人

<400> 61

Ser Asn Asn Gln Arg Pro
1 5

<210> 62
<211> 6
<212> PRT
<213> 智人

<400> 62

Asp Asn Asn Asn Arg Pro
1 5

<210> 63

[0024]

<211> 6
<212> PRT
<213> 智人

<400> 63

Gly Asn Ile Asn Arg Pro
1 5

<210> 64
<211> 13
<212> PRT
<213> 智人

<400> 64

Gln Ser Tyr Asp Ser Asp Leu Ser Gly Pro Tyr Val Leu
1 5 10

<210> 65
<211> 10
<212> PRT
<213> 智人

<400> 65

Ser Ser Tyr Val Gly Ser Asp Leu Val Val
1 5 10

<210> 66
<211> 11
<212> PRT
<213> 智人

<400> 66

Ala Thr Trp Gln Asp Thr Leu Thr Gly Trp Met
1 5 10

<210> 67
<211> 10
<212> PRT
<213> 智人

<400> 67

Ser Ser Tyr Thr Ser Ser Asn Thr Val Val
1 5 10

<210> 68
<211> 11
<212> PRT
<213> 智人

<400> 68

Gln Ser Tyr Asp Ser Ser Leu Ser Gly Ser Val
1 5 10

<210> 69
<211> 12
<212> PRT
<213> 智人

<400> 69

Gln Ser Tyr Asp Ser Ser Leu Ser Gly Asp Val Val
1 5 10

[0025]

<210> 70
<211> 11
<212> PRT
<213> 智人

<400> 70

Ala Ala Trp Asp Asp Ser Leu Asn Gly Arg Val
1 5 10

<210> 71
<211> 11
<212> PRT
<213> 智人

<400> 71

Ser Ser Tyr Thr Ser Ser Ser Thr His Val Val
1 5 10

<210> 72
<211> 11
<212> PRT
<213> 智人

<400> 72

Ala Ala Trp Asp Asp Ser Leu Asn Gly Val Val
1 5 10

<210> 73
<211> 10
<212> PRT
<213> 智人

<400> 73

Ser Ser Tyr Thr Arg Ser Ala Thr Leu Val
1 5 10

<210> 74
<211> 5
<212> PRT
<213> 智人

<400> 74

Asn His Trp Met Thr
1 5

<210> 75
<211> 5
<212> PRT
<213> 智人

<400> 75

Asn His Trp Met Thr
1 5

<210> 76
<211> 5
<212> PRT
<213> 智人

<400> 76

Ser Tyr Ser Met Asn
1 5

[0026]

<210> 77
<211> 5
<212> PRT
<213> 智人

<400> 77

Ser Asn Tyr Met Ser
1 5

<210> 78
<211> 5
<212> PRT
<213> 智人

<400> 78

Ser Tyr Trp Met Ser
1 5

<210> 79
<211> 5
<212> PRT
<213> 智人

<400> 79

Ser Tyr Gly Met His
1 5

<210> 80
<211> 5
<212> PRT
<213> 智人

<400> 80

Asn Tyr Ala Met Ser
1 5

<210> 81
<211> 5
<212> PRT
<213> 智人

<400> 81

Asn Tyr Ala Met Thr
1 5

<210> 82
<211> 5
<212> PRT
<213> 智人

<400> 82

Asn His Trp Met Thr
1 5

<210> 83
<211> 5
<212> PRT
<213> 智人

<400> 83

Gly Tyr Gly Met His
1 5

[0027]

<210> 84
<211> 18
<212> PRT
<213> 智人

<400> 84

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 85
<211> 18
<212> PRT
<213> 智人

<400> 85

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 86
<211> 18
<212> PRT
<213> 智人

<400> 86

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 87
<211> 18
<212> PRT
<213> 智人

<400> 87

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 88
<211> 18
<212> PRT
<213> 智人

<400> 88

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

[0028]

<210> 89
<211> 17
<212> PRT
<213> 智人

<400> 89

Ser Val Ile Tyr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 90
<211> 18
<212> PRT
<213> 智人

<400> 90

Ala Val Ile Ser Tyr Asp Gly Arg Glu Lys Tyr Tyr Ser Asp Ser Val
1 5 10 15

Lys Gly

<210> 91
<211> 18
<212> PRT
<213> 智人

<400> 91

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 92
<211> 18
<212> PRT
<213> 智人

<400> 92

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

<210> 93
<211> 18
<212> PRT
<213> 智人

<400> 93

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
1 5 10 15

Lys Gly

[0029]

<210> 94
<211> 15
<212> PRT
<213> 智人

<400> 94

Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp Pro
1 5 10 15

<210> 95
<211> 15
<212> PRT
<213> 智人

<400> 95

Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp Pro
1 5 10 15

<210> 96
<211> 13
<212> PRT
<213> 智人

<400> 96

Asp Arg Arg Val Arg Gly Ile Tyr Asp Ala Phe Asp Met
1 5 10

<210> 97
<211> 15
<212> PRT
<213> 智人

<400> 97

Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp Pro
1 5 10 15

<210> 98
<211> 15
<212> PRT
<213> 智人

<400> 98

Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp Pro
1 5 10 15

<210> 99
<211> 7
<212> PRT
<213> 智人

<400> 99

Met Gly Arg Ala Phe Asp Ile
1 5

<210> 100
<211> 8
<212> PRT
<213> 智人

<400> 100

Asp Arg Gly Arg Thr Phe Asp Tyr
1 5

[0030]

<210> 101
<211> 15
<212> PRT
<213> 智人

<400> 101

Ala Asp Ser Ser Ser Ala Gly Arg Trp Ala Gly Ser Leu Asp Tyr
1 5 10 15

<210> 102
<211> 15
<212> PRT
<213> 智人

<400> 102

Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp Pro
1 5 10 15

<210> 103
<211> 13
<212> PRT
<213> 智人

<400> 103

Asn Arg Ala Thr Arg Ser Gly Tyr Tyr Tyr Phe Asp Ser
1 5 10

<210> 104
<211> 25
<212> DNA
<213> 人工的

<220>
<223> 引物序列

<400> 104
gaaacagcta tgaaatacct attgc 25

<210> 105
<211> 19
<212> DNA
<213> 人工的

<220>
<223> 引物序列

<400> 105
gcctgagcag tggaagtcc 19

<210> 106
<211> 24
<212> DNA
<213> 人工的

<220>
<223> 引物序列

<400> 106
taggtatttc attatgactg tctc 24

<210> 107
<211> 23
<212> DNA
<213> 人工的

<220>

[0031]

<223> 引物序列

<400> 107
cccagtcacg acgttgtaaa acg 23

<210> 108
<211> 217
<212> PRT
<213> 智人

<400> 108

Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu
65 70 75 80

Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 109
<211> 453
<212> PRT
<213> 智人

<400> 109

[0032]

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
            100                 105                 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
        115                 120                 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
    130                 135                 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145                 150                 155                 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                165                 170                 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
            180                 185                 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
        195                 200                 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
    210                 215                 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
225                 230                 235                 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
                245                 250                 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
            260                 265                 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
        275                 280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
    290                 295                 300
```

[0033]

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
435 440 445

Ser Leu Ser Pro Gly
450

<210> 110
<211> 217
<212> PRT
<213> 智人

<400> 110

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu

[0034]

115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 111
<211> 449
<212> PRT
<213> 智人

<400> 111

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Gly Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

[0035]

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
195 200 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
210 215 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
290 295 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 112
<211> 217

[0036]

<212> PRT
<213> 智人

<400> 112

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 113
<211> 449
<212> PRT
<213> 智人

<400> 113

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

[0037]

```
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Gly Val Ser Trp Gln Gly Ser Arg Thr His Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
            100                 105                 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
        115                 120                 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
    130                 135                 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145                 150                 155                 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
                165                 170                 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
            180                 185                 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
        195                 200                 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
    210                 215                 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
                325                 330                 335
```

[0038]

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 114
<211> 217
<212> PRT
<213> 智人

<400> 114

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
115 120 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
130 135 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val

[0039]

145 150 155 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
165 170 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 115
<211> 449
<212> PRT
<213> 智人

<400> 115

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Ala Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
195 200 205

[0040]

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
210 215 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
290 295 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

<210> 116
<211> 217
<212> PRT
<213> 智人

<400> 116

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

[0041]

```
Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
            20                  25                  30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85                  90                  95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
        115                 120                 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
    130                 135                 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145                 150                 155                 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
                165                 170                 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
            180                 185                 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
        195                 200                 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
    210                 215
```

<210> 117
<211> 449
<212> PRT
<213> 智人

<400> 117

```
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Gly Val Ser Trp Asn Gly Ala Arg Thr His Tyr Ala Asp Ser Val
    50                  55                  60
```

[0042]

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
195 200 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
210 215 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
290 295 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

[0043]

```
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385                 390                 395                 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
                405                 410                 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly
```

<210> 118
<211> 217
<212> PRT
<213> 智人

<400> 118

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Ala
            20                  25                  30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr Gly Asn Asn Lys Arg Pro Ser Gly Val Pro Asp Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
                85                  90                  95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
        115                 120                 125

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
    130                 135                 140

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
145                 150                 155                 160

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
                165                 170                 175

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
```

[0044]

180 185 190

His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
195 200 205

Lys Thr Val Ala Pro Thr Glu Cys Ser
210 215

<210> 119
<211> 449
<212> PRT
<213> 智人

<400> 119

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Gly Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Leu Thr Gly Arg Ser Gly Trp Met Arg Phe Pro Asn Trp Phe Asp
100 105 110

Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190

Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn
195 200 205

Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg
210 215 220

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
225 230 235 240

[0045]

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
290 295 300

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly

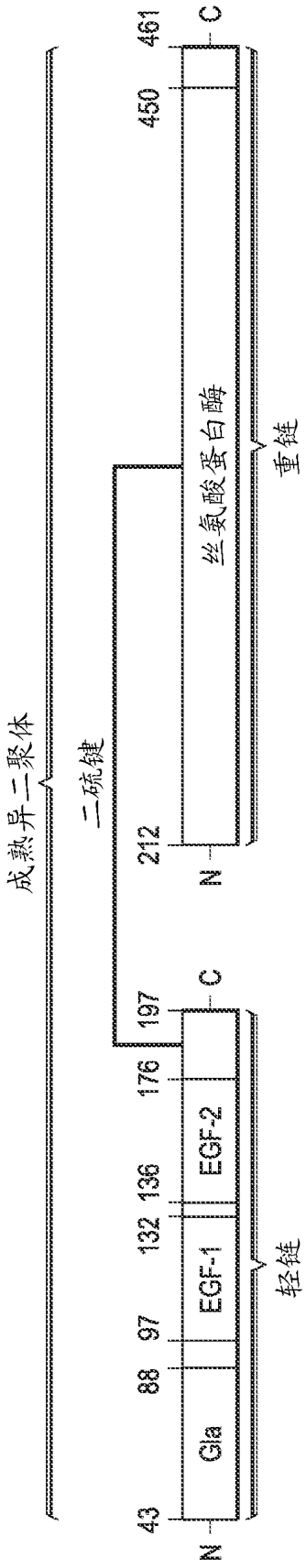

成熟异二聚体
二硫键
丝氨酸蛋白酶
重链
轻链
Gla
EGF-1
EGF-2
N
C
43
88
97
132
136
176
197
212
450
461

图 1

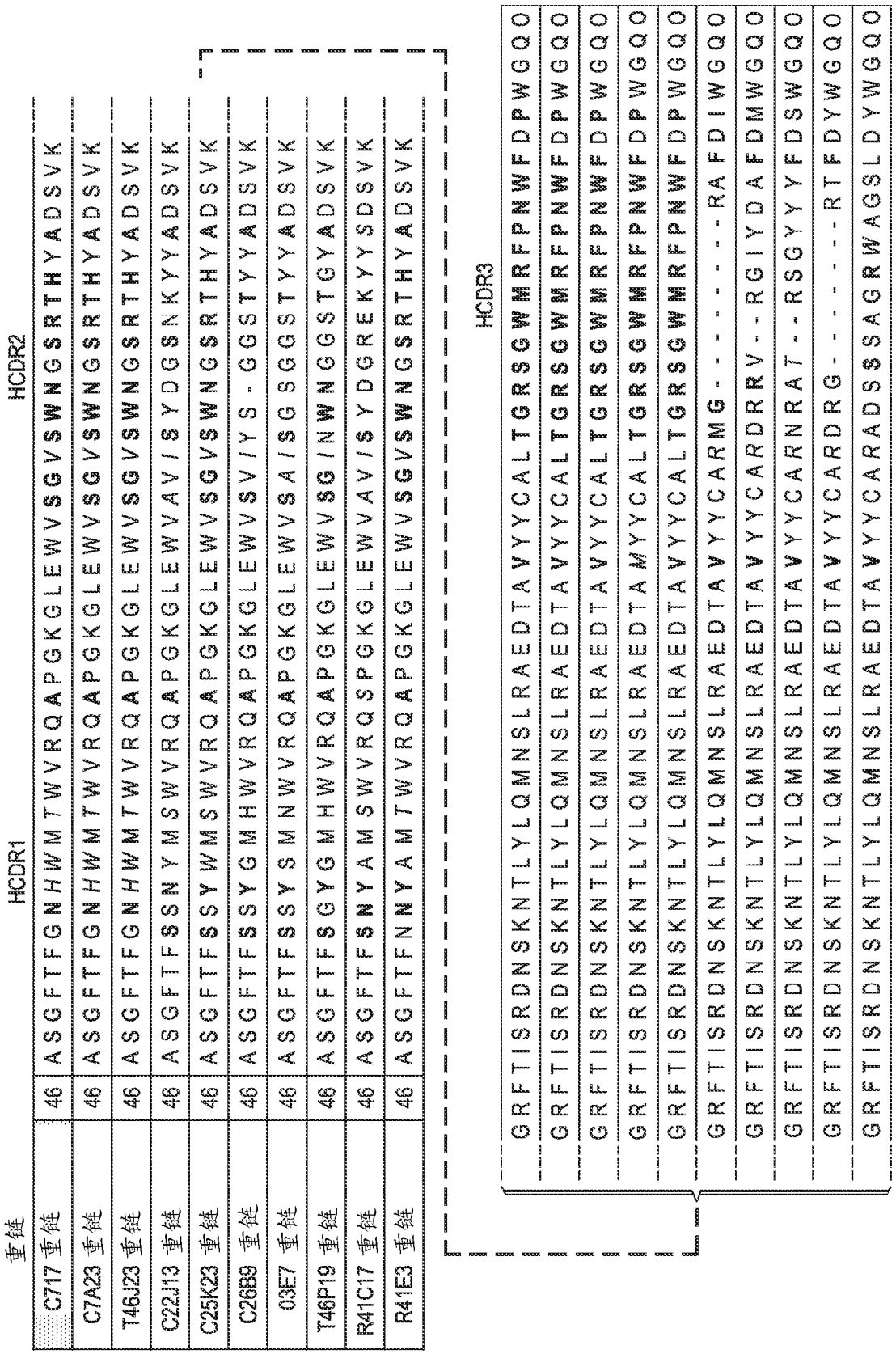

重链
HCDR1
HCDR2
HCDR3
C717 重链 46 ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK
C7A23 重链 46 ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK
T46J23 重链 46 ASGFTFGNHWMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK
C22J13 重链 46 ASGFTFSSNYMSWVRQAPGKGLEWVAVISYDGSNKYYADSVK
C25K23 重链 46 ASGFTFSSYWMSWVRQAPGKGLEWVSGVSWNGSRTHYADSVK
C26B9 重链 46 ASGFTFSSYGMHWVRQAPGKGLEWVSVIYS-GGSTYYADSVK
03E7 重链 46 ASGFTFSSYSMNWVRQAPGKGLEWVSAISGSGGSTYYADSVK
T46P19 重链 46 ASGFTFSGYGMHWVRQAPGKGLEWVSGINWNGGSTGYADSVK
R41C17 重链 46 ASGFTFSNYAMSWVRQSPGKGLEWVAVISYDGREKYYSDSVK
R41E3 重链 46 ASGFTFNNYAMTWVRQAPGKGLEWVSGVSWNGSRTHYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCALTGRSGWMRFPNWFDPWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCALTGRSGWMRFPNWFDPWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARMG-----RAFDIWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRRV--RGIYDAFDMWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNRAT--RSGYYYFDSWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRG------RTFDYWGQQ
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARADSSSAGRWAGSLDYWGQQ

图 2

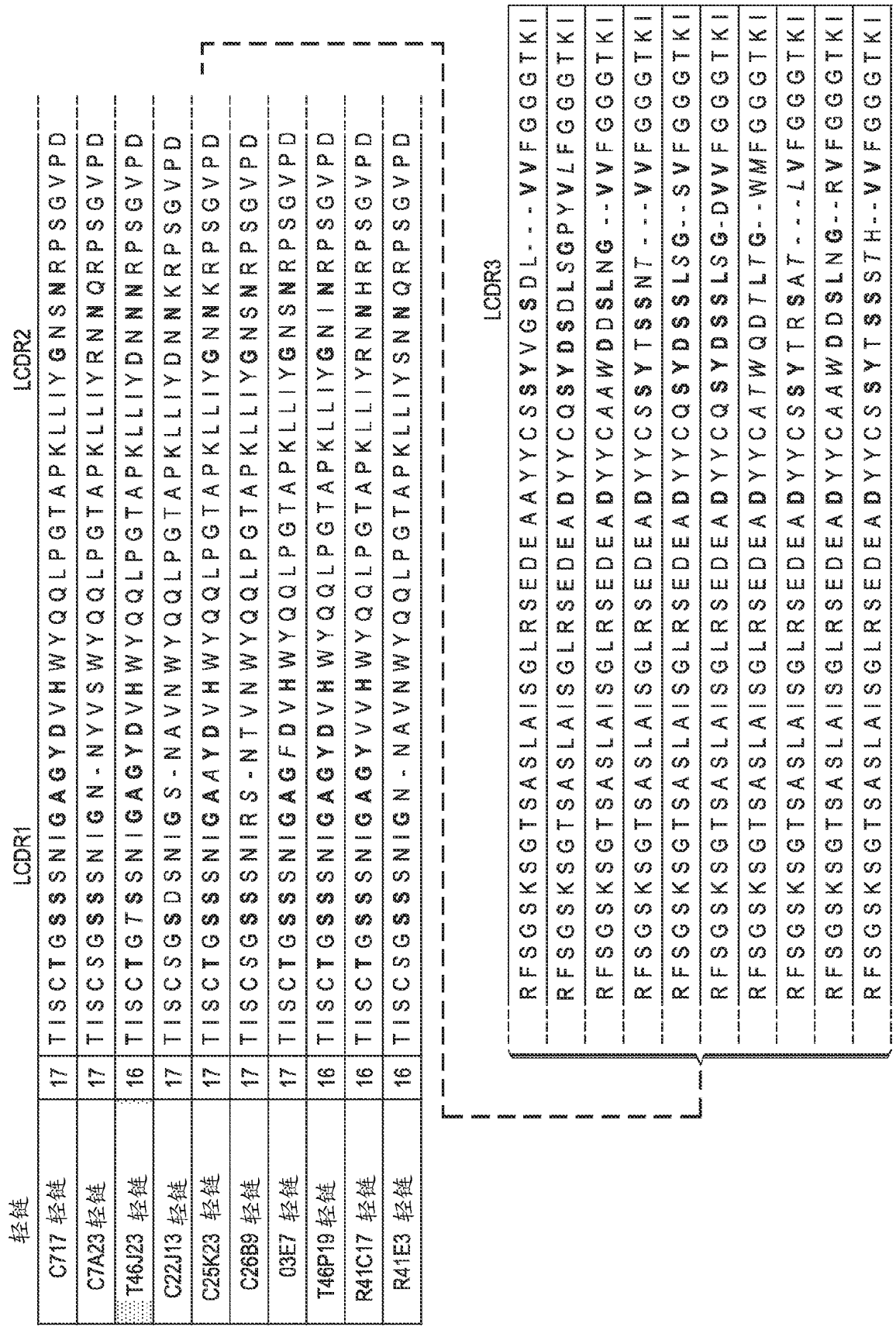

轻链
LCDR1
LCDR2
LCDR3
C717 轻链 17 TISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSNRPSGVPD
C7A23 轻链 17 TISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYRNNQRPSGVPD
T46J23 轻链 16 TISCTGTSSNIGAGYDVHWYQQLPGTAPKLLIYDNNNRPSGVPD
C22J13 轻链 17 TISCSGSDSNIGSNAVNWYQQLPGTAPKLLIYDNNKRPSGVPD
C25K23 轻链 17 TISCTGSSSNIGAAYDVHWYQQLPGTAPKLLIYGNNKRPSGVPD
C26B9 轻链 17 TISCSGSSSNIRSNTVNWYQQLPGTAPKLLIYGNSNRPSGVPD
03E7 轻链 17 TISCTGSSSNIGAGFDVHWYQQLPGTAPKLLIYGNSNRPSGVPD
T46P19 轻链 16 TISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNINRPSGVPD
R41C17 轻链 16 TISCTGSSSNIGAGYVVHWYQQLPGTAPKLLIYRNNHRPSGVPD
R41E3 轻链 16 TISCSGSSSNIGNNAVNWYQQLPGTAPKLLIYSNNQRPSGVPD
RFSGSKSGTSASLAISGLRSEDEAAYYCSSYVGSDL--VVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSDLSGPYVLFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLNG--VVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCSSYTSSNT---VVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSSLSG--SVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCQSYDSSLSG-DVVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCATWQDTLTG--WMFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCSSYTRSAT---LVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLNG--RVFGGGTKI
RFSGSKSGTSASLAISGLRSEDEADYYCSSYTSSST H--VVFGGGTKI

图 2

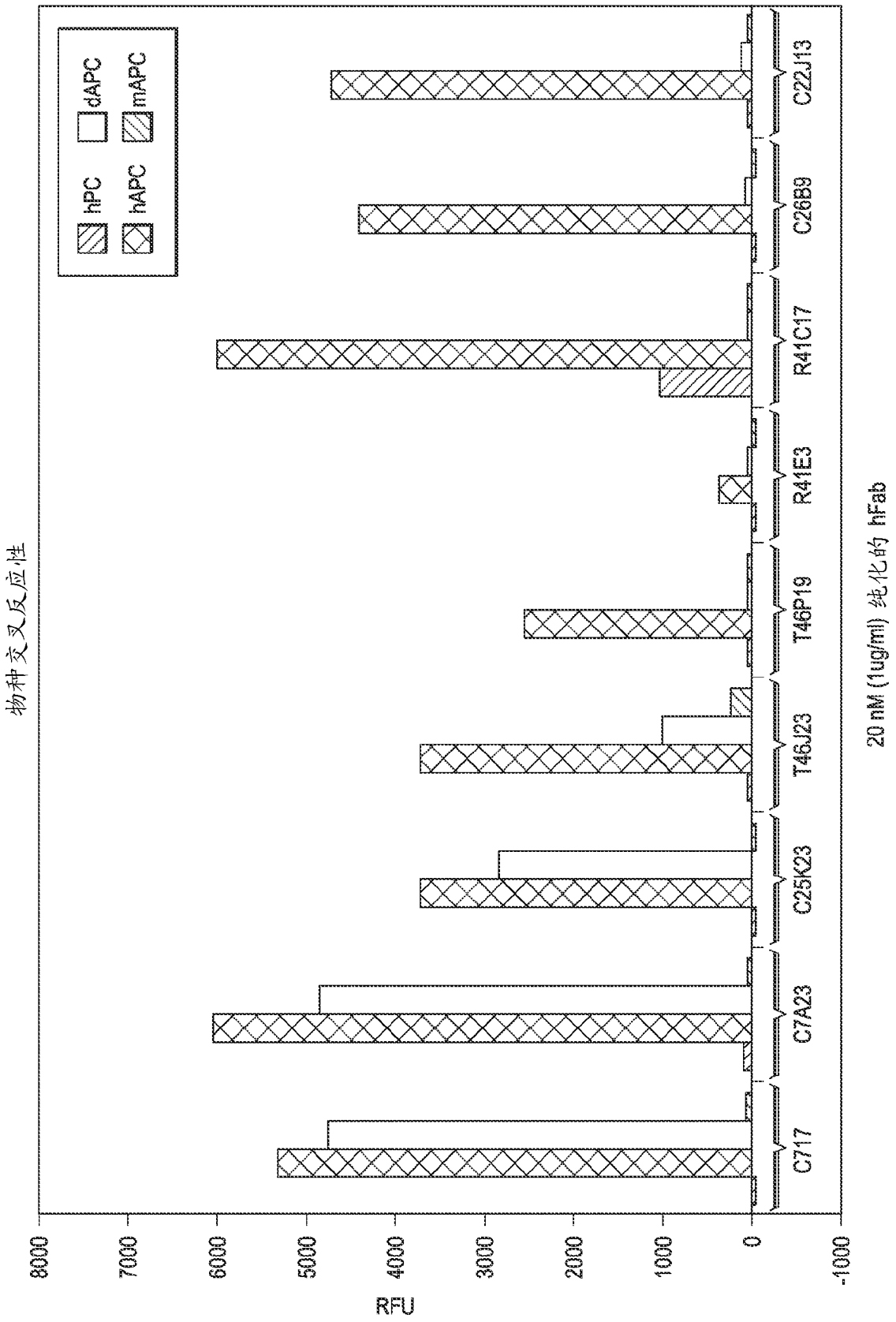

物种交叉反应性
hPC
dAPC
hAPC
mAPC
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
RFU
C717
C7A23
C25K23
T46J23
T46P19
R41E3
R41C17
C26B9
C22J13
20 nM (1ug/ml) 纯化的 hFab

图 3

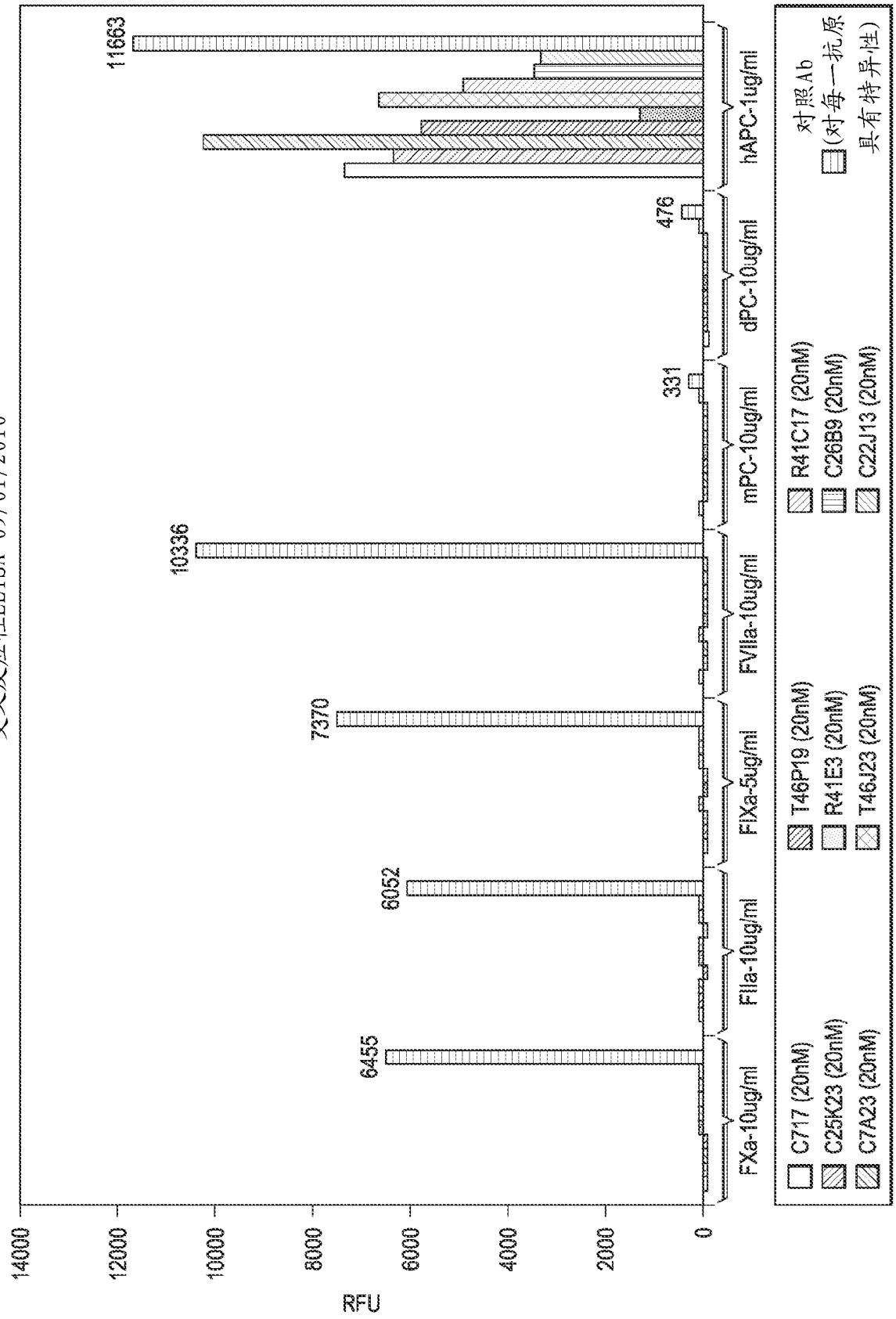

交叉反应性ELISA 09/01/2010
RFU
14000
12000
10000
8000
6000
4000
2000
0
6455
6052
7370
10336
331
476
11663
FXa-10ug/ml
FIIa-10ug/ml
FIXa-5ug/ml
FVIIa-10ug/ml
mPC-10ug/ml
dPC-10ug/ml
hAPC-1ug/ml
C717 (20nM)
C25K23 (20nM)
C7A23 (20nM)
T46P19 (20nM)
R41E3 (20nM)
T46J23 (20nM)
R41C17 (20nM)
C26B9 (20nM)
C22J13 (20nM)
对照Ab
(对每一抗原具有特异性)

图 4

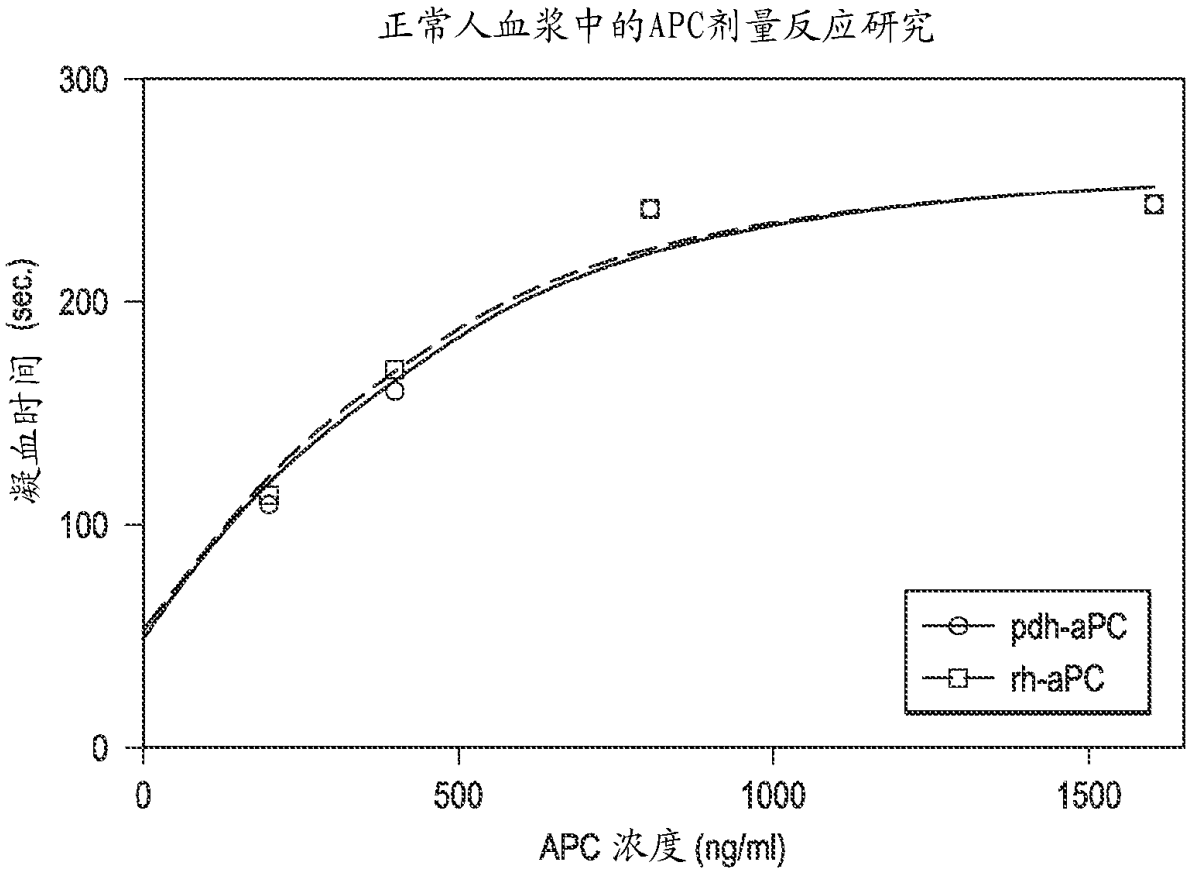

正常人血浆中的APC剂量反应研究
凝血时间 (sec.)
300
200
100
0
0
500
1000
1500
APC 浓度 (ng/ml)
pdh-aPC
rh-aPC

图 5

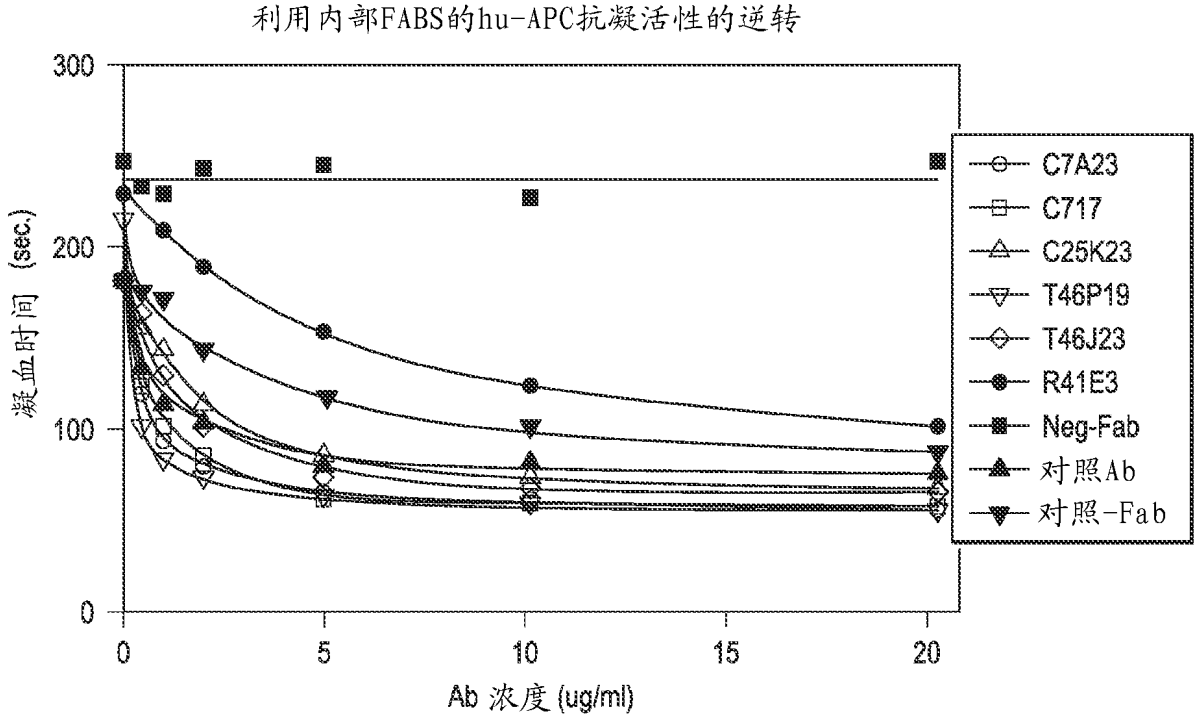

利用内部FABS的hu-APC抗凝活性的逆转
300
200
100
0
凝血时间 (sec.)
0
5
10
15
20
Ab 浓度 (ug/ml)
C7A23
C717
C25K23
T46P19
T46J23
R41E3
Neg-Fab
对照Ab
对照-Fab

图 6

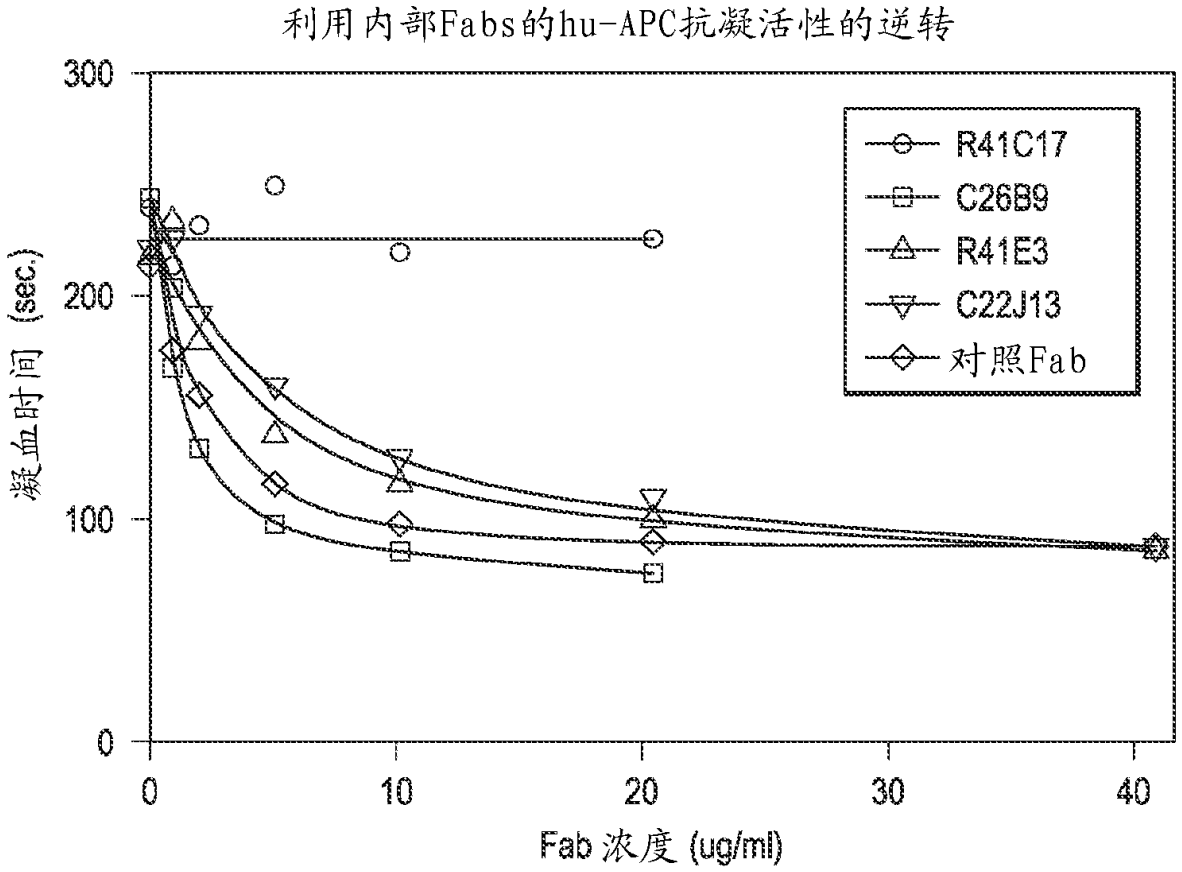

利用内部Fabs的hu-APC抗凝活性的逆转
300
200
100
0
凝血时间 (sec.)
0
10
20
30
40
Fab 浓度 (ug/ml)
R41C17
C26B9
R41E3
C22J13
对照Fab

图 6

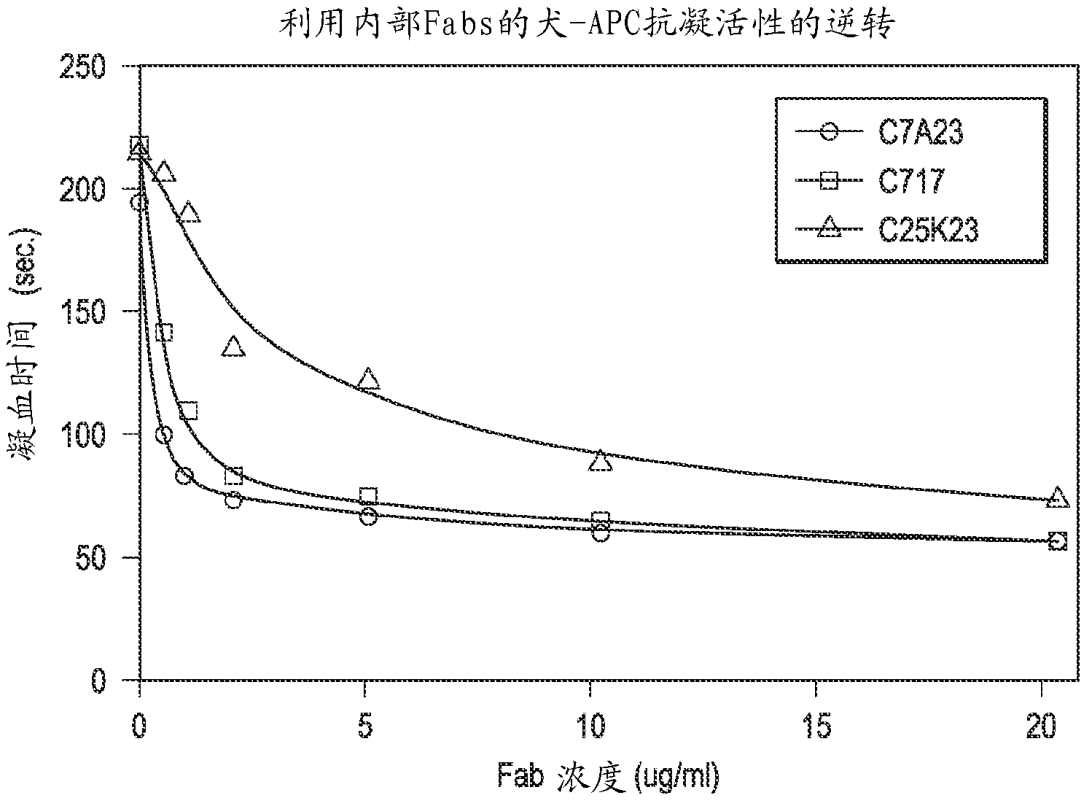
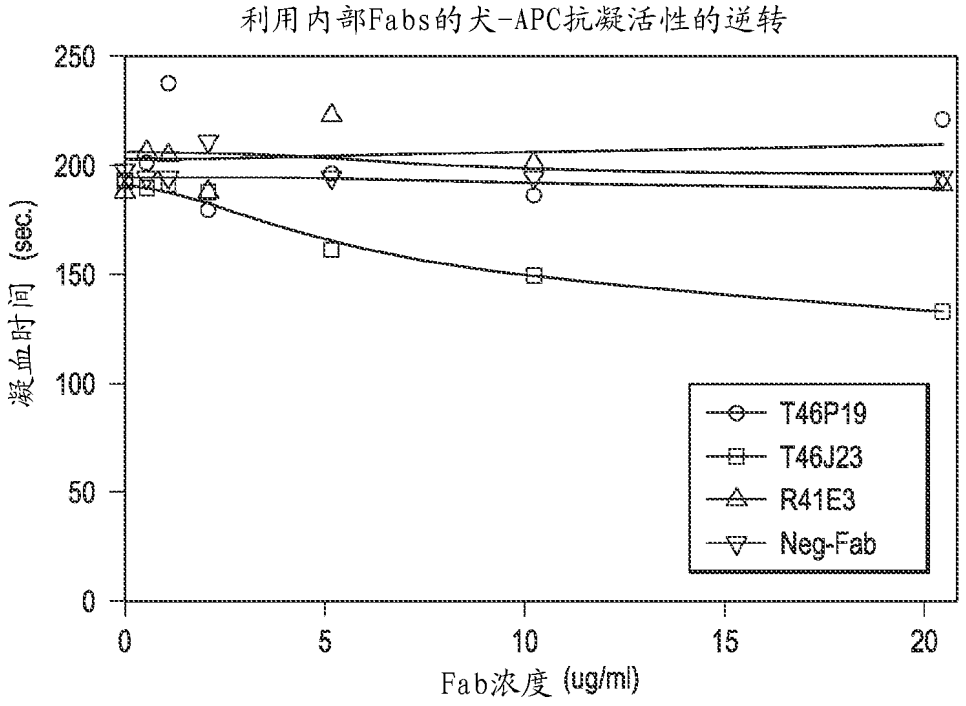

利用内部Fabs的犬-APC抗凝活性的逆转
250
200
150
100
50
0
凝血时间 (sec.)
0
5
10
15
20
Fab 浓度 (ug/ml)
C7A23
C717
C25K23

图 7



犬-APC (400 ng/ml)

Fab浓度 (ug/ml) (Fab:APC)	%抑制					
	C7A23	C717	C25K23	T46P19	T46J23	R41E3
20.4 (60:1)	95.56	95.16	84.61	0.00	41.48	0.00
10.2 (30:1)	91.59	92.14	75.90	0.00	30.50	0.00
5.08 (15:1)	**88.11**	**48.35**	**55.77**	**0.00**	**21.12**	**0.00**
2.04 (6:1)	82.50	80.27	47.25	0.00	3.45	0.00
1.02 (3:1)	75.32	64.44	15.22	0.00	0.62	0.00
0.51 (1.5:1)	64.59	45.19	5.68	0.00	2.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00

图 7

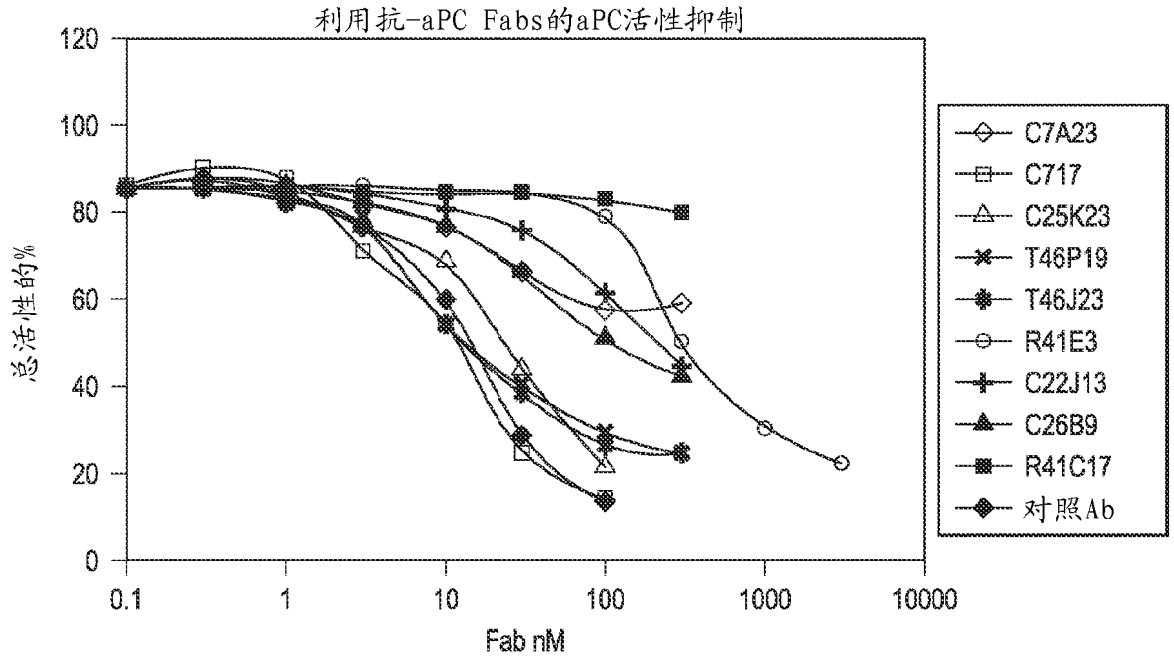

利用抗-aPC Fabs的aPC活性抑制
总活性的%
120
100
80
60
40
20
0
0.1
1
10
100
1000
10000
Fab nM
C7A23
C717
C25K23
T46P19
T46J23
R41E3
C22J13
C26B9
R41C17
对照Ab

图 8

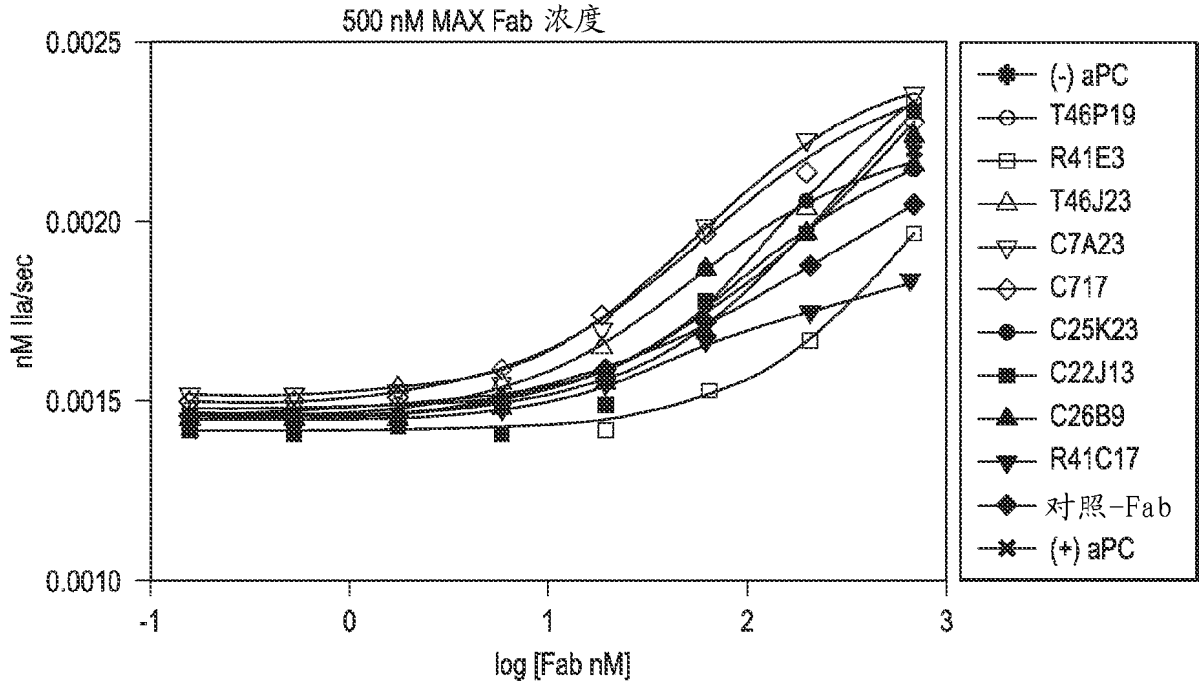

500 nM MAX Fab 浓度
nM IIa/sec
0.0025
0.0020
0.0015
0.0010
-1
0
1
2
3
log [Fab nM]
(-) aPC
T46P19
R41E3
T46J23
C7A23
C717
C25K23
C22J13
C26B9
R41C17
对照-Fab
(+) aPC

图 9

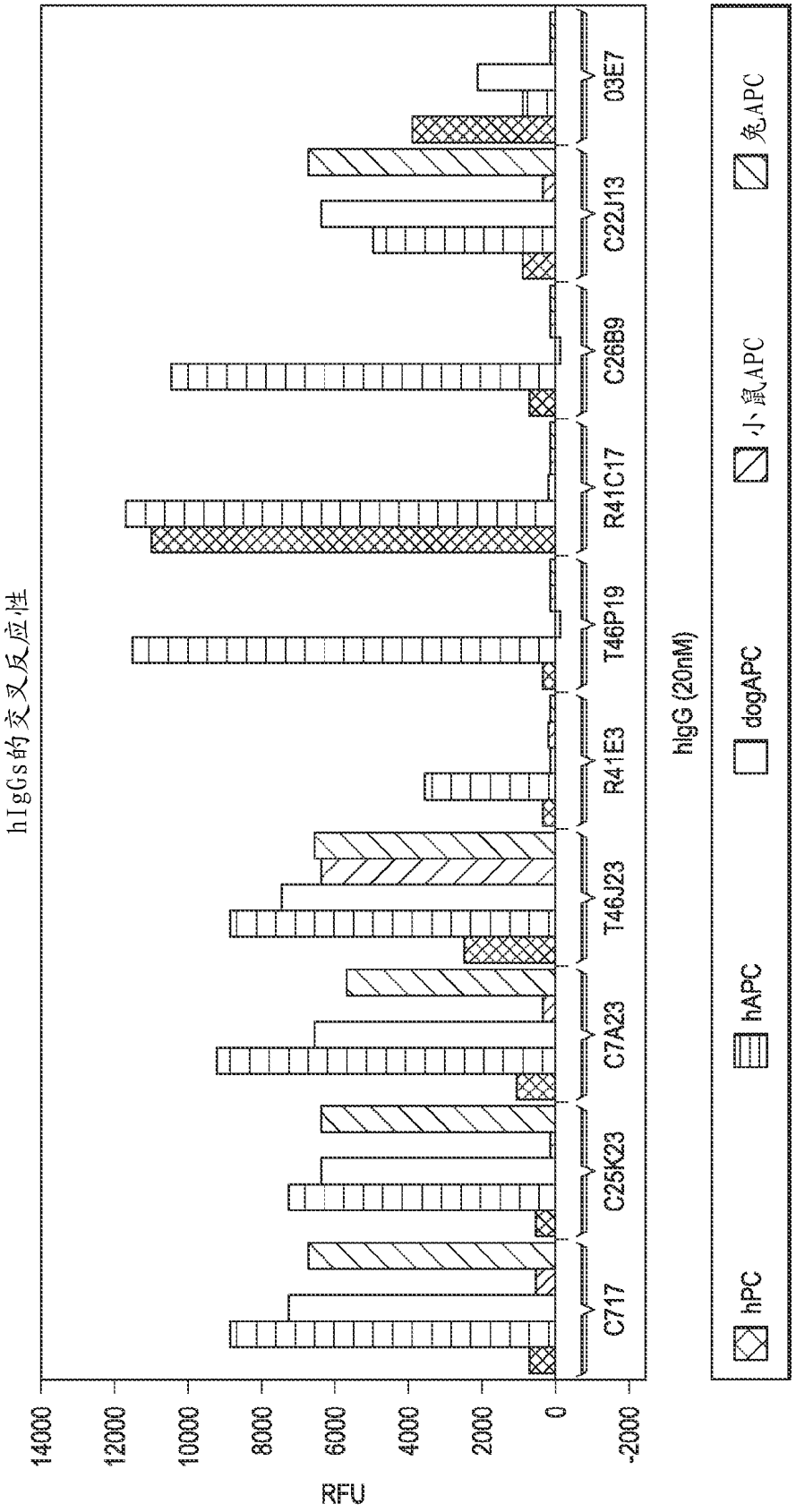

hIgGs的交叉反应性
RFU
14000
12000
10000
8000
6000
4000
2000
0
-2000
C717
C25K23
C7A23
T46J23
R41E3
T46P19
R41C17
C26B9
C22J13
03E7
hIgG (20nM)
hPC
hAPC
dogAPC
小鼠APC
兔APC

图 10

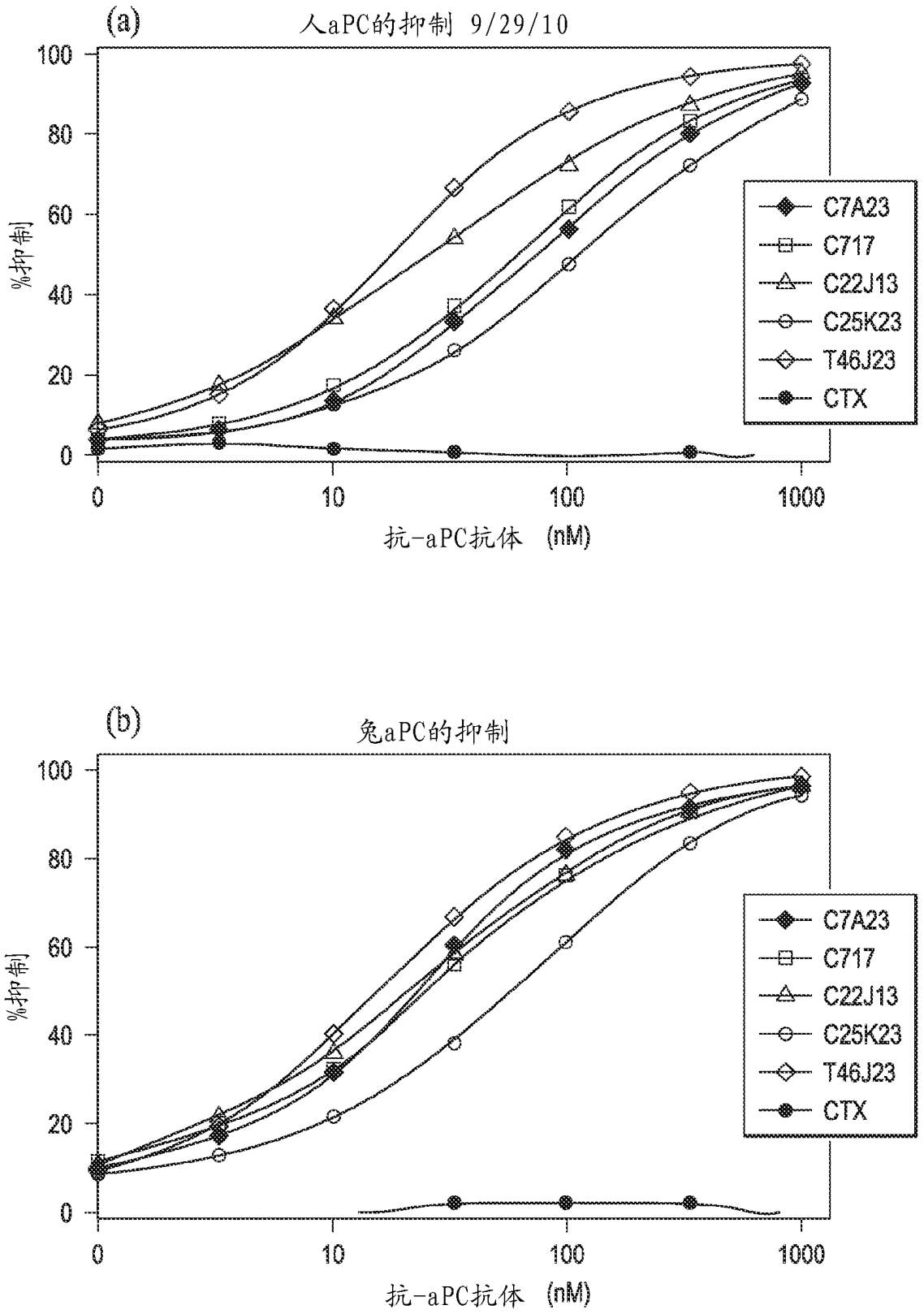
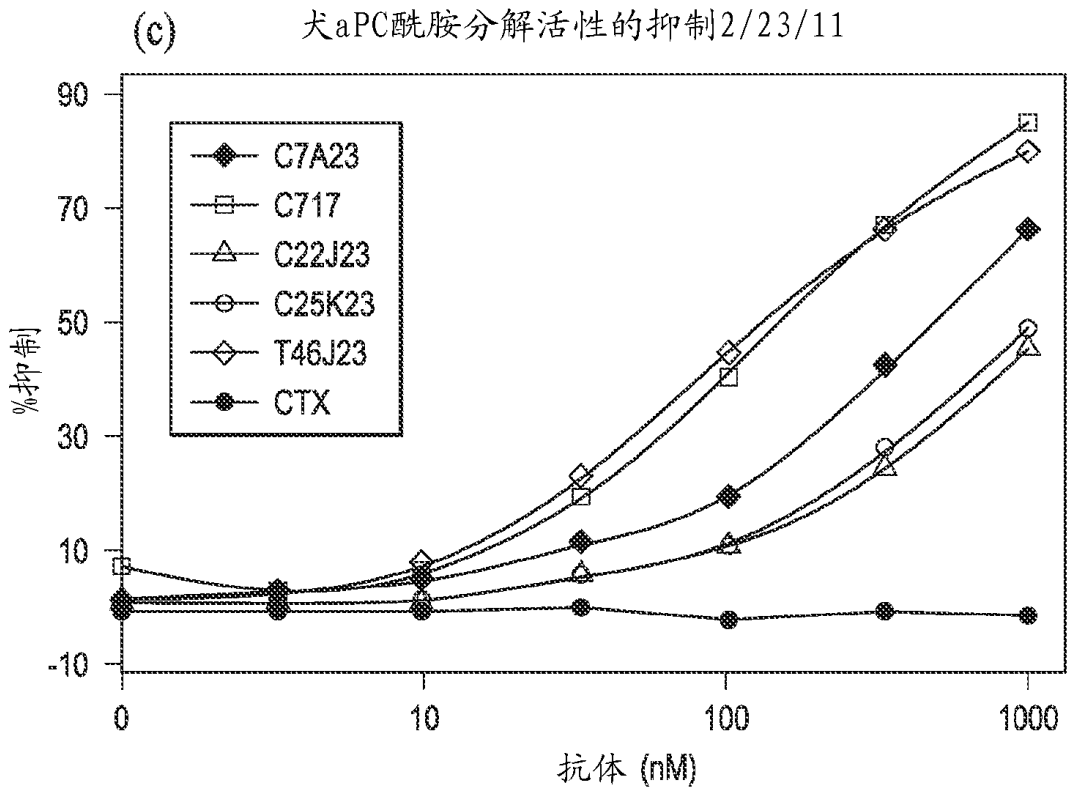

(a)
人aPC的抑制 9/29/10
%抑制
抗-aPC抗体 (nM)
C7A23
C717
C22J13
C25K23
T46J23
CTX
(b)
兔aPC的抑制
%抑制
抗-aPC抗体 (nM)
C7A23
C717
C22J13
C25K23
T46J23
CTX

图 11


(c)
犬aPC酰胺分解活性的抑制2/23/11
C7A23
C717
C22J23
C25K23
T46J23
CTX
%抑制
90
70
50
30
10
-10
0
10
100
1000
抗体 (nM)

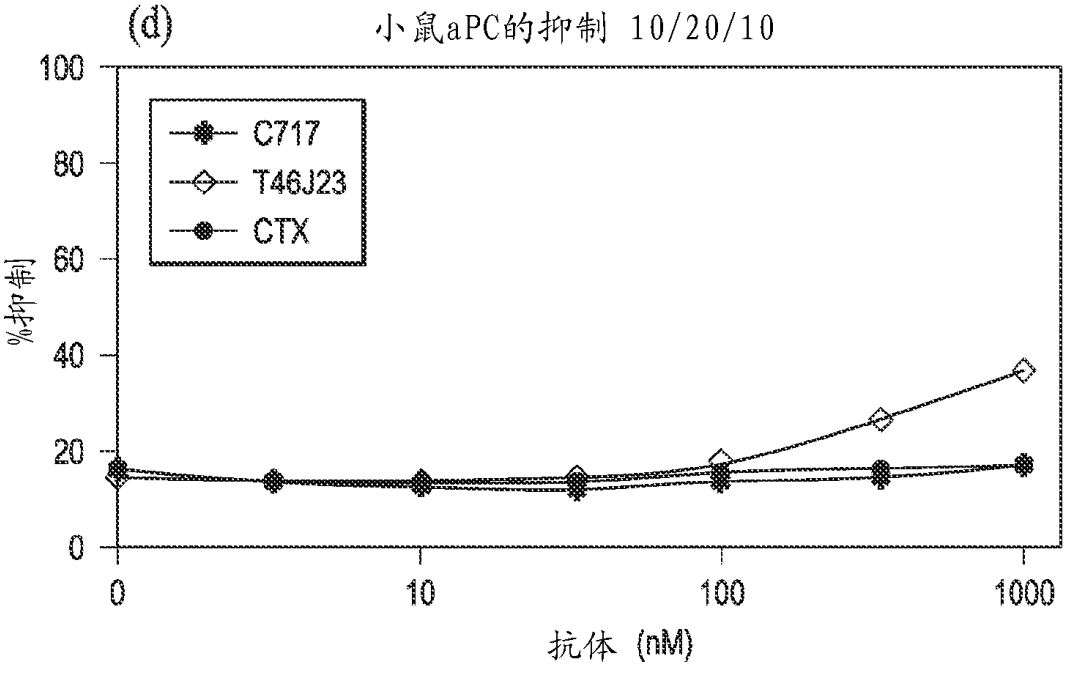

(d)
小鼠aPC的抑制 10/20/10
C717
T46J23
CTX
%抑制
100
80
60
40
20
0
0
10
100
1000
抗体 (nM)

图 11

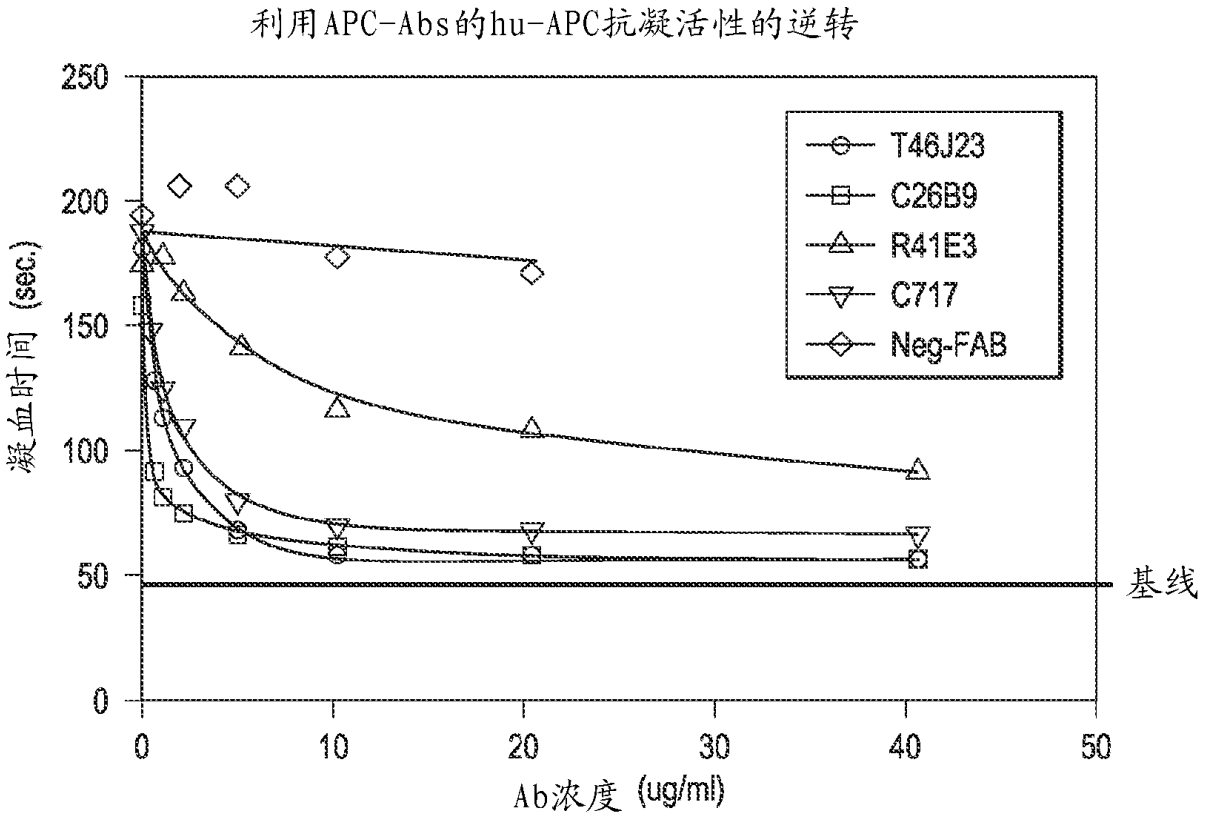

利用APC-Abs的hu-APC抗凝活性的逆转
250
200
150
100
50
0
凝血时间 (sec.)
0
10
20
30
40
50
Ab浓度 (ug/ml)
T46J23
C26B9
R41E3
C717
Neg-FAB
基线

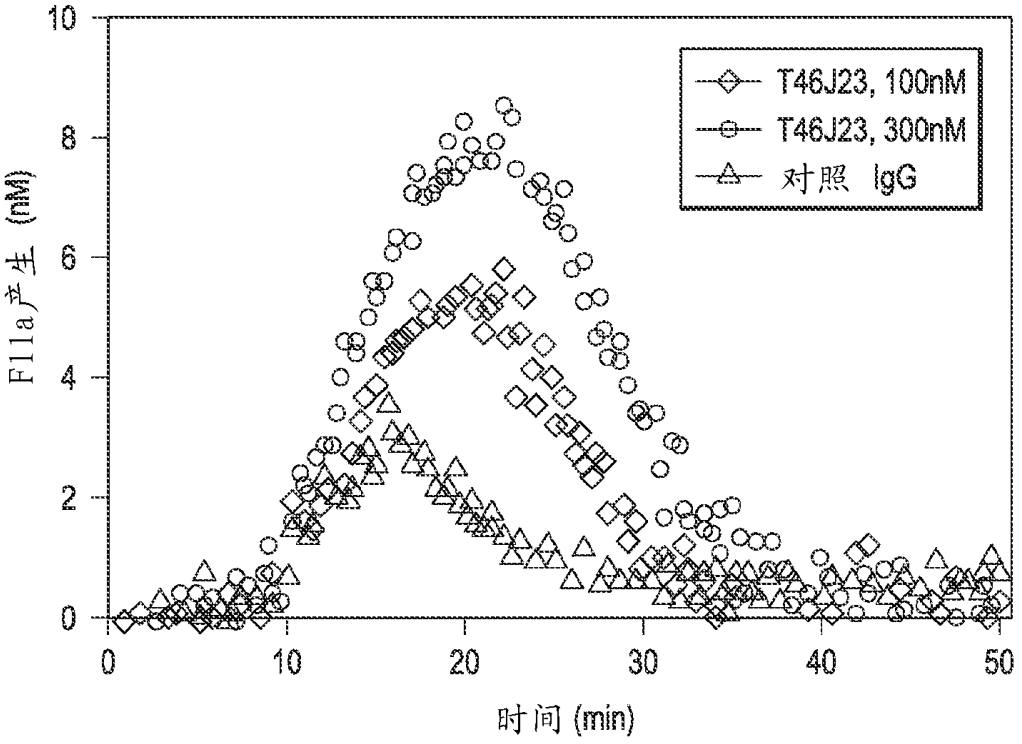
图 12

hu-APC (400 ng/ml)

Fab浓度 (ug/ml) (Ab:APC)	凝血时间					%抑制				
	T46.J23	C26B9	R41E3	C717	Neg-IgG	T46.J23	C26B9	R41E3	C717	Neg-IgG
163.2 (163:1)			75					80.02		
81.6 (81:1)			80.8					75.43		
40.8 (40:1)	53	56	91.5	62.6		98.45	96.64	66.98	92.89	
20.4 (20:1)	57.9	58.2	109.2	67.4	170.5	94.67	94.53	53.00	89.33	16.07
10.2 (10:1)	59.1	62.0	117	73.2	177.8	93.74	90.89	46.84	85.04	10.88
5.08 (5:1)	68.8	66.3	142.4	79.6	205.5	86.24	86.77	26.78	80.30	-8.82
2.04 (2:1)	93.3	73.5	165.1	109	204.9	67.31	79.87	8.85	58.52	-8.39
1.02 (1:1)	114.1	81.5	177.5	124.8	169.8	51.24	72.20	-0.95	46.81	16.57
0.51 (0.5:1)	129.0	91.5	174.4	147.1	158.1	39.72	62.61	1.50	30.30	24.89
0	180.4	156.8	176.3	188	193.1	0.00	0.00	0.00	0.00	0.00
50% FACT	51.0	52.5	49.7	53.0	52.5					

● R41E3在APTT分析中在抑制hu-APC方面不如其它IgG有效

图 12


T46J23, 100nM
T46J23, 300nM
对照 IgG
FIIa产生 (nM)
时间 (min)
0
2
4
6
8
10
0
10
20
30
40
50

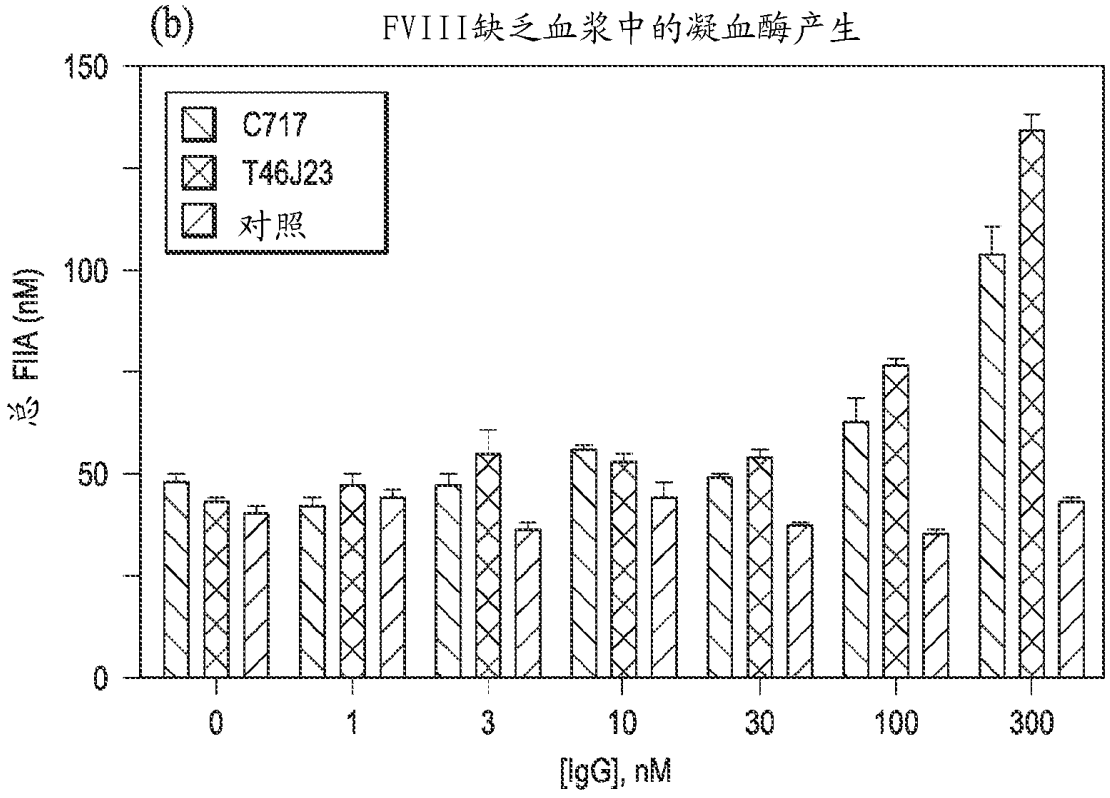
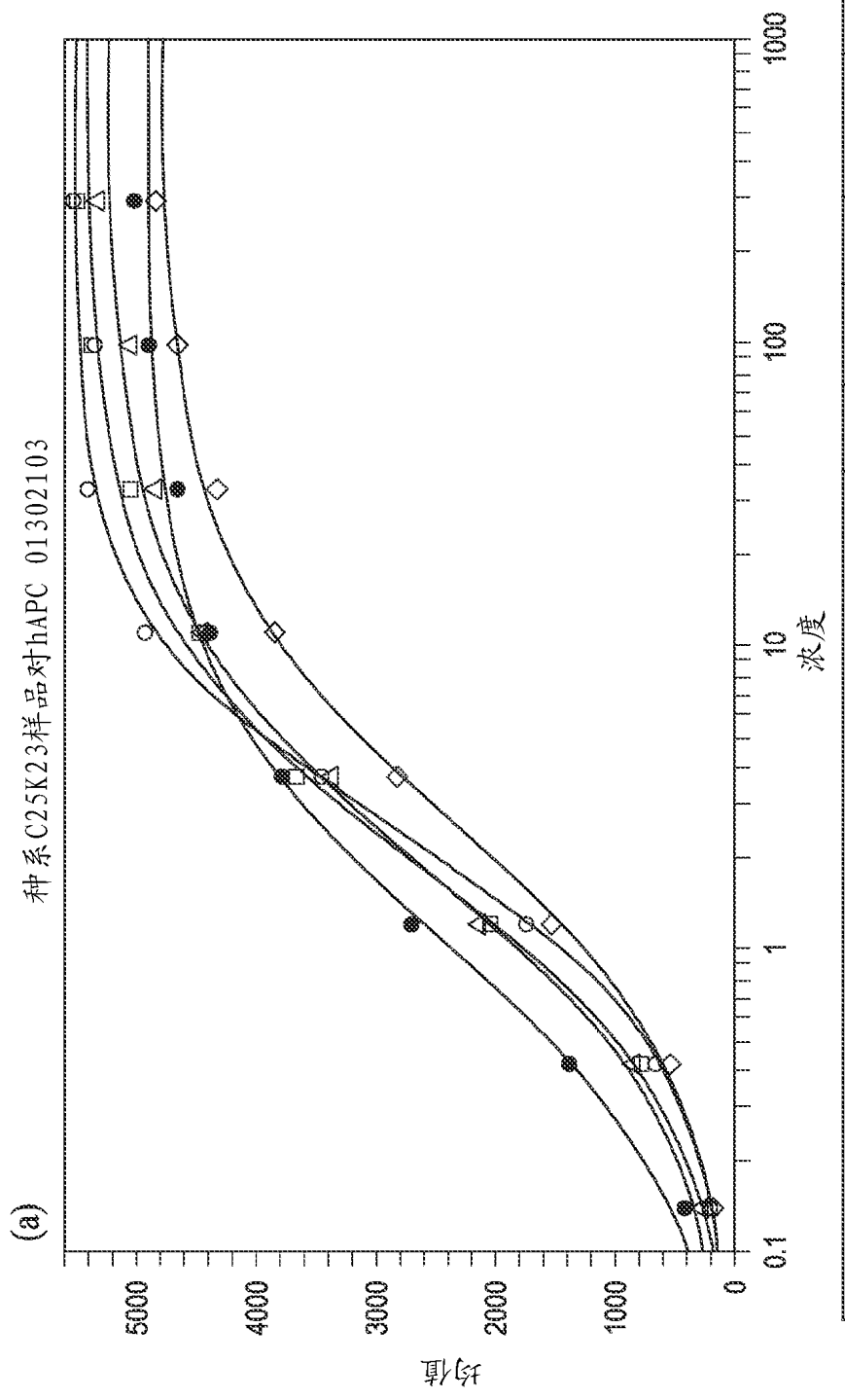
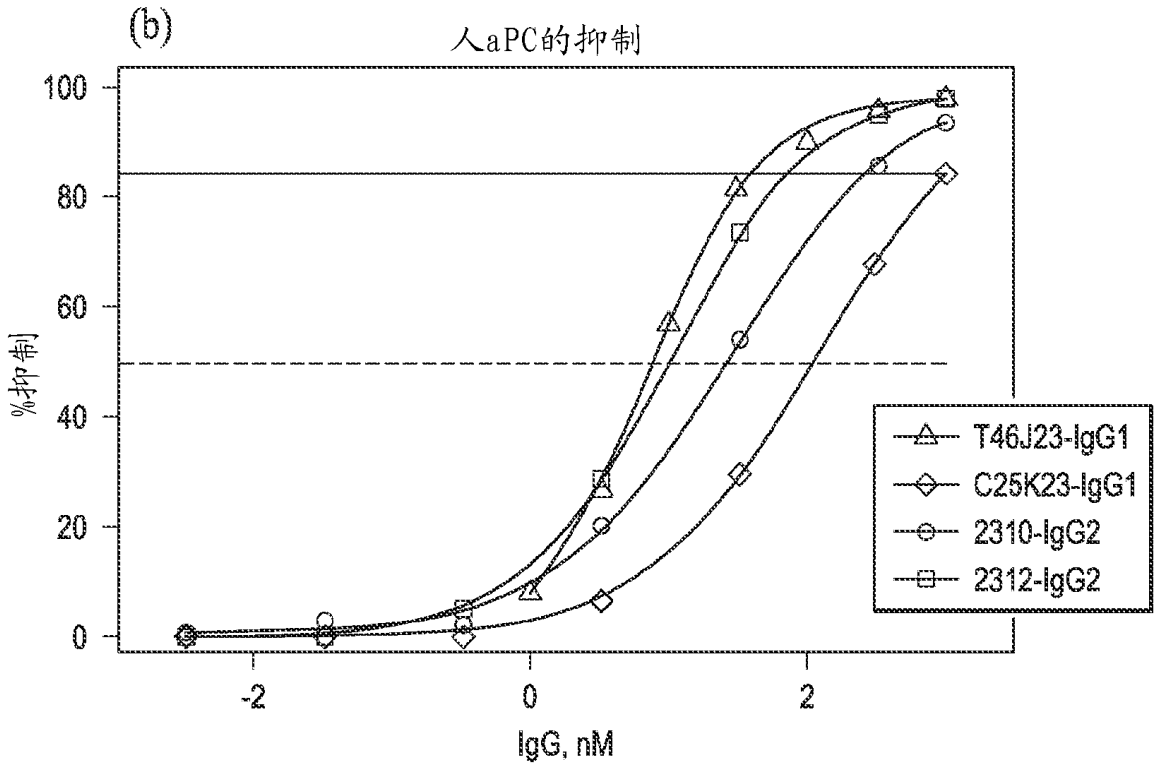

(b)
FVIII缺乏血浆中的凝血酶产生
C717
T46J23
对照
总 FIIA (nM)
[IgG], nM
0
50
100
150
0
1
3
10
30
100
300

图 13



4-P FIT: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
C25K23 对 hAPC (C25K23 hIgG1 对 hAPC: 浓度)	63.3	1.25	2.4	5.51e+03	0.999
TPP-2031 对 hAPC (TPP-2031 对 hAPC: 浓度)	-65.9	1.03	1.98	5.42e+03	0.998
TPP-2309 对 hAPC (TPP-2309 对 hAPC: 浓度)	-53.6	0.971	1.89	5.24e+03	0.999
TPP-2310 对 hAPC (TPP-2310 对 hAPC: 浓度)	-47.2	0.994	2.66	4.8e+03	0.999
TPP-2312 对 hAPC (TPP-2312 对 hAPC: 浓度)	-60.5	0.986	1.06	4.91e+03	0.998

图 14


(b)
人aPC的抑制
%抑制
100
80
60
40
20
0
-2
0
2
IgG, nM
T46J23-IgG1
C25K23-IgG1
2310-IgG2
2312-IgG2

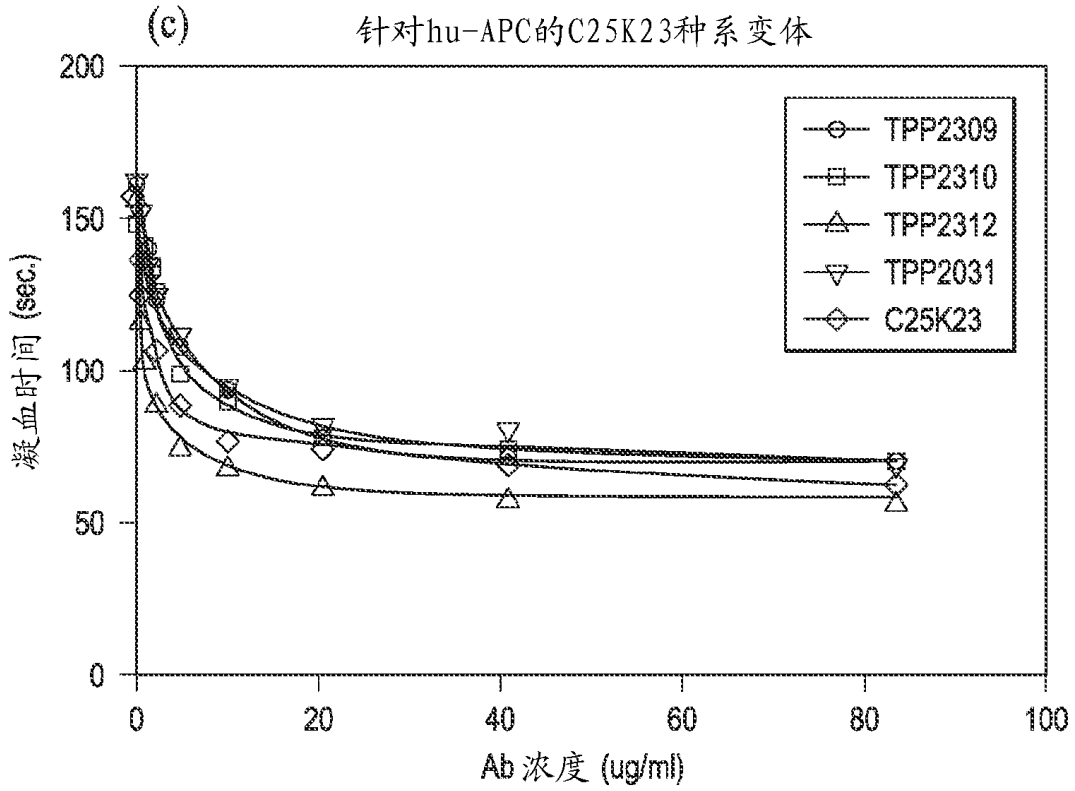

(c)
针对hu-APC的C25K23种系变体
凝血时间 (sec.)
200
150
100
50
0
0
20
40
60
80
100
Ab 浓度 (ug/ml)
TPP2309
TPP2310
TPP2312
TPP2031
C25K23

图 14

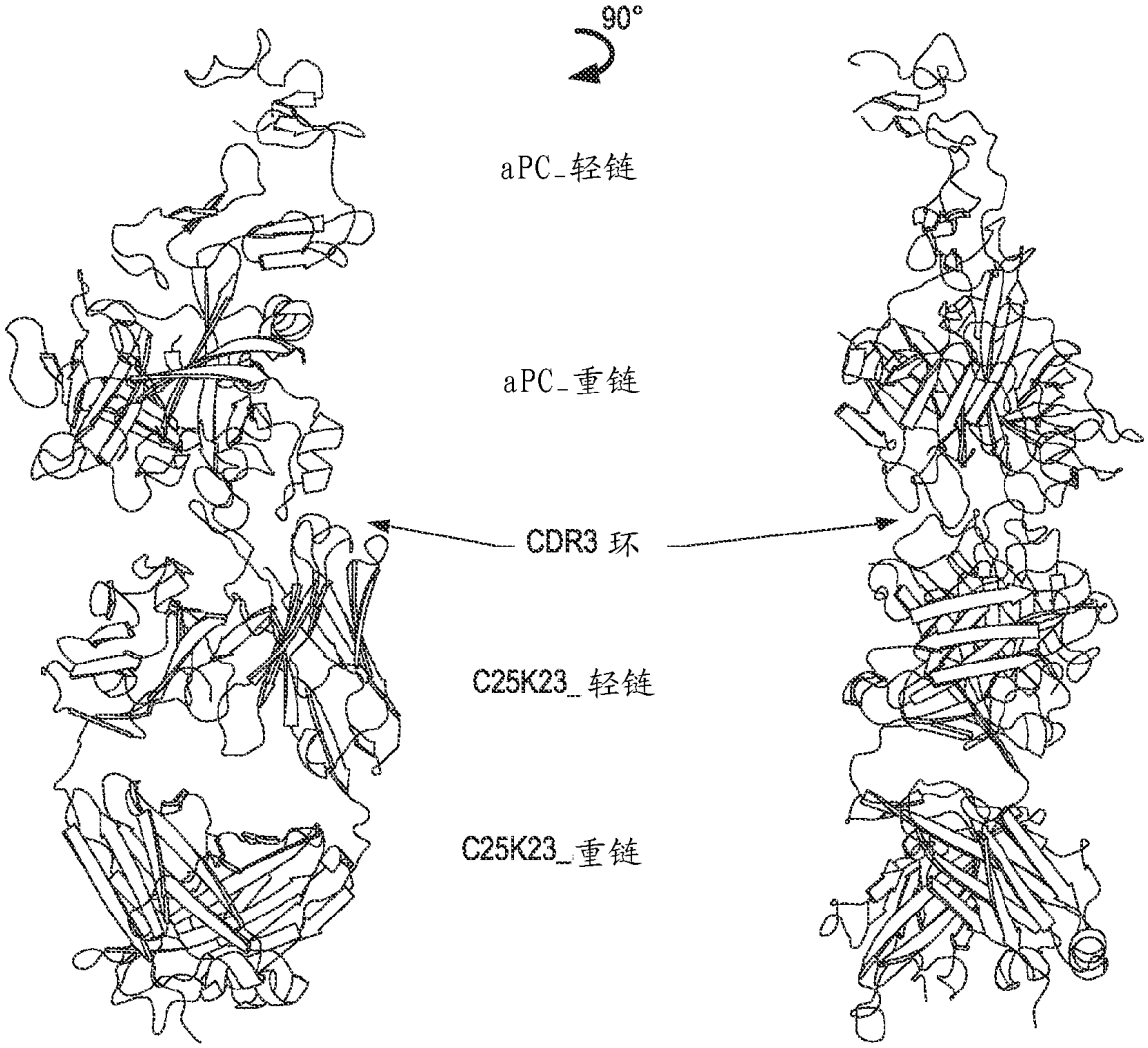


图 15

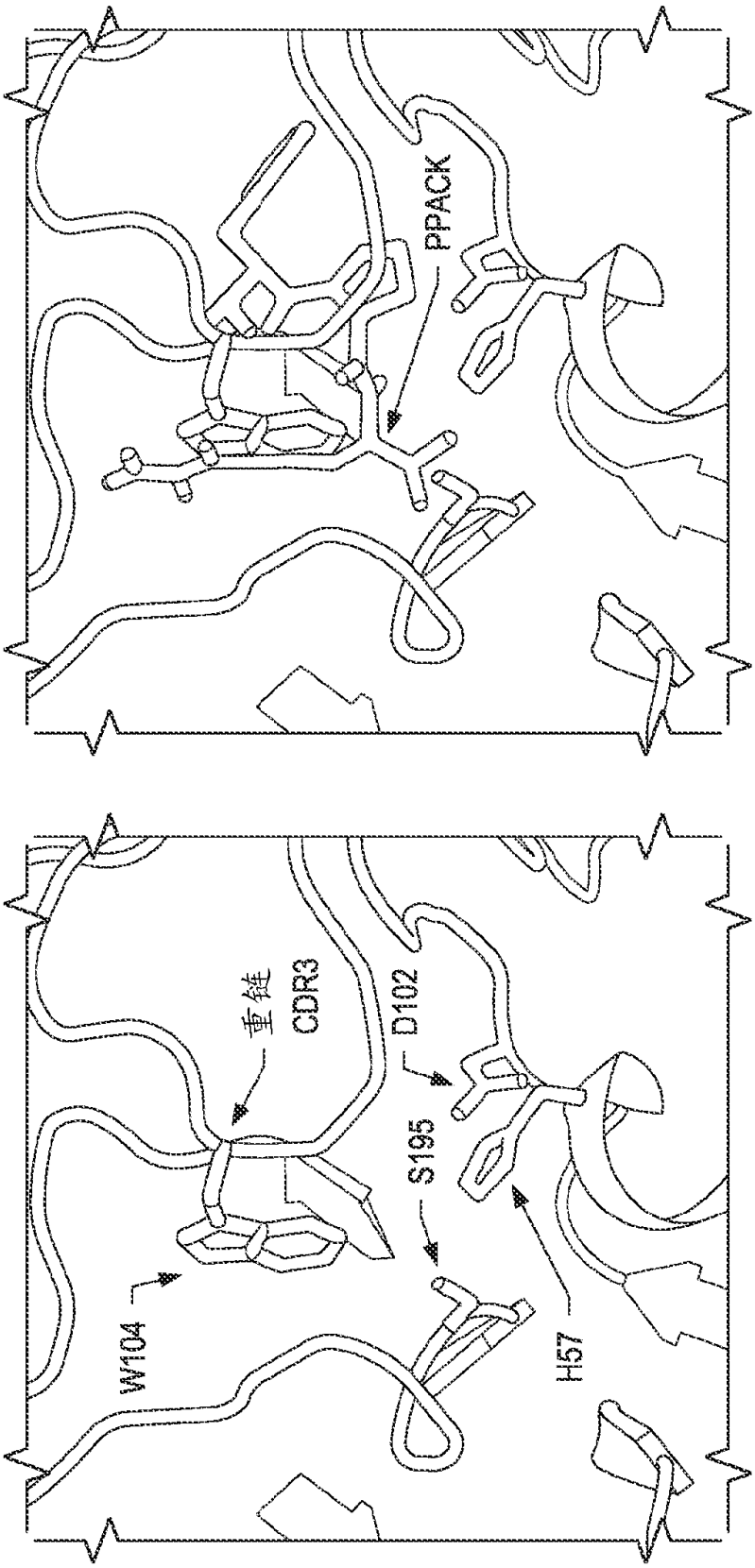


图 16

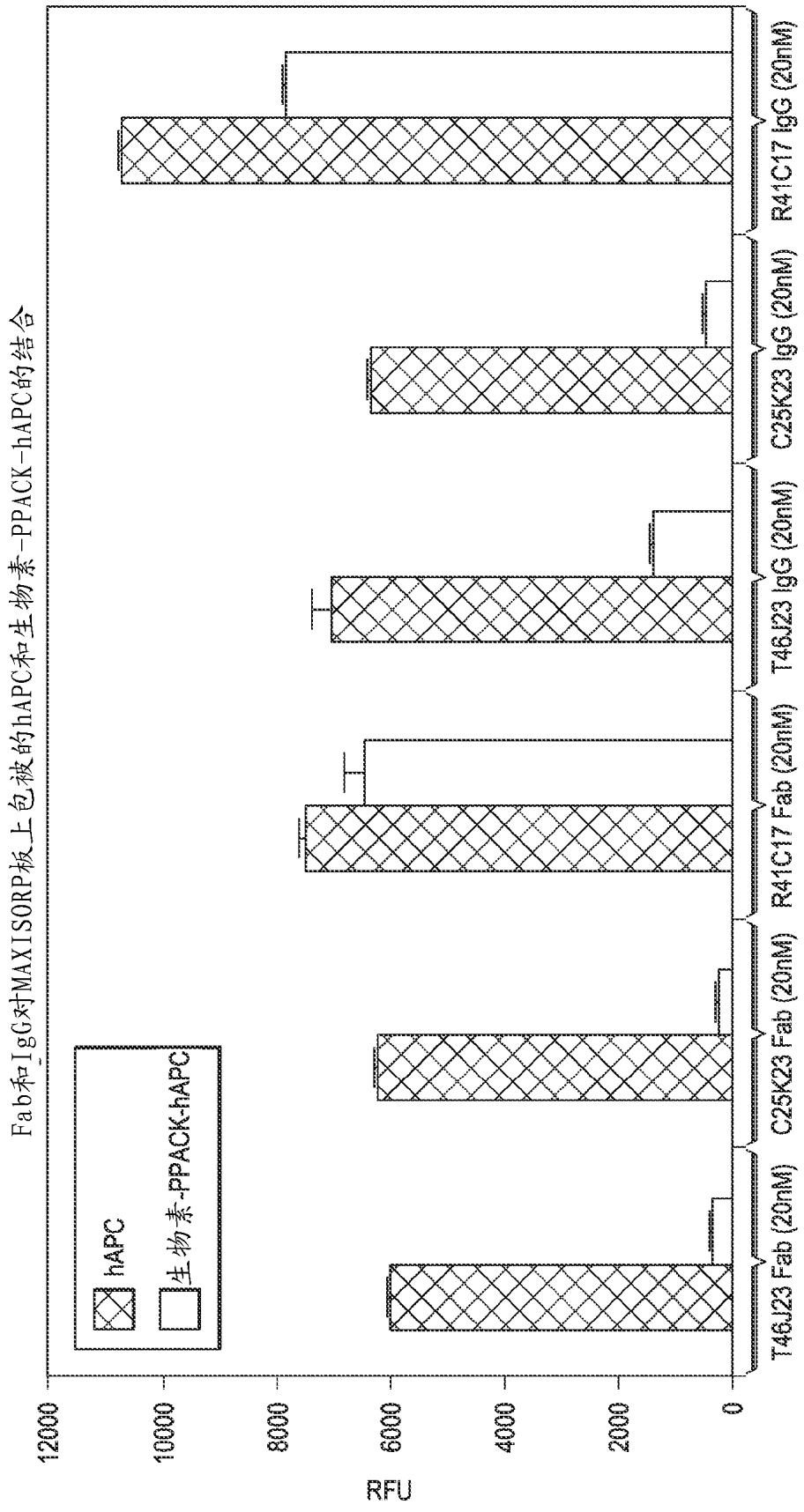

Fab和IgG对MAXISORP板上包被的hAPC和生物素-PPACK-hAPC的结合
hAPC
生物素-PPACK-hAPC
RFU
12000
10000
8000
6000
4000
2000
0
T46J23 Fab (20nM)
C25K23 Fab (20nM)
R41C17 Fab (20nM)
T46J23 IgG (20nM)
C25K23 IgG (20nM)
R41C17 IgG (20nM)

图 17