

MONOCLONAL ANTIBODIES AGAINST ANTITHROMBIN BETA COMPLEXED WITH HEPARIN

Cross-References to Related Applications

5 This application is being filed on 14 March 2014, as a PCT International patent application, and claims priority to U.S. Provisional Patent Application No. 61/784,590, filed March 14, 2013, the entire disclosure of which is hereby incorporated by reference in its entirety.

Sequence Listing Submission

10 The present application includes a Sequence Listing in electronic format as a txt file titled "SEQUENCE-LISTING-17207.0006WOU2" which was created on March 14, 2014 and which has a size of 65.1 kilobytes (KB). The contents of txt file "SEQUENCE-LISTING-17207.0006WOU2" are incorporated by reference herein.

Background

15 Current unmet medical needs in the hemophilia field are mainly: (1) treatment of hemophilia patients with inhibitors (~30% of hemophilia patients); and (2) long acting and efficacious coagulant factors (FVIII/FIX) and/or their replacement (bypass drugs) (WFH report 2012, Paris). The most widely used bypass
20 drug for treating hemophilia patients with inhibitors is rFVII, which has major drawbacks such as risk of thrombogenicity, short half-life in plasma and high production cost. Antibodies against anti-coagulant factors, such as Tissue Factor Protein Inhibitor (TFPI), APC (Activated Protein C) and Antithrombin (AT) represent a new treatment paradigm. These antibodies not only bypass or reduce the
25 need for FVIII or FIX coagulation factors in hemophilia patients with inhibitors, but also exhibit longer plasma half-life (which reduces the dosing frequency) and, thus, increases patient compliance. To date, there have been several antibody-based procoagulant drugs at the preclinical development or research stage, such as anti-TFPI and anti-APC.

30 AT is a major anticoagulant in human plasma. It inhibits thrombin, FXa and other serine proteases functioning in the coagulation pathway. It consists of 432 amino acids, is produced by the liver hepatocyte and has a long plasma half-life of

three days (Collen, Schetz et al. 1977). The amino acid sequence of AT is well-conserved and the homology among cow, sheep, rabbit, mouse and human is 84%-89% (Olson and Bjork 1994). Although the primary physiological targets of AT are thrombin and FXa, AT also inhibits FIXa, FXIa, FXIIa, as well as FVIIa to a lesser extent. AT exerts its inhibition together with heparin. In presence of heparin the inhibition rate of thrombin and FXa by AT increases by 3 to 4 orders of magnitude from $7-11 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ to $1.5 - 4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and from $2.5 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ to $1.25 - 2.5 \text{ M}^{-1} \text{ s}^{-1}$ respectively (Olson, Swanson et al. 2004).

Unlike TFPI and APC which inhibit coagulation solely at the initiating stage and the amplification stage respectively, AT exerts its inhibition on coagulation at both the initiation and amplification stage. Therefore, blocking AT could have more potent pro-coagulant effect than blocking either TFPI or APC alone. Decreased AT levels and activity have been shown to correlate with increased thrombosis in human. Patients with AT deficiency tend to show recurrent venous thrombosis and pulmonary embolisms (van Boven and Lane 1997). Furthermore, homozygous AT knockout mice die in the embryonic stage with an extreme hypercoagulable state (Ishiguro, Kojima et al. 2000). A recent study shows that heterozygous AT knockout hema mice in which AT is reduced by 50% significantly have less blood loss and enhanced thrombin generation in a tail-clip bleeding model (Bolliger, Szlam et al. 2010).

AT is a glycoprotein with two isoforms based on differential glycosylation on Asn135, AT α and AT β (Bjork 1997). AT β lacks glycosylation at Asn135 and is a minor glyco-isoform representing 10% of human plasma AT. Asn135 is located adjacent to the initial heparin attachment site and constitutes part of extended heparin binding site after allosteric activation and D helix extension (dela Cruz, Jairajpuri et al. 2006). The lack of bulky-sized glycan at Asn135 affects AT β activation profoundly in two ways: 1) a faster allosteric activation upon heparin binding required for inhibition of FXa and FIXa; and 2) extra accessible binding sites for higher affinity heparin binding for inhibition of FXa and thrombin by a bridging mechanism. Indeed, under physiological salt concentration, plasma-derived AT β binds to heparin with a K_D of $36 \pm 3 \text{ nm}$ while AT α binds to heparin with a K_D of $500 \pm 50 \text{ nm}$ (Turk IV. et al., 1993). The higher affinity of AT β for heparin leads

to its preferential distribution to the sub-endothelial layer which is enriched in the heparin-like structure – glycosaminoglycan. Consequently, AT β is proposed to play a major and potent role in inhibition of FXa and thrombin at the vascular injury sites (Carlson and Atencio 1982; McCoy AJ, Pei XY. *et al.* 2003; Turk B, Brieditis I. *et al.* 1997; Witmer MR, Hatton MW. 1991; Frebelius S, *et al.* 1996). The importance and stronger potency of AT β relative to that of AT α is also reported in clinical studies. In patients, the severity of AT homozygous mutations defective in heparin-binding is ameliorated by the beta form of AT (Martinez-Martinez, Navarro-Fernandez *et al.* 2012). In another study, a borderline level (~70% of normal AT antigen and activity) of AT is compensated by the 20% ~ 30% AT β in plasma (Bayston, Tripodi *et al.* 1999).

Summary

Monoclonal antibodies to human AT β H (AT β complexed with heparin and/or heparin-like structure) are provided. In at least one embodiment, the anti-AT β H monoclonal antibodies exhibit binding to AT β complexed with Heparin.

In other embodiments, the monoclonal antibodies to AT β H may be optimized, for example to have increased affinity or increased functional activity. Also provided are specific epitopes that may be on human AT β H and are bound by an isolated monoclonal antibody. Further provided are the isolated nucleic acid molecules encoding the same.

Pharmaceutical compositions comprising the anti-AT β H 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 bleeding time by administering an anti-AT β H monoclonal antibody to a patient in need thereof. Methods for producing a monoclonal antibody that binds human AT β H are also provided.

Brief Description of the Drawings

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 or claims in any way.

FIG. 1 shows a schematic representation of AT β bound to heparin and the various binding domains of AT β .

FIGS. 2A-2C show how AT β is distinguished from AT α by lacking of one N-glycan.

FIGS. 3A-3D show AT β with faster binding to heparin and more potent inhibition than AT α .

FIG 4A shows biotinlayted hAT and rAT are functional in inhibition of Fxa generation (Fig. 4A). FIGS 4B-4C show various strategies for antibody discovery by phage display.

FIG. 5 shows a screening method for identifying antibodies capable of functional inhibition of AT β :heparin.

FIGS. 6A and 6B shows alignment of the amino acid sequences of the light chain domain and heavy chain domain, respectively, of antibodies TPP-2009 (SEQ ID NO:1 and SEQ ID NO: 2, respectively), TPP-2015 (SEQ ID NO:3 and SEQ ID NO:4, respectively), TPP-2016 (SEQ ID NO:5 and SEQ ID NO:6 respectively), TPP-2019 (SEQ ID NO:7 and SEQ ID NO:8, respectively), and TPP-2803 (SEQ ID NO:9 and SEQ ID NO:10, respectively).

FIGS. 7A-7C show antibody binding specificity determined by Biacore (FIG. 7A) and ELISA (FIG. 7B) tests, and antibody binding affinity to human At β H (FIG. 7C).

FIG. 8A is a graphical representation of the effect of TPP antibodies on thrombin generation in human HEM-A plasma, and illustrates that antibody presence increases peak thrombin generation in human HEM-A plasma.

FIG. 8B is a table showing antibodies shorten clotting time in human Hema plasma and in human AT-deficient plasma spiked in with At β or At α .

FIG 9 is a graphical representation of the PK of antibody TPP 2009 in HEM-A mice using IV dosing at 0.3, 3 and 30mg/kg, three mice per time point (10 time points over 21 days), and associated PK parameters.

FIGS. 10A and 10B show an experimental protocol for a tail vein transection (TVT) model in HemA and the efficacy of antibody TPP-2009 in the TVT model in HemA mice. FIG. 10B shows the antibody TPP-2009 has potent efficacy in the Tail Vein Transection (TVT) model of HemA mice.

FIGS. 11A and 11B shows a molecular model of the three-dimensional structures of native AT β complexed with/without heparin (FIG. 11A), and fully activated antibody TPP2009 bound to heparin (FIG. 11B) and its predicted epitope structure. Helix D is extended upon heparin binding B.

FIG. 12 shows a TPP2803 exhibited dose-dependent shortening of the clotting time in both normal human plasma and hemophilia patient plasma using the FXa activated clotting assay. CT: clotting time, HEM-A: Hemophilia A plasma.

FIG. 13 shows an experimental design of the heparinized rabbit bleeding model; Experimental groups: Vehicle, PBS; Positive control, Protamine sulfate, (28mg/kg IV); Negative control, M14 IgG2; treatment: 30mg/kg; TPP2803, 3mg/kg; TPP2803, 30mg/kg.

FIG. 14. shows the effect of a control and TPP2803 on bleeding time before and after LMWH and compound administration in a heparinized rabbit bleeding model.

FIG. 15. shows the effect of a control and TPP2803 on delta bleeding time Significantly different from PBS ($p \leq 0.05$; T-test).

FIG. 16. shows the effect of a control and TPP2803 on blood loss before and after LMWH and antibody administration. (Significance by the T-test).

Detailed Description

This disclosure provides antibodies, including monoclonal antibodies and other binding proteins that specifically bind to the activated form of AT β , but exhibit comparatively little or no reactivity against the AT α form, either naïve or activated.

Definitions

For the purpose of interpreting this specification, the following definitions will apply. 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).

Whenever appropriate, terms used in the singular will also include the plural and vice versa. 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 "or" means "and/or" unless stated otherwise. The use of "comprise," "comprises," "comprising," "include," "includes," and "including" are interchangeable and are not limiting. The terms "such as," "for example," and "e.g." also are not intended to be limiting. For example, the term "including" shall mean "including, but not limited to."

As used herein, the term "about" refers to +/- 10% of the unit value provided. As used herein, the term "substantially" refers to the qualitative condition of exhibiting a total or approximate degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, achieve or avoid an absolute result because of the many variables that affect testing, production, and storage of biological and chemical compositions and materials, and because of the inherent error in the instruments and equipment used in the testing, production, and storage of biological and chemical compositions and materials. The term substantially is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

The term "AT β " or "AT β H" as used herein refers to any variant, isoform, and/or species homolog of AT in its form that is naturally expressed by cells and present in plasma and is distinct from AT α . Further, the term "AT β " or "AT β H" as used herein can also refer to an activated form of AT β complexed with heparin or a heparin-like structure.

The term “antibody” as used herein refers to a whole antibody and any antigen binding fragment (i.e., “antigen-binding portion”) or single chain thereof. This term includes a full-length immunoglobulin molecule (e.g., an IgG antibody) that is naturally occurring or formed by normal immunoglobulin gene fragment

5 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-AT β H monoclonal antibody fragment binds to an epitope of AT β H. The antigen-binding

10 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

15 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, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al.,

20 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.

25 USA 85:5879-5883). Such single chain antibodies are also 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.

30 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 refers to a protein that exhibits binding activity similar to a particular

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.

5 The term “anti-AT β antibody” as used herein refers to an antibody that specifically binds to an epitope of AT β associated with heparin or heparin-like. When bound *in vivo* to an epitope of AT β H, the anti-AT β antibodies disclosed herein augment one or more aspects of the blood clotting cascade.

 The terms “inhibits binding” and “blocks binding” (e.g., referring to
10 inhibition/blocking of binding of AT β substrate to AT β H) as used herein 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 about
15 100%.

 In reference to the inhibition and/or blocking of binding of AT β substrate to AT β , the terms inhibition and blocking also include any measurable decrease in the binding affinity of AT β and/or AT β H to a physiological substrate when in contact with an anti-AT β antibody as compared to AT β not in contact with an anti-AT β
20 antibody, e.g., the blocking of the interaction of AT β with its substrates 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%.

 The terms “monoclonal antibody” or “monoclonal antibody composition” as
25 used herein refer to 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” as used herein refers to antibodies displaying a single binding specificity that have variable and constant regions derived from human germline immunoglobulin
30 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*).

The term “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 ATβH is substantially free of antibodies that bind antigens other than ATβH). 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 ATβH can, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., ATβH species homologs). Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals.

The term “specific binding” as used herein refers to antibody binding to a predetermined antigen. An antibody that exhibits specific binding typically binds to an antigen with an affinity of at least about 10^5 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.” 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 10^2 M^{-1} and does not bind to a predetermined antigen with higher affinity than it binds to an irrelevant antigen.

When used herein for an antibody such as an IgG antibody, the term “high affinity” refers to a binding affinity of at least about 10^7 M^{-1} , in at least one embodiment at least about 10^8 M^{-1} , in some embodiments at least about 10^9 M^{-1} , about 10^{10} M^{-1} , about 10^{11} M^{-1} or greater, e.g., up to about 10^{13} M^{-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 10^7 M^{-1} .

The term "isotype" as used herein refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

The terms "Complementarity-determining region" or "CDR" as used herein 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. 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.

The term "competing antibodies" as used herein refers to antibodies that bind to about the same, substantially the same, essentially the same, or even the same epitope as an antibody against A β H 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 A β H. 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.

The term "conservative substitutions" as used herein 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
5 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
10 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.

For nucleic acids and polypeptides, the term "substantial homology" as used
15 herein 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%, about 91%, about 92%, about 93%, about 94%, or about 95%, in at least one
20 embodiment at least about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, or about 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
25 having substantial homology to the specific nucleic acid sequences and amino acid sequences recited herein. 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, that need to be introduced for optimal alignment of
30 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.

5 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/LINNEACommunities/Vector-NTI-Community/Sequence-analysis-and-data-management-software-for-PCs/AlignX-Module-for-Vector-NTI-Advance.reg.us.html>).

Another method for determining the an 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.

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 ATβH

Bleeding disorders where homeostasis is deregulated in hemophilia or in trauma patients where the wound results in a temporary loss of hemostasis, can be treated by AT inhibitors. Antibodies, antigen-binding fragments thereof, and other AT-specific protein scaffolds can be used to provide targeting specificity to inhibit a subset of AT protein functions while preserving the rest. Given the at least 10-fold difference in plasma concentration of ATβ (<12 ug/ml) versus ATα (120 ug/ml), increased specificity of any potential ATβ inhibitor therapeutics is helpful to block ATβ function in the presence of a high circulating excess of ATα. ATβ specific antibodies that block the anti-coagulant function of ATβ can be used as therapeutics for patients with bleeding disorders. Examples of bleeding disorders include hemophilia, hemophilia patients with inhibitors, trauma-induced coagulopathy, severe bleeding patients during sepsis treatment by AT, bleeding resulting from elective surgery such as transplantation, cardiac surgery, orthopedic surgery, and excessive bleeding from Menorrhagia. Anti-ATβH antibodies having long circulating half-life can be useful in treating chronic diseases like hemophilia. ATβH antibody fragments or ATβH-binding protein scaffolds with shorter half-lives can be more effective for acute use (e.g. therapeutic use in trauma). ATβH-binding antibodies were identified by panning and screening human antibody libraries against human ATβ in complex with heparin. The identified antibodies exhibited binding to human ATβH. 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 variable regions of the AT β H-specific monoclonal antibodies are summarized in Table 1A.

Table 1A - Human anti-AT β H (heparin complexed AT β) antibodies

Clone	Light Chain variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
TPP2009	AQSVLTQDPAVSVALGQTVRIT CQGDSLRSYYASWYQQKPGQ APVLVIYGKNNRPSGIPDRFSGS SSGNTASLTITGAQAEDEADYY CNSRDSSGNHLVFGGGTKLTV LGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAW KADGSPVKAGVETTKPSKQSN NKYAASSYLSLTPEQWKSHRS YSCQVTHEGSTVEKTVAPAEC	No.1	EVQLLESGGGLVQPG GSLRLSCAASGFTFS AYRMGWVRQAPGK GLEWVSRIYSSGGRT RYADSVKGRFTISR NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGSFSEALDYWG QGTLVTVSS	No.2
TPP2015	AQDIQMTQSPGTLSPGERAT LSCRASQSVSSSYLA WYQQK GQAPRLLIYGASSRATGIPDRF SGSGTDFTLTISRLEPEDFAVY YCQQYGSSRTFGQGTKVEIRRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEEKHKVYACE VTH QGLSSPVTKS FNRGEC	No.3	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYKMDWVRQAPGK GLEWVSRIGPSGGKT MYADSVKGRFTISR NSKNTLYLQMNSLR AEDTAVYYCAREKA SDLSGTYSSEALDYW GQGTLLTVSS	No.4
TPP2016	AQDIQMTQSPATLSVSPGERAT LSCRASQNINRNLA WYQQKPG RAPRLLIHTASTRAPGVVPRITG SGSGTEFTLTISSELEPEDFAVYF CQQYASPPRTFGQGTKVEIKRT VAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEEK HKVYACEVTH QGLSSPVTKS FNRGEC	No.5	EVQLLESGGGLVQPG GSLRLSCAASGFTFS KYRMDWVRQAPGK GLEWVSRIGPSGGKT TYADSVKGRFTISR NSKNTLYLQMNSLR AEDTAVYYCAREKT SDLSGSYSSEALDYW GQGTLLTVSS	No.6

Clone	Light Chain variable Region	SEQ ID	Heavy Chain Variable Region	SEQ ID
TPP2019	AQDIQMTQSPATLSLSPGERAT LSCRASQRVSSSYLTWYQQKP GQAPRLLIYGASSRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVY YCQQYDSTPPLTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPPREAKVQWKVD NALQSGNSQESVTEQDSKDEST YSL STLTLSKADYEKHKVYACEVT HQLSSPVTKSFNRGEC	No.7	EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYAMYWVRQA PGKGLEWVSR ISPSGGKTHY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARLS QTGYYPHYHY YGMDEVWGQGT TVTVSS	No.8
TPP2803	SSELTQDPAVSVALGQTVRITC QGDSLRSYYASWYQQKPGQAP VLVIYGKNNRPSGIPDRFSGSSS GNTASLTITGAQAEDEADYYC NSRDSSGNHLVFGGGTKLTVL GQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSN KYAASSYLSLTPEQWKSRSYS CQVTHEGSTVEKTVAPAEC	No.9	EVQLLESGGGLVQPG GSLRLSCAASGFTFSS YRMSWVRQAPGKGL EWVSRIYSSGGRTRY ADSVKGRFTISRDNS KNTLYLQMNSLRAE DTAVYYCAREKASD LSGSFSEALDYWGQ GTLTVSS	No.10

In at least some possible embodiments, an isolated monoclonal antibody binds to human AT β H and inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

In at least some possible embodiments, an isolated monoclonal antibody binds to human AT β H and inhibits anticoagulant activity, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In at least some possible embodiments an isolated monoclonal antibody binds to human AT β H and inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In at least some possible embodiments, the antibody comprises heavy and light chain variable regions comprising:

- 5 (a) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 1;
- (b) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 4, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 3;
- 10 (c) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 5; or
- (d) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 8, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 7; or
- 15 (e) a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 9.

Table 1B shows heavy and light chain amino acid sequences for humanized IgG mAbs.

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Table 1B - Heavy and Light Chain Amino Acid Sequences for humanized IgG mAbs.

TPP2009 hIgG Light_Chain AQSVLTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHLVFGGG TKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD GSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTV EKTVAPECS SEQ ID NO: 51
TPP2009 hIgG Heavy_chain EVQLLESGGGLVQPGGSLRLSCAASGFTFSAYRMGWVRQAPGKGLEWVSRI YSSGGRTRYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAREKAS DLSGSFSEALDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSGF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG SEQ ID NO: 52
TPP-2015 hIgG light_chain AQDIQMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGGTDFTLISRLEPEDFAVYYCQQYGSSRTFGQGTK VEIRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC SEQ ID NO: 53

TPP-2015|hIgG|heavy_chain

EVQLLES GGGLVQPGGSLRLSCAASGFTFSKYKMDWVRQAPGKGLEWVSR
IGPSGGKTM YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREKA
SDLSGTYSEALDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPSSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SG SEQ ID
NO: 54

TPP-2016|hIgG|light_chain, Kappa

AQDIQMTQSPATLSVSPGERATLSCRASQNINRNLA WYQQKPGRAPRLIHT
ASTRAPGVPVRITGSGSGTEFTLTISSELPEDFAVYFCQQYASPPRTFGQGTK
VEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGEC SEQ ID NO: 55

TPP-2016|hIgG|heavy_chain

EVQLLES GGGLVQPGGSLRLSCAASGFTFSKYRMDWVRQAPGKGLEWVSRI
GPSGGKTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREKTS
DLSGSYSEALDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVS VLT VLV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL P
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF
FLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG SEQ ID
NO: 56

TPP-2019|hIgG|light_chain, Kappa

AQDIQMTQSPATLSLSPGERATLSCRASQRVSSSYLTWYQQKPGQAPRLLIY
GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDSTPPLTFGGG
TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC SEQ ID NO: 57

TPP-2019|hIgG|heavy_chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYAMYWVRQAPGKGLEWVSRI
SPSGGKTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARLSQT
GYYPHYHYYGMDVWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG SEQ
ID NO: 58

Table 1C – shows TPP2803 IgG2, Germlined and converted to IgG2.

TPP2803 IgG2 light chain G2, Lambda, amino acid sequence shown in Table 1C is
SEQ ID NO:59 and TPP2803 heavy chain amino acid sequence shown in Table 1C

5 is SEQ ID NO: 60.

Table 1C - TPP2803 IgG2, Germlined and converted to IgG2

>TPP-2803|hIgG2 |light_chain, lambda

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKN
NRPSGIPDRFSGSSSGNTASLTITGAQAEDADYYCNSRDSSGNHLVFGGGT
KLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGS
PVKAGVETTKPSKQSN NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK
TVAPAECS
SEQ ID NO: 59

>TPP-2803|hIgG2 |heavy_chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYRMSWVRQAPGKGLEWVSRI
YSSGGRTRYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAREKAS
DLSGSFSEALDYWGQGT LVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLV
KDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTPSSNFGTQTY
TCNV D HKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMIS
RTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS
VLT VVHQDWLNGKEYKCKVSNKGLPAIEKTI SKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPMLDSDGSFFLY
SKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG SEQ ID NO: 60

Table 2A provides a summary of the SEQ ID NOS: for the CDR regions (“CDR1,” “CDR2,” and “CDR3”) of heavy and light chains of monoclonal antibodies that bind to human ATβH.

5

Table 2A - Sequence Identifiers for CDR Regions of Human Anti-ATβH Antibodies

Clones	Light Chain Variable Region			Heavy Chain Variable Region		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
TPP2009	21	26	31	36	41	46
TPP2015	22	27	32	37	42	47
TPP2016	23	28	33	38	43	48
TPP2019	24	29	34	39	44	49
TPP2803	25	30	35	40	45	50

10 Table 2B provides sequences of the SEQ ID NOS: for the CDR regions (“CDR1,” “CDR2,” and “CDR3”) of heavy and light chains of monoclonal antibodies that bind to human ATβH.

Table 2B – Sequences for CDR Regions of Human Anti-ATβH Antibodies

Clone CDR	Sequence-Identifier	Amino Acid Sequence
TPP2009 LCDR1	SEQ ID NO: 21	QGDSLRSYYAS

Clone CDR	Sequence-Identifier	Amino Acid Sequence
TPP2015 LCDR1	SEQ ID NO: 22	RASQSVSSSYLA
TPP2016 LCDR1	SEQ ID NO: 23	RASQNINRNLA
TPP2019 LCDR1	SEQ ID NO: 24	RASQRVSSSYLT
TPP2803 LCDR1	SEQ ID NO: 25	QGDSLRSYYAS
TPP2009 LCDR2	SEQ ID NO: 26	GKNNRPS
TPP2015 LCDR2	SEQ ID NO: 27	GASSRAT
TPP2016 LCDR2	SEQ ID NO: 28	TASTRAP
TPP2019 LCDR2	SEQ ID NO: 29	GASSRAT
TPP2803 LCDR2	SEQ ID NO: 30	GKNNRPS
TPP2009 LCDR3	SEQ ID NO: 31	NSRDSSGNHLV
TPP2015 LCDR3	SEQ ID NO: 32	QQYGSSRT
TPP2016 LCDR3	SEQ ID NO: 33	QQYASPPRT
TPP2019 LCDR3	SEQ ID NO: 34	QQYDSTPPLT
TPP2803 LCDR3	SEQ ID NO: 35	NSRDSSGNHLV
TPP2009 HCDR1	SEQ ID NO: 36	AYRMG
TPP2015 HCDR1	SEQ ID NO: 37	KYKMD
TPP2016 HCDR1	SEQ ID NO: 38	KYRMD
TPP2019 HCDR1	SEQ ID NO: 39	RYAMY
TPP2803 HCDR1	SEQ ID NO: 40	SYRMS
TPP2009 HCDR2	SEQ ID NO: 41	RIYSSGGRTRYADSVKG
TPP2015 HCDR2	SEQ ID NO: 42	RIGPSGGKTM YADSVKG
TPP2016 HCDR2	SEQ ID NO: 43	RIGPSGGKTT YADSVKG
TPP2019 HCDR2	SEQ ID NO: 44	RISPSGGKTH YADSVKG
TPP2803 HCDR2	SEQ ID NO: 45	RIYSSGGRTR YADSVKG
TPP2009 HCDR3	SEQ ID NO: 46	AREKASDLSGSFSEALDY
TPP2015 HCDR3	SEQ ID NO: 47	AREKASDLSG TYSEALDY
TPP2016 HCDR3	SEQ ID NO: 48	AREKTSDLSG SYSEALDY
TPP2019 HCDR3	SEQ ID NO: 49	ARLSQTGYYP HYHYYGMDV
TPP2803 HCDR3	SEQ ID NO: 50	AREKASDLSG SFSEALDY

In at least some possible embodiments, an isolated monoclonal antibody is provided that binds to human ATβH, wherein the antibody comprises a CDR3 comprising an amino acid sequence of any one of SEQ ID NOS: 46-50. These

5 CDR3s are from a heavy chain 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: 36-40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 41-45; or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 36-40 and a CDR2 comprising an amino acid sequence selected from the group consisting of
5 SEQ ID NOS: 41-45.

In at least some possible embodiments, antibodies 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 ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3
10 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31-35. In further embodiments, the antibody further comprises (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25, (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-30, or (c) both a CDR1 comprising an
15 amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-30.

In at least some possible embodiments, the antibody comprises a CDR3 from a heavy chain and a light chain of the antibodies identified from screening and
20 panning. Provided is an isolated monoclonal antibody, wherein said antibody binds to ATβH and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 46-50 and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31-35. In a further embodiment, the antibody
25 further comprises: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 36-40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 41-45; (c) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21-25; and/or (d) a CDR2 comprising an amino acid sequence selected from
30 the group consisting of SEQ ID NOS: 26-30.

In some embodiments, 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: 21, 26, and 31 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 36, 41, and 46;
- 5 (b) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 22, 27, and 32 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 37, 42, and 47;
- 10 (c) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 23, 28, and 33 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 38, 43, and 48;
- 15 (d) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 24, 29, and 34 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 39, 44, and 49;
- 20 (e) a light chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 25, 30, and 35 and a heavy chain variable region comprising an amino acid sequence comprising SEQ ID NOS: 40, 45, and 50.

Also provided is an isolated monoclonal antibody that binds to At β H and inhibits anticoagulant activity, wherein said antibody comprises an amino acid sequence having at least about 89%, about 90%, about 91%, about 92%, about 93%,
 25 about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 99.5% identity to an amino acid sequence selected from the group consisting of the amino acid sequences set forth in SEQ ID NOS: 1-10.

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 AT β H
 30 of human, mouse, rat, rabbit, guinea pig, monkey, pig, dog, cat or other mammalian species.

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, and IgD, and an IgE antibody.

5 In one embodiment, provided is an isolated fully human monoclonal antibody to human ATIII.

Optimized Variants of Anti-AT β H Antibodies

10 In some embodiments, the antibodies can be panned, screened and optimized, for example to increase affinity to AT β H, to further decrease any affinity to AT α , to improve cross-reactivity to different species, or to improve blocking activity of AT β H. 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.

15 Also provided are monoclonal antibodies that may have increased or high affinity to AT β H. In some embodiments, the anti-AT β H antibodies may have a binding affinity of at least about 10^8M^{-1} , in some other embodiments may have at least about 10^9M^{-1} , about 10^{10}M^{-1} , about 10^{11}M^{-1} or greater, e.g., up to about 10^{13}M^{-1} or greater.

20 In some embodiments, additional amino acid modifications can be introduced to reduce divergence from the germ line sequence. In other embodiments, amino acid modifications can be introduced to facilitate antibody production for large scale production processes.

25 In some embodiments, provided are isolated anti-AT β H monoclonal antibodies that specifically bind to human AT β , which antibodies may comprise one or more amino acid modifications. In some embodiments, the antibody may comprise about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20 or more modifications.

30

Epitopes

Also provided is an isolated monoclonal antibody that can bind to a predicted epitope of human AT β H, wherein the epitope comprises one or more of residues from human AT β H as shown in FIG. 11.

- 5 In some embodiments, the epitope comprises the N135 site of human AT β H. In other embodiments, the site can comprise part of the amino acid residue sequence of RCL loop of human AT β H.

- Also provided are antibodies that can compete with any of the antibodies described herein for binding to human AT β H. For example, such a competing
10 antibody can bind to one or more epitopes described above.

Nucleic Acids, Vectors and Host Cells

- Also provided are isolated nucleic acid molecules encoding any of the monoclonal antibodies described herein. Thus, provided is an isolated nucleic acid
15 molecule encoding an antibody that binds to human AT β H. Table 3 shows the nucleotide sequences of some anti-AT β H antibodies.

Table 3 - Nucleotide sequence of anti-AT β H antibodies.

	Light Chain	Heavy Chain
TPP2009	GCACAGAGCGTCTTG	GAAGTTCAATTGTTAGAGTCTGGTGG
	ACTCAGGACCCTGCT	CGGTCTTGTTGAGCCTGGTGGTCTTT
	GTGTCTGTGGCCTTG	ACGTCTTTCTTGCGCTGCTTCCGGATT
	GGACAGACAGTCAG	CACTTTCTCTGCTTACCGTATGGGTTG
	GATCACATGCCAAGG	GGTTCGCCAAGCTCCTGGTAAAGGTT
	AGACAGCCTCAGAA	TGGAGTGGGTTTCTCGTATCTATTCTT
	GCTATTATGCAAGCT	CTGGTGGCCGTACTCGTTATGCTGACT
	GGTACCAGCAGAAG	CCGTTAAAGGTCGCTTCACTATCTCTA
	CCAGGACAGGCCCT	GAGACAACCTCTAAGAATACTCTCTAC
	GTACTTGTCATCTAT	TTGCAGATGAACAGCTTAAGGGCTGA
	GGTAAAAACAACCG	GGACACGCGCCGTGTATTACTGTGCGA
	GCCCTCAGGGATCCC	GAGAGAAAGCGTCGGATCTATCGGGG
	AGACCGATTCTCTGG	AGTTTTTCTGAGGCCCTTGACTACTGG
	CTCCAGCTCAGGAAA	GGCCAGGGAACCCCTGGTCACCGTCTC
	CACAGCTTCCTTGAC	AAGCGCCTCCACCAAGGGCCCATCGG
	CATCACTGGGGCTCA	TCTTCCCGCTAGCACCCAGCAGCAAG
	GGCGGAAGATGAGG	AGCACCAGCGGCGGAACAGCCGCCCT
	CTGACTATTACTGTA	GGGCTGCCTGGTGAAAGACTACTTCC
	ACTCCCGGGACAGCA	CCGAGCCCCGTGACCGTGTCTTGGAAAC
	GTGGTAACCATCTGG	TCTGGCGCCCTGACCAGCGGAGTGCA

	Light Chain	Heavy Chain
	TATTCGGCGGAGGGA CCAAGCTGACCGTCC TAGGTCAGCCCAAGG CTGCCCCCTCGGTCA CTCTGTTCCCGCCCT CCTCTGAGGAGCTTC AAGCCAACAAGGCC ACACTAGTGTGTCTG ATCAGTGACTTCTAC CCGGGAGCTGTGACA GTGGCCTGGAAGGCA GATGGCAGCCCCGTC AAGGCGGGAGTGGA GACCACCAAACCCCTC CAAACAGAGCAACA ACAAGTACGCGGCCA GCAGCTACCTGAGCC TGACGCCCCGAGCAGT GGAAGTCCACAGA AGCTACAGCTGCCAG GTCACGCATGAAGGG AGCACCGTGGAGAA GACAGTGGCCCCCTGC AGAATGCTCT (SEQ ID NO: 11)	TACCTTCCCCGCCGTGCTGCAGAGCA GCGGCCTGTACAGCCTGAGCAGCGTG GTGACAGTGCCCAGCAGCAGCCTGGG AAGCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAGGTG GACAAGAAGGTGGAACCCAAGAGCT GCGACAAGACCCACACCTGTCCCCC TGCCCTGCCCCCTGAACTGCTGGGCGG ACCCAGCGTGTTCTGTTCACCCCAAA GCCCAAGGACACCCTGATGATCAGCC GGACCCCCGAAGTGACCTGCGTGGTG GTGGACGTGTCCACGAGGACCCAGA AGTGAAGTTTAATTGGTACGTGGACG GCGTGGAAGTGCATAACGCCAAGACC AAGCCCAGAGAGGAACAGTACAACA GCACCTACCGGGTGGTGTCCGTGCTG ACCGTGCTGCACCAGGACTGGCTGAA CGGCAAAGAGTACAAGTGCAAGGTCT CCAACAAGGCCCTGCCTGCCCCCATC GAGAAAACCATCAGCAAGGCCAAGG GCCAGCCCCGCGAGCCTCAGGTGTAC ACACTGCCCCCAGCCGGGATGAGCT GACCAAGAACCAGGTGTCCCTGACCT GTCTGGTGAAAGGCTTCTACCCACAGC GATATCGCCGTGGAATGGGAGAGCAA CGGCCAGCCCCGAGAACAATTACAAGA CCACCCCCCTGTGCTGGACAGCGAC GGCTCATTCTTCCTGTACTCCAAGCTG ACCGTGGACAAGAGCCGGTGGCAGCA GGGCAACGTGTTTACGCTGCAGCGTGA TGCACGAGGCCCTGCACAATCACTAC ACCCAGAAGTCCCTGAGCCTGAGCCC CGGC (SEQ ID NO: 12)
TPP2015	GCACAAGACATCCAG ATGACCCAGTCTCCA GGCACCCCTGTCTTTG TCTCCAGGGGAAAGA GCCACCCCTCTCCTGC AGGGCCAGTCAGAGT GTTAGCAGCAGCTAC TTAGCCTGGTACCAG CAGAAACCTGGCCAG GCTCCCAGGCTCCTC ATCTATGGTGCATCC AGCAGGGCCACTGGC ATCCCAGACAGGTTT	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTTACGCCTGGTGGTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACAAGATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCTT CTGGTGGCAAGACTATGTATGCTGAC TCCGTTAAAGGTCGCTTCACTATCTCT AGAGACAACTCTAAGAATACTCTCTA CTTGACAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGAGAGAAAGCGTCGGATCTATCGGG GACTTATTCTGAGGCCCTTGACTACTG

	Light Chain	Heavy Chain
	AGTGGCAGTGGGTCT GGGACAGACTTCACT CTCACCATCAGCAGA CGGAGCCTGAAGATT TTGCAGTGTATTACT GTCAGCAGTATGGTA GCTCAACGTTTCGGCC AAGGGACCAAGGTG GAAATCAGACGAACT GTGGCTGCAATCTGT CTTCATCTTCCCGCC ATCTGATGAGCAGTT GAAATCTGGAAGTGC CTCTGTTGTGTGCCT GCTGAATAACTTCTA TCCCAGAGAGGCCAA AGTACAGTGGAAGGT GGATAACGCCCTCCA ATCGGGTAACTCCCA GGAGAGTGTACAG AGCAGGACAGCAAG GACAGCACCTACAGC CTCAGCAGCACCCCTG ACGCTGAGCAAAGC AGACTACGAGAAAC ACAAAGTCTACGCCT GCGAAGTCACCCATC AGGGCCTGAGCTCGC CCGTCACAAAGAGCT TCAACAGGGGAGAG TGT (SEQ ID NO: 13)	GGGCCAGGGAACCCTGGTCACCGTCT CAAGCGCCTCCACCAAGGGCCCATCG GTCTTCCCGCTAGCACCCAGCAGCAA GAGCACCAGCGGCGGAACAGCCGCCC TGGGCTGCCTGGTGAAAGACTACTTC CCCGAGCCCGTGACCGTGTCTTGAA CTCTGGCGCCCTGACCAGCGGAGTGC ATACCTTCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGT GGTGACAGTGCCCAGCAGCAGCCTGG GAACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGT GGACAAGAAGGTGGAACCCAAGAGC TGCGACAAGACCCACACCTGTCCCCC CTGCCCTGCCCTGAACTGCTGGGCG GACCCAGCGTGTTCTGTTCCTCCCAA AGCCCAAGGACACCCTGATGATCAGC CGGACCCCCGAAGTGACCTGCGTGGT GGTGGACGTGTCCACAGAGGACCCAG AAGTGAAGTTTAATTGGTACGTGGAC GGCGTGGAAGTGCATAACGCCAAGAC CAAGCCCAGAGAGGAACAGTACAAC AGCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTC TCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAGCCTCAGGTGTA CACACTGCCCCCAGCCGGGATGAGC TGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAAGGCTTCTACCCAG CGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCGAGAACAATTACAAG ACCACCCCCCTGTGCTGGACAGCGA CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTACAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 14)
TPP2016	GCACAAGACATCCAG ATGACCCAGTCTCCA GCCACCCTGTCTGTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGAAT ATTAATAGAACTTG	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTTCAGCCTGGTGGTTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTAAGTACCGTATGGATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCGGTCCCT CTGGTGGCAAGACTACTTATGCTGAC

	Light Chain	Heavy Chain
	GCCTGGTACCAGCAG AAGCCTGGCCGGGCT CCCAGACTCCTCATC CATAACGCATCCACT AGGGCCCCCTGGTGTC CCAGTCAGGATCACT GGCAGTGGGTCTGGA ACAGAGTTCACCTCTC ACCATCAGCAGCCTG GAACCTGAAGATTTT GCAGTGTATTTCTGT CAGCAGTATGCTAGC CCACCTCGGACGTTT GGCCAAGGGACCAA GGTGGAAATCAAGC GAACTGTGGCTGCAC CATCTGTCTTCATCTT CCCGCCATCTGATGA GCAGTTGAAATCTGG AACTGCCTCTGTTGT GTGCCTGCTGAATAA CTTCTATCCCAGAGA GGCCAAAGTACAGTG GAAGGTGGATAACG CCCTCCAATCGGGTA ACTCCCAGGAGAGTG TCACAGAGCAGGAC AGCAAGGACAGCAC CTACAGCCTCAGCAG CACCCTGACGCTGAG CAAAGCAGACTACG AGAAACACAAAGTCT ACGCCTGCGAAGTCA CCCATCAGGGCCTGA GCTCGCCCCGTCACAA AGAGC TTCAACAGGGGAGA GTGT (SEQ ID NO: 15)	TCCGTTAAAGGTCGCTTCACTATCTCT AGAGACAACCTCTAAGAATACTCTCTA CTTGCAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGAGAGAAAAACGTCCGATCTATCGGG GAGTTATTCTGAGGCCCTTGACTACTG GGGCCAGGGAACCCTGGTACACCGTCT CAAGCGCCTCCACCAAGGGCCCCATCG GTCTTCCCGCTAGCACCCAGCAGCAA GAGCACCAGCGGCGGAACAGCCGCCC TGGGCTGCCTGGTGAAAGACTACTTC CCCGAGCCCCGTGACCGTGTCTGGAA CTCTGGCGCCCTGACCAGCGGAGTGC ATACCTTCCCCGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGT GGTGACAGTGCCCAGCAGCAGCCTGG GAACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGT GGACAAGAAGGTGGAACCCAAGAGC TCGACAAGACCCACACCTGTCCCCC CTGCCCTGCCCTGAACTGCTGGGCG GACCCAGCGTGTTCCTGTTCCCCCAA AGCCCAAGGACACCCTGATGATCAGC CGGACCCCCGAAGTGACCTGCGTGGT GGTGGACGTGTCCCACGAGGACCCAG AAGTGAAGTTTAATTGGTACGTGGAC GGCGTGGAAGTGCATAACGCCAAGAC CAAGCCCAGAGAGGAACAGTACAAC AGCACCTACCGGGTGGTGTCCGTGCT GACCGTGTGTCACCAAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTG TCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAGCCTCAGGTGTA CACACTGCCCCCAGCCGGGATGAGC TGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAAGGCTTCTACCCAG CGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCAGAGAACAATTACAAG ACCACCCCCCTGTGCTGGACAGCGA CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTTACGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 16)

	Light Chain	Heavy Chain
TPP2019	GCACAAGACATCCAG ATGACCCAGTCTCCA GCCACCCTGTCTTTG TCTCCAGGGGAAAGA GCCACCCTCTCCTGC AGGGCCAGTCAGCGT GTTAGCAGCAGCTAC TTAACCTGGTACCAG CAGAAACCTGGCCAG GCTCCAGGCTCCTC ATCTATGGTGCATCC AGCAGGGCCACTGGC ATCCCAGACAGGTTC AGTGGCAGTGGGTCT GGGACAGACTTCACT CTCACCATCAGCAGA CTGGAGCCTGAAGAT TTTGCAGTTTATTACT GTCAGCAGTATGATA GTACGCCTCCGCTCA CCTTCGGCGGAGGGA CCAAGGTGGAGATCA AACGAACTGTGGCTG CACCATCTGTCTTCA TCTTCCCGCCATCTG ATGAGCAGTTGAAAT CTGGAACTGCCTCTG TTGTGTGCCTGCTGA ATAACTTCTATCCCA GAGAGGCCAAAGTA CAGTGGAAAGGTGGAT AACGCCCTCCAATCG GGTAACTCCCAGGAG AGTGTACAGAGCAG GACAGCAAGGACAG CACCTACAGCCTCAG CAGCACCTGACGCT GAGCAAAGCAGACT ACGAGAAACACAAA GTCTACGCCTGCGAA GTCACCCATCAGGGC CTGAGCTCGCCGTC ACAAAGAGCTTCAAC AGGGGAGAGTGT (SEQ ID NO: 17)	GAAGTTCAATTGTTAGAGTCTGGTGG CGGTCTTGTTTCAGCCTGGTGGTCTTT ACGTCTTTCTTGCGCTGCTTCCGGATT CACTTTCTCTCGTTACGCTATGTATTG GGTTCGCCAAGCTCCTGGTAAAGGTT TGGAGTGGGTTTCTCGTATCTCTCCTT CTGGTGGCAAGACTCATTATGCTGAC TCCGTAAAGGTTCGCTTCACTATCTCT AGAGACAACTCTAAGAATACTCTCTA CTTGCAGATGAACAGCTTAAGGGCTG AGGACACGGCCGTGTATTACTGTGCG AGACTGTCTCAAACCTGGTTATTACCTT CACTACCACTACTACGGTATGGACGT CTGGGGCCAAGGGACCACGGTCACCG TCTCAAGCGCCTCCACCAAGGGCCCA TCGGTCTTCCCGCTAGCACCCAGCAG CAAGAGCACCCAGCGGCGGAACAGCC GCCCTGGGCTGCCTGGTGAAAGACTA CTTCCCCGAGCCCGTGACCGTGTCTTG GAACTCTGGCGCCCTGACCAGCGGAG TGCATACCTTCCCCGCCGTGCTGCAGA GCAGCGGCCTGTACAGCCTGAGCAGC GTGGTGACAGTGCCCAGCAGCAGCCT GGGAACCCAGACCTACATCTGCAACG TGAACCACAAGCCCAGCAACACCAAG GTGGACAAGAAGGTGGAACCCAAGA GCTGCGACAAGACCCACACCTGTCCC CCCTGCCCTGCCCTGAACTGCTGGGC GGACCCAGCGTGTTCCTGTTCCCCCA AAGCCCAAGGACACCTGTATGATCAG CCGGACCCCCGAAGTGACCTGCGTGG TGGTGGACGTGTCCACAGGACCCA GAAGTGAAGTTTAATTGGTACGTGGA CGGCGTGGAAGTGCATAACGCCAAGA CCAAGCCCAGAGAGGAACAGTACAAC AGCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTC TCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCCCGCGAGCCTCAGGTGTA CACACTGCCCCCAGCCGGGATGAGC TGACCAAGAACCAGGTGTCCCTGACC TGTCTGGTGAAAGGCTTCTACCCAG CGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCCGAGAACAATTACAAG ACCACCCCCCTGTGCTGGACAGCGA

	Light Chain	Heavy Chain
		CGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGAGCCGGTGGCAGC AGGGCAACGTGTTTCAGCTGCAGCGTG ATGCACGAGGCCCTGCACAATCACTA CACCCAGAAAGTCCCTGAGCCTGAGCC CCGGC (SEQ ID NO: 18)
TPP2803	AGCGAATTGACTCAG GACCCTGCTGTGTCT GTGGCCTTGGGACAG ACAGTCAGGATCACA TGCCAAGGAGACAG CCTCAGAAGCTATTA TGCAAGCTGGTACCA GCAGAAGCCAGGAC AGGCCCTGTACTTG TCATCTATGGTAAAA ACAACCGGCCCTCAG GGATCCCAGACCGAT TCTCTGGCTCCAGCT CAGGAAACACAGCTT CCTTGACCATCACTG GGGCTCAGGCGGAA GATGAGGCTGACTAT TACTGTAACTCCCGG GACAGCAGTGGTAAC CATCTGGTATTTCGGC GGAGGGACCAAGCT GACCGTCCTAGGTCA GCCCAAGGCTGCCCC CTCGGTCACTCTGTT	GAAGTGCAGCTGCTGGAAAGCGGCGG AGGCCTGGTGCAGCCTGGCGGATCTC TGAGACTGAGCTGTGCCGCCAGCGGC TTCACCTTCAGCAGCTACAGAATGAG CTGGGTGCGCCAGGCCCTGGCAAGG GACTGGAATGGGTGTCCCGGATCTAC AGCAGCGGCGGCAGAACAGATACGC CGACAGCGTGAAGGGCCGGTTCACCA TCTCCCGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCTGCG GGCCGAGGACACCCCGTGTACTATT GCGCCAGAGAGAAGGCCAGCGACCTG AGCGGCAGCTTTAGCGAGGCCCTGGA TTATTGGGGCCAGGGCACACTCGTGA CCGTGTCTAGCGCCAGCACAAAGGGC CCCAGCGTGTTCCTCTGGCCCCCTTGT AGCAGAAGCACCAGCGAGTCTACAGC CGCCCTGGGCTGCCTCGTGAAGGACT ACTTTCCCGAGCCCGTGACAGTGTCTT GGAAGTCTGGCGCCCTGACAAGCGGC GTGCACACCTTTCCAGCCGTGCTGCA GAGCAGCGGCCTGTACTCTCTGAGCA GCGTCGTGACTGTGCCAGCAGCAAC TTCGGCACCCAGACCTACACCTGTAA

	Light Chain	Heavy Chain
	CCCGCCCTCCTCTGA GGAGCTTCAAGCCAA CAAGGCCACACTAGT GTGTCTGATCAGTGA CTTCTACCCGGGAGC TGTGACAGTGGCCTG GAAGGCAGATGGCA GCCCCGTCAAGGCGG GAGTGGAGACCACC AAACCCCTCAAACAG AGCAACAACAAGTA CGCGGCCAGCAGCTA CCTGAGCCTGACGCC CGAGCAGTGGAAGTC CCACAGAAGCTACAG CTGCCAGGTCACGCA TGAAGGGAGCACCGT GGAGAAGACAGTGG CCCCTGCAGAATGCT CT (SEQ ID NO: 19)	CGTGGACCACAAGCCCAGCAACACCA AGGTGGACAAGACCGTGGAACGGAA GTGCTGCGTGGAATGCCCCCCTTGTCC TGCCCCCTCCAGTGGCTGGCCCTTCCGT GTTCCTGTTCCCCCCTAAAGCCCAAGG ACACCCTGATGATCAGCCGGACCCCG AAGTGACCTGCGTGGTGGTGGATGTG TCCCACGAGGACCCCGAGGTGCAGTT CAATTGGTACGTGGACGGCGTGGAAG TGCACAACGCCAAGACCAAGCCCAGA GAGGAACAGTTCAACAGCACCTTCCG GGTGGTGTCCGTGCTGACCGTGGTGC ATCAGGACTGGCTGAACGGCAAAGAG TACAAGTGCAAGGTGTCCAACAAGGG CCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGACCAAAGGCCAGCCCCGC GAGCCCCAGGTGTACACACTGCCTCC AAGCCGGGAAGAGATGACCAAGAAC CAGGTGTCCCTGACCTGTCTCGTGAA AGGCTTCTACCCCTCCGATATCGCCGT GGAATGGGAGAGCAACGGCCAGCCC GAGAACAACTACAAGACCACCCCCC CATGCTGGACAGCGCGGCTCATTCCTC CTGTACAGCAAGCTGACAGTGGACAA GTCCCGGTGGCAGCAGGGCAACGTGT TCAGCTGCAGCGTGATGCACGAAGCC CTGCACAACCACTACACCCAGAAGTC CCTGAGCCTGAGCCCTGGC (SEQ ID NO: 20)

In some embodiments, isolated nucleic acid molecules encode an antibody that binds to AT β H and inhibits anticoagulant activity but has minimal binding to AT α , wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.

In some embodiments, isolated nucleic acid molecules encode an antibody that binds to AT β H and inhibits anticoagulant activity but has minimal binding to AT α , wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.

In other embodiments, isolated nucleic acid molecules encode an antibody that binds to AT β and inhibits anticoagulant activity of AT β , wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10 or a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9 and one or more amino acid modifications in the heavy chain variable region or light chain variable region.

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 AT β H

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, an exemplary method for producing a monoclonal antibody that binds with human AT β H can comprise: (a) transfecting a nucleic acid molecule encoding a monoclonal antibody into a host cell; (b) culturing the host cell so to express the monoclonal antibody in the host cell, and (c) optionally isolating and purifying the produced monoclonal antibody, wherein the nucleic acid molecule comprises a nucleotide sequence encoding a monoclonal antibody.

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" refers to an antibody gene that 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, alternatively, 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). 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" includes 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, non-viral

regulatory sequences can be used, such as the ubiquitin promoter or β -globin promoter.

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).

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" encompasses 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, including mammalian 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. 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.

5

Use of Partial Antibody Sequences to Express Intact Antibodies

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, the corresponding germline leader sequence is used 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 advantages such as elimination or inclusion of particular restriction sites, or optimization of particular codons. 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; and 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. 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. 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. 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. In another aspect, the structural features of a human anti-AT β H antibody are used to create structurally related human anti-AT β H antibodies that retain the function of binding

to AT β . For example, 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-AT β H antibodies.

5

Pro-coagulant efficacy of Anti-AT β H mAbs

Pro-coagulant efficacy of anti-AT β H mAbs was investigated using various assays.

Table 4 shows pro-coagulant efficacy of Anti-AT β H mAbs TPP2009 and TPP2803 in plasma from various animal species in the FXa-activated clotting assay.

10

Table 4 - Pro-coagulant efficacy of TPP2009 and TPP2803 in plasma from various animal species.

Species	Normal Plasma (EC ₅₀ nM)		HEM A Plasma (EC ₅₀ in nM)		HEM A Plasma (EC ₅₀ in nM)
	2009	2803	2009	2803	2009
FXa-Activated Clotting Assay					dPT
Human	10.5	2.4	4.7	2.7	9.0
Mouse	NDR	ND	ND	ND	NDR
Rat	NDR	ND	ND	ND	ND
Rabbit	25.7	ND	10.5	ND	NDR
Beagle	NDR	ND	ND	ND	NDR
Cyno	21.5	ND	4.3	ND	13.9

NDR: no dose response, ND: not determined, dPT: diluted prothrombin time, HEM-A: Hemophilia A plasma.

15

Anti-AT β H mAbs TPP2009 and TPP2803 both exhibited pro-coagulant efficacy in human normal plasma and hemophilia A plasma in the FXa-activated clotting assay. Specifically, TPP2009 exhibited pro-coagulant efficacy in human

normal plasma and hemophilia A plasma in the FXa-activated clotting assay with EC_{50} 's of 10.5 nM and 4.7 nM, respectively. TPP2803 exhibited pro-coagulant efficacy in human normal plasma and hemophilia A plasma in the FXa-activated clotting assay with EC_{50} 's of 2.4 and 2.7 nM, respectively.

5 In addition, Anti-AT β H mAb TPP2803 exhibited dose-dependent shortening of the clotting time in both normal human plasma and hemophilia patient plasma using the FXa activated clotting assay, as shown in FIG. 12. CT: clotting time, HEM-A: Hemophilia A plasma.

10 The heparinized rabbit bleeding model outlined in FIG. 13 was employed to demonstrate in vivo pro-coagulant efficacy of Anti-AT β H mAb TPP2803. The effect of a control and TPP2803 on bleeding time before and after LMWH and compound administration in a heparinized rabbit bleeding model are shown in FIG. 14. Bleeding time after administration of LMWH and Test Article (either 3mg/kg or 30 mg/kg TPP2803) was significantly reduced compared to bleeding time after
15 LMWH in PBS. Delta bleeding time after administration of either Test Article (either 3mg/kg or 30 mg/kg TPP2803), or positive control protamine sulfate, was significantly different from PBS ($p \leq 0.05$; * significance by T-test) as shown in FIG. 15. The effect of a control and TPP2803 on blood loss before and after LMWH and antibody administration is shown in FIG. 16. TPP2803 (3 mg/kg) and positive
20 control protamine sulfate both exhibited a significant change in blood loss (*Significance by the T-test) after administration of LMWH.

Pharmaceutical Compositions

25 Also provided are pharmaceutical compositions comprising therapeutically effective amounts of anti-AT β H monoclonal antibody and a pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" as used herein refers to 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
30 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 monoclonal antibody.

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, the composition of the carrier includes isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride.

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

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 ATβH with its substrate, which can include Factor Xa or Factor IIa. 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-AT β H 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-AT β H monoclonal antibody to a patient in need thereof.

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

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 at least some embodiments, a method for treating genetic and acquired deficiencies or defects in coagulation comprises 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 at least some embodiments, a method for treating genetic and acquired deficiencies or defects in coagulation comprises 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 co-administered. Also provided 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.

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.

5 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.

10 The compositions can be administered to patients in need as a bolus or by continuous infusion. For example, a bolus administration of an antibody as a Fab fragment can be in an amount from about 0.0025 to about 100 mg/kg body weight, about 0.025 to about 0.25 mg/kg, about 0.010 to about 0.10 mg/kg or about 0.10 to about 0.50 mg/kg. For continuous infusion, an inventive antibody present as an Fab
15 fragment can be administered at about 0.001 to about 100 mg/kg body weight/minute, about 0.0125 to about 1.25 mg/kg/min, about 0.010 to about 0.75 mg/kg/min, about 0.010 to about 1.0 mg/kg/min, or about 0.10 to about 0.50 mg/kg/min for a period of about 1-24 hours, about 1-12 hours, about 2-12 hours, about 6-12 hours, about 2-8 hours, or about 1-2 hours. For administration of an
20 inventive antibody present as a full-length antibody (with full constant regions), dosage amounts can be about 1-10 mg/kg body weight, about 2-8 mg/kg, or about 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.

25 Additionally, the compositions can be administered to patients via subcutaneous injection. For example, a dose of about 10 to about 100 mg anti-AT β H antibody can be administered to patients via subcutaneous injection weekly, biweekly or monthly. As used herein, "therapeutically effective amount" means an amount of an anti-AT β H monoclonal antibody or of a combination of such antibody
30 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 thereof. The precise amount will depend upon numerous factors, including 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.

- 5 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 - Human and rabbit AT α and AT β purification

- 10 AT α and AT β were purified from human and rabbit plasma by affinity chromatography on heparin-sepharose according to methods previously described (Carlson and Atencio 1982; Peterson and Blackburn 1985) at Enzyme Research laboratory (South Bend, IN). Briefly, the supernatant from a dextran sulphate/calcium chloride precipitation was applied to a heparin-sepharose affinity column (Pharmacia). AT α and AT β were separated with a NaCl gradient: AT α and
15 AT β were eluted at 0.8 M and 1.3 M NaCl, respectively. Anion-exchange chromatography (HiTrap-Q, Pharmacia) was employed for further purification of AT β . Purity and glycan profile of AT α and AT β were evaluated by protein SDS-PAGE and LC-MS.

20 **Example 2 - Determination of the number and position of glycans on AT α and AT β by mass-spectrometry analysis**

- Due to the distinct number of glycans, AT α and AT β were differentiated based on their mass by Agilent 6520 LC-MS system which is equipped with duo-ESI (or nano ChipCube) source, MassHunter acquisition software and qualitative
25 analysis software including Bioconfirm. Glycosylation sites were determined by a bottom-up method in which proteins are digested by trypsin and Arg-c followed by target MSMS to identify the glycosylated and mono-glycosylated peptide sequences. Data was collected in two experiments: Fragmentor voltage 175v and 430v.

Example 3 - AT antigen biotinylation

Human and rabbit AT α and AT β were labeled with biotins on the surface lysine residues by NHS-biotin. For lysine biotinylation, proteins were first desalted into PBS/Ca⁺⁺ buffer (Life Technologies Corporation, Carlsbad, CA) to remove any
5 amines that might be inhibitory to the biotinylation reaction. Concentrations of desalted proteins were determined by OD280 on the NanoDrop. Protein were then incubated for 1 hour at room temperature (RT) with Sulfo-NHS-Biotin (Pierce Thermo Scientific, Rockford, IL) at the 1:5 and/or 1:3 molar ratio of AT:NHS-biotin (i.e. biotin in excess). Free biotin was removed by overnight dialysis into PBS/Ca⁺⁺
10 buffer. The amount of biotin in the biotinylated proteins was quantified using Biotin Quantitation Kit (Pierce Thermo Scientific, Rockford, IL). Biotinylated AT α and AT β were analyzed by SDS-PAGE, and biotinylation was confirmed by Western blot analysis using streptavidin-HRP (Pierce Thermo Scientific, Rockford, IL) as probe. The functional activities of biotinylated AT were evaluated by FXa inhibition
15 assay. By comparison of the biotinylated AT α and AT β with unbiotinylated AT α and AT β , only slight reductions in AT inhibition activity were observed after biotinylation, indicating the biotinylated AT β and AT α prepared in this way would be representative and could be used in panning for AT β H binders as selective anti-coagulant blockers.

20

Example 4 - Human monoclonal antibody discovery by phage display and panning

A four-arm panning strategy was designed to discover Fabs specifically against AT β H from a human Fab library (Dyax Fab310). The library was first
25 depleted with biotinylated heparin/Fondaparinux-bound AT α and biotinylated AT α and then was panned against heparin/Fondaparinux-bound AT β and biotinylated AT β on streptavidin beads, respectively. For each round of panning, the heparin-bound AT α (AT α H) was included in the binding buffer as a competitor. To keep hAT β in active conformation (heparin bound form), heparin was added to the wash
30 buffer in all three rounds of panning. After panning, pooled clones were screened for hAT β and hAT β H specific binding and counter-screened for hAT α by ELISA. These clones were also examined for differential binding to rabbit AT β over rabbit

AT α . Clones showing differential binding to both hAT β H and rAT β H over hAT α and rAT α were further subject to FXa –deinhibition assay with hAT β spike-in. Positive hits (Fabs) were reformatted into IgG1, expressed in HEK293 cells and purified by protein-A column. These purified IgG1s were extensively tested in AT-
 5 depleted human plasma and hemA patient plasma for TGA assay (Thrombin Generation Assay) and dPT (diluted Prothrombin Time) assay to measure the clotting time.

Example 5 - ELISA (Enzyme-Linked Immunosorbent Assay)

10 2 μ g/ml biotinylated AT antigens in PBS were coated on Streptavidin Microplates (Greiner, 781997) with or without heparin (50ug/ml, heparin-Natrium-5000, Apotheke, Fa. Ratiopharm). After overnight antigen coating at 4°C, plates were washed with PBST +/- heparin and blocked with 5% milk in PBST +/- heparin at 37°C for one hour. After removal of blocking buffer, 20ug/ml Fab or 4ug/ml IgG
 15 in blocking buffer (5% milk in PBST +/- heparin) was then added to the plates and plates were incubated at room temperature for 1 hour. Plates were then washed three times. Anti-human IgG POD (Sigma, A0170) in blocking buffer was added to plates and plates were incubated at room temperature for 30 minutes. Amplex red (In vitrogen, Cat#A22170) was used for detection at 1:1000 together with H₂O₂. After
 20 30 min incubation, plates were read at Ex535, Em 590 in a fluorescent plate reader.

Example 6 - FXa de-inhibition assay – AT with heparin

Heparin was incubated with AT β or AT α to form stable ATH complexes. Antibody was then added to the AT β H or AT α H complexes. In the meantime, 10 μ l
 25 of 200ng/ml FXa (HTI) and 20 μ l of 50 μ g/ml Fluophen FXa fluorogenic substrate (Hyphen Biomed) were mixed in a separate plate. The antibody-ATH mixture was added to the FXa/substrate solution quickly and fluorescent kinetic measurement was started immediately at Ex360nm and Em465nm. All necessary dilutions is made in 100mM NaCl, 20mM Tris, 2.5mM CaCl₂, 0,1% BSA, 0,1% PEG8000.

30

Example 7 - Thrombin Generation Assay (TGA) in FVIII deficiency human plasma

A 1:2 serial dilution of AT β H antibody was made in HemA human plasma starting from 1 μ M of final concentration to 0.015 μ M. Heparin was added in each antibody solution at a final concentration of 50nM. An 80 μ l of the antibody-heparin-plasma mixture was then added to each well containing 20 μ l of reconstitute PPP reagent or calibrator in a 96 well TGA plate. The plate was placed in the TGA instrument and the machine automatically dispensed 20 μ l of FluCa (Fluo substrate + CaCl₂) into each well. The reaction was allowed to run 60 min. Plasma alone was used as the negative control.

Example 8 - Thrombin Generation Assay (TGA) in AT-depleted human plasma with spiked-in AT α and AT β respectively

Antibodies were added to human AT-deficient plasma spiked with 15nM of AT α or AT β . Heparin was then pipetted into each reaction at a final concentration of 50nM. 80 μ l of plasma samples containing ATH-specific antibody, heparin and AT α or AT β were added into wells of a 96 well TGA plate with 20 μ l of PPP reagent or calibrator. Plates were placed in the TGA instrument, and then 20 μ l of FluCa (Fluo substrate + CaCl₂) was dispensed into each well. Reactions were allowed to continue for 60 min.

Example 9 - Diluted Prothrombin Time assay (dPT) in human hemA plasma and AT deficient plasma

A serial dilution of anti-AT β H hmAbs was made in hemA plasma starting at 250 nM with 0.1 U/mL of heparin. The mixture of antibody, plasma and heparin was incubated at room temperature for 20-30min. Then 50 μ L of this mixture was added to a 50 μ L of diluted Innovin (1/2000) (Dade Behring), incubated for 4 min at 37°C, followed by adding 50 μ L of 25 mM CaCl₂ (HemSil). dPT test program was set on ACL Top coagulometer with acquisition time of 360 seconds. For dTP in AT deficient plasma, AT-DP was spiked in with either AT α or AT β at a final concentration of 0.2 μ M with 0.1 U/ml of heparin. Anti-AT β H mAbs was added to AT-DP/heparin/AT α or AT-DP/heparin/AT β mixtures at a final concentration of

0.25 μ M and incubated at room temperature for 20-30min. For each reaction, a 50 μ L of plasma/antibody/heparin mixture was added to 50 μ L of diluted Innovin (1/4000), incubate 4 min at 37°C, followed by adding 50 μ L of 25 mM CaCl₂ (HemSil) as above.

5

Example 10 - Antibody purification

Pre-washed protein A agarose beads were incubated with antibody in binding buffer (volume ratio: 1:1) with rotation overnight at 4°C. Beads were then packed into a column and washed with 1 X PBS until O.D.₂₈₀ < 0.05. Residual solution was drained. Antibodies were eluted with elution buffer and collected into tubes containing neutralizing buffer. Eluted fractions were dialyzed against 1 x PBS overnight at 4°C with at least twice buffer changes. IgG concentration was measured at 280nm by nanodrop. The antibody purity was examined by either ELISA, SDS-PAGE or SSC.

15

Example 11 - Antibody binding affinity study by Biacore

Antibody affinity measurement was performed on a Biacore T100 or T200 processing unit. Anti-human Fc antibody or streptavidin was immobilized on a CM5 chip. hAT β H or biotinylated hmAb antibodies were injected and captured on the chip. AT β or AT α at different concentration with/without heparin were injected. Only AT and ATH bound to the antibodies generate binding constants. The binding results were reported as Equilibrium Dissociation Constants (KD) in nanoMoles. When AT/heparin complex was analyzed, heparin at 1 μ M is included in the running buffer.

25

Example 12 - Heparinized rabbit bleeding model

Experimental design of the heparinized rabbit bleeding model is outlined in FIG. 13. Following preparation of rabbit jugular veins (right vein: venous stasis; left vein: cannulation), low molecular weight heparin (LMWH) is administered to the rabbit (1800U/kg) IV in PBS vehicle at time 0. After 10 minutes, the test article is administered. Experimental groups include Vehicle, PBS; Positive control, Protamine sulfate, (28mg/kg IV); Negative control, M14 IgG2; treatment: 30mg/kg;

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TPP2803, 3mg/kg; TPP2803, 30mg/kg. Five minutes after administration of test article, an ear puncture (3-5 mm) is performed and thrombus formation in situ (stasis) is monitored over a 30 min period. Blood from the incision is removed every 15 seconds with a filter paper until the bleeding stops.

- 5 The foregoing disclosure and examples are not intended to narrow the scope of the claims in any way. It should be understood that various modifications and changes can be made, and equivalents can be substituted, to the foregoing embodiments and teachings without departing from the true spirit and scope of the claims appended hereto. The specification and examples are, accordingly, to be
- 10 regarded in an illustrative sense rather than in a restrictive sense. Furthermore, the disclosure of all articles, books, patent applications, patents, and other material referred to herein are incorporated herein by reference in their entireties.

Claims

We claim:

1. A monoclonal antibody capable of binding the antithrombin (β) heparin complex (AT β H), wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (AYRMG) of SEQ ID NO: 2, a CDR2 sequence of amino acids 50 to 66 (RIYSSGGRTRYADSVKG) of SEQ ID NO: 2, and a CDR3 sequence of amino acids 97 to 114 (AREKASDLSGSFSEALDY) of SEQ ID NO: 2; and
- wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 24 to 34 (QGDSLRSYYAS) of SEQ ID NO: 1, a CDR2 sequence of amino acids 50 to 56 (GKNNRPS) of SEQ ID NO: 1; and a CDR3 sequence of amino acids 89 to 99 (NSRDSSGNHLV) of SEQ ID NO: 1.
2. A monoclonal antibody capable of binding AT β H, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (KYKMD) of SEQ ID NO: 4, a CDR2 sequence of amino acids 50 to 66 (RIGPSGGKTM YADSVKG) of SEQ ID NO: 4, and a CDR3 sequence of amino acids 97 to 114 (AREKASDLSG TYSEALDY) of SEQ ID NO: 4; and
- wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 26 to 37 (RASQSVSSSYLA) of SEQ ID NO: 3, a CDR2 sequence of amino acids 53 to 59 (GASSRAT) of SEQ ID NO: 3, and a CDR3 sequence of amino acids 92 to 99 (QQYGSSRT) of SEQ ID NO: 3.
3. A monoclonal antibody capable of binding AT β H, wherein the heavy chain of said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (KYRMD) of SEQ ID NO: 6, a CDR2 sequence of amino acids 50 to 66 (RIGPSGGKTT YADSVKG) of SEQ ID NO: 6, and a CDR3 sequence of amino acids 97 to 114 (AREKTSDL SG SYSEALDY) of SEQ ID NO: 6; and
- wherein the light chain of said antibody comprises: a CDR1 sequence of amino acids 26 to 36 (RASQNINRNLA) of SEQ ID NO: 5, a CDR2 sequence of amino acids 52 to

58 (TASTRAP) of SEQ ID NO: 5, and a CDR3 sequence of amino acids 91 to 99 (QQYASPPRT) of SEQ ID NO: 6.

4. A monoclonal antibody capable of binding AT β H, wherein the heavy chain of
5 said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (RYAMY) of
SEQ ID NO: 8, a CDR2 sequence of amino acids 50 to 66 (RISPSGGKTH
YADSVKG) of SEQ ID NO: 8, and a CDR3 sequence of amino acids 97 to 115
(ARLSQTGYYP HYHYYGMDV) of SEQ ID NO: 8; and

wherein the light chain of said antibody comprises: a CDR1 sequence of
10 amino acids 26 to 37 (RASQRVSSSYLT) of SEQ ID NO: 7, a CDR2 sequence of
amino acids 53 to 59 (GASSRAT) of SEQ ID NO: 7; and a CDR3 sequence of
amino acids 92 to 101 (QQYDSTPPLT) of SEQ ID NO: 7.

5. A monoclonal antibody capable of binding AT β H, wherein the heavy chain of
15 said antibody comprises: a CDR1 sequence of amino acids 31 to 35 (SYRMS) of
SEQ ID NO: 10, a CDR2 sequence of amino acids 50 to 66 (RIYSSGGRT
YADSVKG) of SEQ ID NO: 10, and a CDR3 sequence of amino acids 97 to 114
(AREKASDL SG SFSEALDY) of SEQ ID NO: 10; and

wherein the light chain of said antibody comprises: a CDR1 sequence of
20 amino acids 23 to 33 (QGDSLRSYYAS) of SEQ ID NO: 9, a CDR2 sequence of
amino acids 49 to 55 (GKNNRPS) of SEQ ID NO: 9; and a CDR3 sequence of
amino acids 88 to 96 (NSRDSSGNH) of SEQ ID NO: 9.

6. An isolated monoclonal antibody that binds to AT β H and inhibits anticoagulant
25 activity, wherein said antibody comprises a heavy chain variable region comprising
an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6,
8, and 10, and amino acid sequences having substantial homology to SEQ ID NOS:
2, 4, 6, 8, and 10.

30 7. The isolated monoclonal antibody of claim 6, further comprising a light chain
variable region comprising an amino acid sequence selected from the group

consisting of SEQ ID NOS: 1, 3, 5, 7, and 9, and amino acid sequences having substantial homology to SEQ ID NOS: 1, 3, 5, 7, and 9.

8. An isolated monoclonal antibody that binds to A β H and inhibits anticoagulant activity, wherein said antibody further comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9, and amino acid sequences having substantial homology to SEQ ID NOS: 1, 3, 5, 7, and 9.
9. An isolated monoclonal antibody that binds to A β H and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 46, 47, 48, 49, and 50.
10. The isolated monoclonal antibody of claim 9, further comprising: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 36, 37, 38, 39, and 40; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ. ID NO: 41, 42, 43, 44, and 45; or (c) both a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ. ID NO: 36, 37, 38, 39, and 40 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41, 42, 43, 44, and 45.
11. An isolated monoclonal antibody that binds to A β H and inhibits anticoagulant activity, wherein said antibody comprises a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 31, 32, 33, 34, and 35.
12. The isolated monoclonal antibody of claim 11, further comprising: (a) a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 21, 22, 23, 24, and 25; (b) a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ. ID NOS: 26, 27, 28, 29, and 30; or (c) both CDR1 comprising an amino acid sequence selected from the group consisting of SEQ. ID NOS: 21, 22, 23, 24, and 25 and a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 26, 27, 28, 29, and 30.

13. An isolated monoclonal antibody that binds to the active site of AT β H.
14. An isolated monoclonal antibody that binds to AT β H and provides anticoagulant
5 activity, wherein the isolated monoclonal antibody exhibits minimal binding to AT
and wherein said antibody is a fully human antibody.
15. The isolated monoclonal antibody of any one of claims 1-14, wherein the
antibody is selected from the group consisting of an IgG1, an IgG2, an IgG3, an
10 IgG4, an IgM, an IgA1, an IgA2, a secretory IgA, an IgD, an IgE antibody, and an
antibody fragment.
16. An isolated monoclonal antibody that binds to human AT β H.
- 15 17. The isolated monoclonal antibody of claims 16, wherein the antibody further
binds to a nonhuman species of AT β H.
18. The isolated monoclonal antibody of any one of claims 1-14, wherein blood
clotting time in the presence of the antibody is shortened.
- 20 19. An antibody which would compete with the isolated monoclonal antibody of any
one of claims 1-14.
20. A pharmaceutical composition comprising a therapeutically effective amount of
25 the
monoclonal antibody of any one of claims 1-14 and a pharmaceutically acceptable
carrier.
21. A method for treating a genetic or acquired deficiency or a defect in coagulation
30 comprising administering a therapeutically effective amount of a pharmaceutical
composition of any one of claims 1-14 to a patient.

22. A method for treating coagulopathy comprising administering a therapeutically effective amount of a pharmaceutical composition of claim 20 to a patient.
23. The method of claims 21 or 22, wherein the coagulopathy is hemophilia A,
5 hemophilia B, or hemophilia C.
24. The method of claims 21 or 22, wherein the coagulopathy is selected from the group consisting of trauma-induced coagulopathy and severe bleeding.
- 10 25. The method of claim 22, further comprising administering a clotting factor.
26. The method of claim 25, wherein the clotting factor is selected from the group consisting of Factor VIIa, Factor VIII, and Factor IX.
- 15 27. A method for shortening bleeding time comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 20 to a patient.
28. An isolated nucleic acid molecule encoding an antibody that binds to ATβH and
20 inhibits anticoagulant activity, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, and 9.
29. An isolated nucleic acid molecule encoding an antibody that binds to ATβH and
25 inhibits anticoagulant activity, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, and 10.
30. The method of claim 21, wherein the defect in coagulation is hemophilia A,
30 hemophilia B or hemophilia C.