

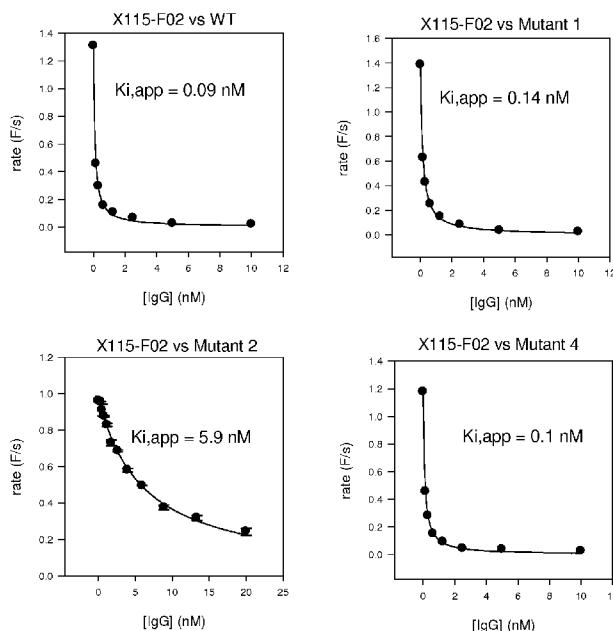
(43) International Publication Date
25 September 2014 (25.09.2014)(51) International Patent Classification:
A61K 39/395 (2006.01)(21) International Application Number:
PCT/US2014/027100(22) International Filing Date:
14 March 2014 (14.03.2014)(25) Filing Language:
English(26) Publication Language:
English(30) Priority Data:
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ANTI-PLASMA KALLIKREIN ANTIBODIES

Figure 4(57) **Abstract:** Disclosed herein are antibodies capable of binding to plasma kallikrein and inhibit its activity. Such antibodies interact with one or more critical residues in the catalytic domain of the plasma kallikrein. The antibodies may also contain specific heavy chain complementarity determining region 3 (CDRs) motifs and optionally specific residues at certain positions within both the heavy chain variable region and the light chain variable region.



Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
- *with sequence listing part of description (Rule 5.2(a))*

Anti-Plasma Kallikrein Antibodies

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of the filing date of U.S. Provisional Application No. 61/791,822, filed March 15, 2013, the entire contents of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 Plasma kallikrein is a serine protease component of the contact system and a potential drug target for different inflammatory, cardiovascular, infectious (sepsis) and oncology diseases (Sainz I. M. et al., *Thromb Haemost* 98, 77-83, 2007). The contact system is activated by either factor XIIa upon exposure to foreign or negatively charged surfaces or on endothelial cell surfaces by prolylcarboxypeptidases (Sainz I. M. et al., *Thromb Haemost* 98, 15 77-83, 2007). Activation of the plasma kallikrein amplifies intrinsic coagulation via its feedback activation of factor XII and enhances inflammation via the production of the proinflammatory nonapeptide bradykinin. As the primary kininogenase in the circulation, plasma kallikrein is largely responsible for the generation of bradykinin in the vasculature. A 20 genetic deficiency in the C1-inhibitor protein (C1-INH), the major natural inhibitor of plasma kallikrein, leads to hereditary angioedema (HAE). Patients with HAE suffer from acute attacks of painful edema often precipitated by unknown triggers (Zuraw B. L. et al., *N Engl J Med* 359, 1027-1036, 2008).

25 Through the use of pharmacological agents or genetic studies in animal models, the plasma kallikrein-kinin system (plasma KKS) has been implicated in various diseases. Thus, it is of great interest to identify agents that inhibit plasma kallikrein activity, thereby effective in treating diseases associated with plasma kallikrein.

SUMMARY OF THE INVENTION

30 The present invention is based on the determination of crystal structures of a complex formed by the catalytic domain of human plasma kallikrein (PKal) and the Fab fragment of DX2930 (an antibody specifically binds human PKal and effectively inhibits its activity), and

the identification of residues in both plasma kallirein (PKal) and the antibody that are critical to the interaction between the two molecules and/or to the inhibition of the pKal activity.

Accordingly, the present disclosure features anti-PKal antibodies capable of inhibiting its activity (*e.g.*, by at least 50%), pharmaceutical compositions comprising such, and uses of the pharmaceutical compositions for treating diseases and disorders associated with plasma kallikrein.

In one aspect, the present disclosure provides an isolated antibody that binds human plasma kallikrein (PKal), wherein the antibody interacts with one or more of amino acid residues in the human PKal and inhibits its activity by at least 50%. The amino acid residues in the PKal that interact with the antibody can be V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, G438, L439, W445, Y475, K476, V477, S478, E479, G480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, G576, S578, T596, S597, W598, G599, E600, G601, C602, A603, R604, Q607, P608, G609, V610, and Y611 as indicated in Figure 2 (boldfaced and underlined).

In some examples, the anti-PKal antibody can bind an epitope of the PKal, the epitope comprising one of the following segments in PKal (Figure 2): V410-C419, H434-L439, Y475-G480, F524-K528, Y552-Y555, D572-S578, T596-R604, or Q607-Y611.

In other examples, the antibody preferentially binds the PKal as relative to a mutant of the PKal (*e.g.*, an inactive mutant) that contains one or more mutations at positions R551, Q553, Y555, T558, and R560 (*e.g.*, Mutant 2 shown in Figure 5).

In another aspect, the present disclosure provides an isolated antibody that binds human plasma kallikrein, wherein the antibody comprises a heavy chain variable region that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3). The HC CDR3 in the antibody comprises the motif X₉₉R₁₀₀X₁₀₁G₁₀₂X₁₀₃P₁₀₄R₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁ (SEQ ID NO: 58), in which X₉₉ is R or Q, X₁₀₁ is T, I, R, S, or P, X₁₀₃ is V, I, or L, X₁₀₆ is R or W, X₁₀₇ is D or N, X₁₀₈ is A, S, D, E, or V, X₁₀₉ is F or L, X₁₁₀ is D, E, or N, and X₁₁₁ is I, N, M, or S.

In some examples, X_{99} can be Q and X_{101} can be I, R, S, or P. In other examples, X_{106} can be W and X_{111} can be N, M, or S. Alternatively or in addition, X_{101} can be I, X_{108} can be E, and X_{103} can be I or L. In yet other examples, X_{101} can be I and X_{103} can be I or L, or X_{103} can be I or L and X_{110} can be D, E, or N.

5 In some embodiments, the heavy chain variable region of the anti-PKal antibody described herein includes H_{31} in the HC CDR1. Alternatively or in addition, the heavy chain variable region includes F_{27} , F_{29} , or both in the framework region 1 (FR1).

10 The anti-PKal antibody described herein can further comprise a light chain variable region that comprises complementarity determining region 1 (LC CDR1), complementarity determining region 2 (LC CDR2), and complementarity determining region 3 (LC CDR3). In some embodiments, the LC CDR2 includes K_{50} , L_{54} , E_{55} , S_{56} , or a combination thereof. Alternatively or in addition, the light chain variable region further includes G_{57} in the framework region 3 (FR3). When necessary, the light chain variable includes N_{45} in the framework region 2 (FR2).

15 Any of the anti-PKal antibodies described herein can inhibit the activity of PKal by at least 50% (e.g., at least 80%, 90%, 95%, or 99%). In some instances, the antibody has an apparent K_i ($K_{i,app}$) lower than about 1 nM (e.g., lower than about 0.1 nM, or lower than about 0.05 nM). Alternatively or in addition, the anti-PKal antibody described herein can have a binding affinity (K_D) for the PKal of less than 10^{-6} M (e.g., less than 10^{-7} M, 10^{-8} M, or 10^{-9} M).

20 The anti-PKal antibodies described herein can be a full-length antibody or an antigen-binding fragment thereof. Alternatively or in addition, the antibody can be a human antibody or a humanized antibody.

25 Also within the scope of the present disclosure are pharmaceutical compositions for use in treating various diseases and disorders associated with plasma kallikrein, or for use in manufacturing a medicament for treating the diseases and disorders. The pharmaceutical compositions each comprise one or more anti-PKal antibodies as described herein and a pharmaceutically acceptable carrier.

30 Further, described herein are methods for treating a disease associated with plasma kallikrein, comprising administering to a subject in need thereof an effective amount of the

pharmaceutical composition, which comprises one or more of the anti-PKal antibodies described herein. In some examples, the subject is a human patient diagnosed with, suspected of having, or at risk for the disease.

The details of one or more embodiments of the invention are set forth in the 5 description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

15 Figure 1 shows the amino acid sequence of the heavy chain variable region (V_H) and light chain variable region (V_L) of a parent antibody, M0162-A04, from which DX2930 was derived, and their alignment with the corresponding germline V_H and V_L genes as indicated. Variations in M0162-A04 as compared to the germline sequences are indicated (boldfaced).

20 Figure 2 shows the amino acid sequence (SEQ ID NO:40) of the catalytic domain of human plasma kallikrein (residues 391-638 of the full length humanPKal). The boldfaced and underlined residues refer to those that are involved in the interaction with the Fab fragment of DX2930 as identified by the crystal structure discussed in Example 1 below.

Figure 3 is a graph showing the apparent K_i ($K_{i, app}$) of a number of antibody mutants derived from M0162-A04 against human PKal.

25 Figure 4 is a graph showing the apparent K_i ($K_{i, app}$) of clone X115-F02 (see Table 1 below) against wild-type PKal and a number of PKal mutants.

Figure 5 shows the amino acid sequences of a number of PKal mutants (catalytic domain), which were produced in Pichia cells.

DETAILED DESCRIPTION OF THE INVENTION

DX-2930 is a fully human IgG derived from parent clone M0162-A04. The amino acid sequences of the V_H and V_L of M0162-A04 are shown in Figure 1. Their alignment with the corresponding germline VH gene (VH3_3-23) and VL gene (VK1_L12) is also shown in Figure 1. Compared to the HC CDR3 of M0162-A04, the HC CDR3 of DX-2930 includes the variations of T101I, I103V, and A108E (see Table 2 below; the HC CDR3 of DX-2930 being identical to M0199-A08). The Chothia Numbering Scheme is used in the present disclosure. <http://www.bioinf.org.uk/abs/>.

Table 1 below provides structural information of DX-2930, its parent antibody M0162-A04, and variants thereof. See also US20120201756 and US20110200611.

Table 1. Structural Properties of DX-2930 and Related Variants

Name	Properties
M162-A04	<ul style="list-style-type: none"> • This is the parent antibody of DX-2930 that was discovered in the initial phage display selection efforts ($K_{i,app} = 2.5$ nM).. • This antibody differs from DX-2930 at 3 critical amino acids in the CDR3 of the heavy chain and the germlined positions.
M199-A08	<ul style="list-style-type: none"> • Fab discovered following the affinity maturation of M0162-A04 using the Hv-CDR3 spiking method ($K_{i,app} \sim 0.06$ nM). • This antibody shares the same amino acids in the variable region with DX-2930 but was not germlined and does not contain a Fc fragment.
X115-F02	<ul style="list-style-type: none"> • Fully human IgG, kappa light chain • 1 amino acid in the light chain was mutated to their germline sequence. • The DNA sequence of X115-F02 was optimized for expression in CHO cells • Expressed transiently in 293T cells following subcloning into the pRH1-CHO vector
DX-2930 (X124-G01)	<ul style="list-style-type: none"> • Fully human IgG, kappa light chain • 1 amino acid in the light chain and 2 amino acids in the heavy were mutated to their germline sequence. • The DNA sequence of DX-2930 was optimized for expression in CHO cells and cloned into the pEh1 vector for stable expression using the glutamate

	<p>synthase system.</p> <ul style="list-style-type: none">• The Fc of DX-2930 was modified to remove the C-terminal lysine residue, in order to obtain a more homogeneous product.
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5 Crystal structures (with different resolutions) of a complex formed between the Fab fragment of DX-2930 and the catalytic domain of human plasma kallikrein (PKal) was determined. Based on the structural information provided by the crystal structures, a number of interacting residues in both the catalytic domain of human PKal and the antibody (in both V_H and V_L) were identified. The interacting residues in the PKal are important targets for developing antibodies capable of inhibiting the PKal activity. Similarly, the interacting residues in the antibody also provide important structural information for designing anti-PKal antibodies with high inhibitory activity.

10 Further, affinity maturation analysis was performed to develop high affinity anti-PKal antibodies, using clone M0162-A04 as the parent. Results obtained from affinity maturation matches with the structural information provided by the crystal structures. Based on the structural information and the affinity maturation results, specific VH and VL motifs/residues were identified for designing anti-PKal antibodies with high inhibitory activities.

15 Accordingly, described herein are antibodies capable of binding to plasma kallikrein (e.g., human plasma kallikrein; PKal) and inhibiting its activity, and uses thereof for treating diseases and disorders associated with plasma kallikrein. Such antibodies interact with one or more critical residues in the catalytic domain of the PKal and/or comprise specific motifs/residues in either the heavy chain variable region (e.g., HC CDR1 or HC CDR3) or the 20 light chain variable region (e.g., LC CDR2), or both.

Antibodies Binding to PKal

25 The present disclosure provides isolated antibodies that bind PKal, particularly the catalytic domain of the PKal, such as human PKal. The term “isolated antibody” used herein refers to an antibody substantially free from naturally associated molecules, i.e., the naturally associated molecules constituting at most 20% by dry weight of a preparation containing the antibody. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, and HPLC.

An antibody (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only 5 intact (i.e., full-length) polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, multispecific antibodies (e.g., bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that 10 comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending 15 on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called 20 alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The antibodies described herein are capable of binding to a PKal, particularly the catalytic domain of a PKal (e.g., human PKal), thereby inhibiting the activity of PKal. In some instances, the antibodies described herein can inhibit the activity of PKal by at least 50%, e.g., 60%, 70%, 80%, 90%, 95%, or higher. The inhibition constant (Ki) provides a 25 measure of inhibitor potency; it is the concentration of inhibitor required to reduce enzyme activity by half and is not dependent on enzyme or substrate concentrations. The inhibitory activity of an anti-PKal antibody can be determined by routine methods, such as the method described in Example 2 below.

30 In some examples, the inhibitory activity of an anti-PKal antibody is determined by

the apparent K_i ($K_{i,app}$) value. The $K_{i,app}$ value of an antibody obtained at different substrate concentrations by measuring the inhibitory effect of different concentrations of the antibody on the extent of the reaction (e.g., enzyme activity); fitting the change in pseudo-first order rate constant as a function of inhibitor concentration to the Morrison equation (Equation 1) 5 yields an estimate of the apparent K_i value. For a competitive inhibitor, the K_i is obtained from the y-intercept extracted from a linear regression analysis of a plot of $K_{i,app}$ versus substrate concentration.

$$v = v_o - v_o \left(\frac{(K_{i,app} + I + E) - \sqrt{(K_{i,app} + I + E)^2 - 4 \cdot I \cdot E}}{2 \cdot E} \right) \quad \text{Equation 1}$$

In some examples, the anti-PKal antibodies described herein have a $K_{i,app}$ value lower 10 than 1 nM, e.g., 0.5 nM, 0.2 nM, 0.1 nM, 0.09 nM, 0.08 nM, 0.07 nM, 0.06 nM, 0.05 nM, 0.04 nM, 0.03 nM, 0.02 nM, 0.01 nM, or lower. The $K_{i,app}$ value of an antibody can be estimated following the methods known in the art and described herein (Example 2).

The antibodies described herein can be murine, rat, human, or any other origin 15 (including chimeric or humanized antibodies). In some examples, the antibody comprises a modified constant region, such as a constant region that is immunologically inert, e.g., does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC). ADCC activity can be assessed using methods disclosed in U.S. Pat. No. 5,500,362. In other embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or 20 UK Patent Application No. 9809951.8.

Any of the antibodies described herein can be either monoclonal or polyclonal. A “monoclonal antibody” refers to a homogenous antibody population and a “polyclonal antibody” refers to a heterogeneous antibody population. These two terms do not limit the source of an antibody or the manner in which it is made.

In one example, the antibody used in the methods described herein is a humanized 25 antibody. Humanized antibodies refer to forms of non-human (e.g. murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or antigen-binding

fragments thereof that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or 5 rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized 10 antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a 15 human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

Humanized antibodies may also involve affinity maturation.

20 In another example, the antibody described herein is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies 25 derived from one species of mammals (e.g., a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or the constant region.

30 In some embodiments, the anti-PKal antibodies described herein have a suitable

binding affinity to a PKal or the catalytic domain thereof. As used herein, "binding affinity" refers to the apparent association constant or K_A . The K_A is the reciprocal of the dissociation constant (K_D). The antibody described herein may have a binding affinity (K_D) of at least 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} M, or lower. An increased binding affinity corresponds to a 5 decreased K_D . Higher affinity binding of an antibody to a first target relative to a second target can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first target than the K_A (or numerical value K_D) for binding the second target. In such cases, the antibody has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 10 1000, 10,000 or 10^5 fold.

Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or 15 spectroscopy (e.g., using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound binding protein as a function of target protein concentration. The concentration of bound binding protein 20 ([Bound]) is related to the concentration of free target protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[\text{Bound}] = [\text{N}][\text{Free}] / (\text{Kd} + [\text{Free}])$$

It is not always necessary to make an exact determination of K_A , though, since 25 sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A , and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an in vitro or in vivo assay.

30

Antibodies Targeting Specific Residues in Human Plasma Kallikrein

5 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, or 45) in the catalytic domain of human PKal, including V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, G438, L439, W445, Y475, K476, V477, S478, E479, G480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, G576, S578, T596, S597, W598, G599, E600, G601, C602, A603, R604, Q607, P608, G609, V610, and Y611 (numbers based on the full length prekallikrein amino acid sequence). The positions of these residues are indicated in Figure 2 (boldfaced and underlined). These residues are identified as important to the pKal activity, according to the crystal structures described in Example 1 below.

10 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, 8, 10, 15, 20, or 23) in the catalytic domain of human PKal, including L418, C419, H434, C435, D437, G438, L439, Y475, D483, F524, D572, A573, C574, K575, G576, S578, T596, S597, W598, G599, E600, G601, and C602 (numbers based on the full length prekallikrein amino acid sequence).

15 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, or 8) in the catalytic domain of human PKal, including K476, V477, S478, E479, G480, Y552, D554, and Y555 (numbers based on the full length prekallikrein amino acid sequence).

20 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, 8, or 10) in the catalytic domain of human PKal, including V410, L412, T413, A414, Q415, R416, E527, K528, A603, and R604 (numbers based on the full length prekallikrein amino acid sequence).

25 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, or 6) in the catalytic domain of human PKal, including W445, Q607, P608, G609, V610, and Y611 (numbers based on the full length prekallikrein amino acid sequence).

30 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, 8, or 9) in the catalytic domain of human PKal, including F524, D572, A573, C574, K575, G576, S578, G601, and C602 (numbers based on the full length prekallikrein amino acid sequence).

In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3, 5, or 8) in the catalytic domain of human PKal, including L418, C419, H434, C435, D437, G438, Y475, and D483 (numbers based on the full length prekallikrein amino acid sequence).

5 In some embodiments, the anti-PKal antibodies interact with one or more of the residues (e.g., at least 3 or 4) in the catalytic domain of human PKal, including S597, W598, G599, and E600 (numbers based on the full length prekallikrein amino acid sequence).

Interacting means that the distance between two residues in a complex formed by two binding partners is lower than a predetermined value, e.g., < 6 Å, < 4 Å, or < 2 Å. For 10 example, an interacting residue in one binding partner can have has at least 1 atom within a given threshold (e.g., < 6 Å, < 4 Å, or < 2 Å) of at least 1 atom from a residue of the other binding partner on the complexed structure. Interacting does not require actual binding. Interacting residues are suggested as involved in antibody recognition.

15 In some embodiments, the antibodies described herein bind human PKal at an epitope comprising one or more of the residues listed above. An “epitope” refers to the site on a target compound that is bound by an antibody such as a Fab or full length antibody. An epitope can be linear, which is typically 6-15 aa in length. Alternatively, the epitope can be conformational.

20 In some examples, the anti-PKal antibodies described herein binds an epitope that comprises the following segments: V410-C419, H434-L439, Y475-G480, F524-K528, Y552-Y555, D572-S578, T596-R604, or Q607-Y611.

25 In some examples, the antibody disclosed herein specifically binds PKal or an epitope therein. An antibody that "specifically binds" (used interchangeably herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody “specifically binds” to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically 30 (or preferentially) binds to human PKal or an epitope therein is an antibody that binds this

target antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens or other epitopes in the same antigen. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, 5 "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

In one example, the anti-PKal antibodies described herein preferentially bind wild-type as compared to a mutant that includes mutations at one or more of R551, Q553, Y555, 10 T558, and R560, e.g., Mutant 2 described in Example 3. Such antibodies may bind wild-type PKal at a much higher affinity as compared to the mutant (e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1,000-fold higher). Alternatively or in addition, the antibodies exhibit a much higher inhibitory activity against the wild-type pKal as relative to the mutant (e.g., at least 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1,000-fold higher). 15

In other examples, the anti-PKal antibodies described herein binds active PKal, including wild-type pKal and functional variant thereof. The antibody can preferentially bind an active PKal as relative to its binding to an inactive mutant.

Anti-Plasma Kallikrein Antibodies Having Specific Motifs and/or Residues

In some embodiments, the anti-PKal antibody described herein comprises a V_H and a V_L, each of which comprises three CDRs flanked by framework regions (FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4; see Figure 1). The CDR3 of the heavy chain can comprise the motif: X₉₉R₁₀₀X₁₀₁G₁₀₂X₁₀₃P₁₀₄R₁₀₅X₁₀₆X₁₀₇X₁₀₈X₁₀₉X₁₁₀X₁₁₁, in which X₉₉ is R or Q, X₁₀₁ is T, I, R, S, or P, X₁₀₃ is V, I, or L, X₁₀₆ is R or W, X₁₀₇ is D or N, X₁₀₈ is A, S, D, E, or V, X₁₀₉ is F or L, X₁₁₀ is D, E, or N, and X₁₁₁ is I, N, M, or S. In some examples, X₉₉ is Q and X₁₀₁ is I, R, S, or P. Alternatively or in addition, X₁₀₆ is W and X₁₁₁ is N, M, or S. In other examples, 25 X₁₀₁ is I, X₁₀₈ is E, and X₁₀₃ is I or L; or X₁₀₁ is I and X₁₀₃ is I or L. In yet other examples, X₁₀₃ is I or L and X₁₁₀ is D, E, or N.

In addition, such an anti-pKal antibody can include one or more other residues that 30 are identified based on the crystal structures discussed herein as being involved in interacting

with the catalytic domain of human PKal. These residues can be located in the V_H or the V_L chain. Examples include E1, V2, F27, T28, F29, and S30 in the FR1 of the V_H , H₃₁ in the HC CDR1; S31 and W32 in the LC CDR1, Y49 in the FR1 of the V_L chain, K50, T53, L54, and E55, and S56 in LC CDR2, and G57 and V58 the FR3 of the V_L chain.

5 The anti-PKal antibodies as described above can use any germline heavy chain and light chain V genes as the framework. Heavy chain V genes include, but are not limited to, IGHV1-2, IGHV1-3, IGHV1-8, IGHV1-18, IGHV1-24, IGHV1-45, IGHV1-46, IGHV1-58, IGHV1-69, IGHV2-5, IGHV2-26, IGHV2-70, IGHV3-7, IGHV3-9, IGHV3-11, IGHV3-13, IGHV3-15, IGHV3-20, IGHV3-21, IGHV3-23, IGHV3-30, IGHV3-33, IGHV3-43, IGHV3-48, IGHV3-49, IGHV3-53, IGHV3-64, IGHV3-66, IGHV3-72, IGHV3-73, IGHV3-74, IGHV4-4, IGHV4-28, IGHV4-31, IGHV4-34, IGHV4-39, IGHV4-59, IGHV4-61, IGHV4-B, IGHV5-51, IGHV6-1, and IGHV7-4-1.

10 In some examples, the antibody uses a κ light chain. Light chain VK genes include, but are not limited to, V genes for IGKV1-05, IGKV1-06, IGKV1-08, IGKV1-09, IGKV1-12, IGKV1-13, IGKV1-16, IGKV1-17, IGKV1-27, IGKV1-33, IGKV1-37, IGKV1-39, IGKV1D-16, IGKV1D-17, IGKV1D-43, IGKV1D-8, IGKV2-24, IGKV2-28, IGKV2-29, IGKV2-30, IGKV2-40, IGKV2D-26, IGKV2D-29, IGKV2D-30, IGKV3-11, IGKV3-15, IGKV3-20, IGKV3D-07, IGKV3D-11, IGKV3D-20, IGKV4-1, IGKV5-2, IGKV6-21, and IGKV6D-41. In other examples, the antibody uses a λ light chain, e.g., any of IGLV1-15 IGLV10.

15 The antibody also can use any germline heavy J segment (e.g., heavy chain IGJH1-IGJH6) and light chain J segment (e.g., IGJK1, IGJK2, IGJK3, IGJK4, or IGJK5), which can subject to variations, such as deletions at the C-terminus, N-terminus, or both.

20 Germline antibody gene/segment sequences are well known in the art. See, e.g., <http://www.vbase2.org/vbstat.php>.

25 In some examples, the anti-PKal antibody described herein uses VH3_3-23 and/or VK1_L12 as the framework for the heavy chain and/or the light chain. It may include substantially similar HC CDR1, HC CDR2, and/or HC CDR3, and LC CDR1, LC CDR2, and/or LC CDR3 as those in M0162-A04 (Figure 1), e.g., containing up to 5, 4, 3, 2, or 1 30 amino acid residue variations as compared to the corresponding CDR region in M0162-A04.

5 In other examples, the anti-PKal antibody comprises a V_H chain that includes a V_H CDR1, V_H CDR2, and V_H CDR3 at least 75% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the corresponding V_H CDRs of M0162-A04, and a V_L chain that includes a V_L CDR1, V_L CDR2, and V_L CDR3 at least 75% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the corresponding V_L CDRs of M0162-A04.

10 Alternatively, the anti-PKal antibody comprises a V_H chain at least 75% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the V_H chain (mature or precursor) of M0162-A04 and/or a V_L chain at least 75% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the V_L chain (mature or precursor) of M0162-A04.

15 The “percent identity” of two amino acid sequences is determined using the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-68, 1990, modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al. J. Mol. Biol.* 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

20 In some instances, conservative mutations can be introduced into the CDRs in M0162-A04, e.g., at positions where the residues are not likely to be involved in interacting with PKal as determined based on the crystal structure. As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. 25 Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino 30

acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

In some embodiments, the anti-PKal antibodies described here are not those described in US 20110200611, which is incorporated by reference herein.

5 In some embodiments, the anti-PKal antibodies described herein bind to the same epitope as DX-2930 and/or compete for binding with DX-2930, with the proviso that the anti-PKal antibody is not DX-2930. In some embodiments, the anti-Pkal antibodies described herein bind to the sequence SWGE (SEQ ID NO: 48) and/or DACKG (SEQ ID NO: 49) in PKal. In some embodiments, the anti-Pkal antibodies described herein do not bind to the sequence SWGE (SEQ ID NO: 48) and/or DACKG (SEQ ID NO: 49) in Pkal. In some 10 embodiments, the anti-Pkal antibodies described herein bind to the sequence DGL, SEG, TSWGEG (SEQ ID NO: 50) and/or DACKG (SEQ ID NO: 49) in Pkal. In some embodiments, the anti-Pkal antibodies described herein do not bind to the sequence DGL, SEG, TSWGEG (SEQ ID NO: 50) and/or DACKG (SEQ ID NO: 49) in Pkal. In some 15 embodiments, the anti-Pkal antibodies described herein do not bind to the sequence LVTNEECQKRYQDYKITQQ (SEQ ID NO: 51), WVTGWGFSKEKGEI (SEQ ID NO: 52), ACKGDSGGPL (SEQ ID NO: 53), SWGDI (SEQ ID NO: 54), HDIALIKL (SEQ ID NO: 55), TPFSQIKEIIHQNY (SEQ ID NO: 56), and/or AHCFDGLPLQDVWRIY (SEQ ID NO: 57).

20 In some embodiments, the anti-PKal antibody described herein binds to an epitope located in the active domain of PKal (the whole epitope or a portion thereof) and is different from that DX-2930. The epitope of such an antibody may have overlapping residues with those of the epitope of DX-2930. Alternatively, there can be no overlapping residues between the two epitopes.

25 The sequences of the full length heavy chain and light chain of DX-2930 are shown below.

DX-2930 Heavy Chain Amino Acid Sequence (451 amino acids)

30 EVQLLESGGGLVQPGGSLRLSCAASGFTFS **HYIMM**WVRQAPGKGLEWVS **GIYSSGGITVYAD**

5 **SVKG**RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAY**RRIGVPRDEFDI**WGQGTMVTVSSAS
TKG P SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFP AVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVFKFNWYVDGVEVHNAKTPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDI AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG (SEQ ID NO: 46)

10 **DX-2930 Light Chain Amino Acid Sequence (213 amino acids)**

15 DIQMTQSPSTLSASVGDRVTITC**RASQSISSWLA**WYQQKPGKAPKLLIY**KASTLES**GVPSRF
SGSGSGTEFTLTISSLQPDFATYYC**QQYNTYWT**FQG GTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL S STTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 47)

15 In the above sequences, the constant regions are italicized and the CDR regions are in boldface and underlined.

Antibody Preparation

20 Antibodies capable of binding PKal as described herein can be made by any method known in the art. See, for example, Harlow and Lane, (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York.

25 In some embodiments, antibodies specific to a target antigen (e.g., a human PKal or the catalytic domain thereof) can be made by the conventional hybridoma technology. The full-length target antigen or a fragment thereof, optionally coupled to a carrier protein such as KLH, can be used to immunize a host animal for generating antibodies binding to that antigen. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of mouse, humanized, and 30 human antibodies are known in the art and are described herein. It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human hybridoma cell lines. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or intradermally with an amount of immunogen, including 35 as described herein.

Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C. (1975)

Nature 256:495-497 or as modified by Buck, D. W., et al., In Vitro, 18:377-381 (1982).

Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization.

Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art.

5 After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another 10 alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the anti-PKal monoclonal antibodies described herein. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

15 Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies capable of interfering with the PKal activity. Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures 20 such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a target antigen or a fragment containing the target amino acid sequence 25 conjugated to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl, or R1N=C=NR, where R and R1 are different 30 alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

If desired, an antibody (monoclonal or polyclonal) of interest (e.g., produced by a hybridoma) may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. In an alternative, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity (affinity maturation), or other characteristics of the antibody. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to the target antigen and greater efficacy in inhibiting the activity of PKal. It will be apparent to one of skill in the art that one or more polynucleotide changes can be made to the antibody and still maintain its binding specificity to the target antigen.

In other embodiments, fully human antibodies can be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XenomouseTM from Amgen, Inc. (Fremont, Calif.) and HuMAb-MouseTM and TC MouseTM from Medarex, Inc. (Princeton, N.J.). In another alternative, antibodies may be made recombinantly by phage display or yeast technology. See, for example, U.S. Pat. Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., (1994) Annu. Rev. Immunol. 12:433-455, and . Alternatively, the phage display technology (McCafferty et al., (1990) Nature 348:552-553) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors.

Antigen-binding fragments of an intact antibody (full-length antibody) can be prepared via routine methods. For example, F(ab')2 fragments can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')2 fragments.

Genetically engineered antibodies, such as humanized antibodies, chimeric antibodies, single-chain antibodies, and bi-specific antibodies, can be produced via, e.g., conventional recombinant technology. In one example, DNA encoding a monoclonal antibodies specific to a target antigen can be readily isolated and sequenced using 5 conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into one or more expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do 10 not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA can then be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., (1984) Proc. Nat. Acad. Sci. 81:6851, or by covalently joining to the 15 immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, genetically engineered antibodies, such as "chimeric" or "hybrid" antibodies; can be prepared that have the binding specificity of a target antigen.

Techniques developed for the production of "chimeric antibodies" are well known in 20 the art. See, e.g., Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81, 6851; Neuberger et al. (1984) Nature 312, 604; and Takeda et al. (1984) Nature 314:452.

Methods for constructing humanized antibodies are also well known in the art. See, 25 e.g., Queen et al., Proc. Natl. Acad. Sci. USA, 86:10029-10033 (1989). In one example, variable regions of VH and VL of a parent non-human antibody are subjected to three-dimensional molecular modeling analysis following methods known in the art. Next, framework amino acid residues predicted to be important for the formation of the correct CDR structures are identified using the same molecular modeling analysis. In parallel, human VH and VL chains having amino acid sequences that are homologous to those of the parent non-human antibody are identified from any antibody gene database using the parent 30 VH and VL sequences as search queries. Human VH and VL acceptor genes are then

selected.

The CDR regions within the selected human acceptor genes can be replaced with the CDR regions from the parent non-human antibody or functional variants thereof. When necessary, residues within the framework regions of the parent chain that are predicted to be 5 important in interacting with the CDR regions (see above description) can be used to substitute for the corresponding residues in the human acceptor genes.

A single-chain antibody can be prepared via recombinant technology by linking a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. Preferably, a flexible linker is incorporated between 10 the two variable regions. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778 and 4,704,692) can be adapted to produce a phage or yeast scFv library and scFv clones specific to a PKal can be identified from the library following routine procedures. Positive clones can be subjected to further screening to identify those that inhibits PKal activity.

15 Antibodies obtained following a method known in the art and described herein can be characterized using methods well known in the art. For example, one method is to identify the epitope to which the antigen binds, or “epitope mapping.” There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, 20 gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, *Using Antibodies, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. In an additional example, epitope mapping can be used to determine the sequence to which an antibody binds. The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a 25 conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch (primary structure linear sequence). Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an antibody. In another example, the epitope to which the antibody binds can be determined in a systematic screening by using 30 overlapping peptides derived from the target antigen sequence and determining binding by

the antibody. According to the gene fragment expression assays, the open reading frame encoding the target antigen is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the antigen with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then

5 transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled antigen fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be

10 tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant of a target antigen in which various fragments of the PKal polypeptide have

15 been replaced (swapped) with sequences from a closely related, but antigenically distinct protein (such as another member of the neurotrophin protein family). By assessing binding of the antibody to the mutant PKal (e.g., those mutants described in Example 2 below), the importance of the particular antigen fragment to antibody binding can be assessed.

Alternatively, competition assays can be performed using other antibodies known to bind to the same antigen to determine whether an antibody binds to the same epitope as the other antibodies. Competition assays are well known to those of skill in the art.

Any of the suitable methods known in the art, e.g., the epitope mapping methods as described herein, can be applied to determine whether the anti-PKal antibody binds one or more of the specific residues/segments in the PKal as described herein. Further, the interaction of the antibody with one or more of those defined residues in PKal can be determined by routine technology. For example, a crystal structure can be determined following the method disclosed in Example 1 below and the distances between the residues in PKal and one or more residues in the antibody can be determined accordingly. Based on such distance, whether a specific residue in PKal interacts with one or more residues in the antibody can be determined. Further, suitable methods, such as competition assays and target

mutagenesis assays can be applied to determine the preferential binding of a candidate anti-PKal antibody to the PKal as compared to another target such as a mutant PKal.

Pharmaceutical Compositions

5 One or more of the above-described anti-PKal antibodies can be mixed with a pharmaceutically acceptable carrier (excipient), including buffer, to form a pharmaceutical composition for use in alleviating a disease or disorder that is associated with PKal. “Acceptable” means that the carrier must be compatible with the active ingredient of the composition (and preferably, capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. Pharmaceutically acceptable excipients (carriers) including buffers, which are well known in the art. See, e.g., Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover. In one example, a pharmaceutical composition described herein contains more than one anti-PKal antibodies that recognize different epitopes/residues of the target antigen.

15 The pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions. (Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such

as TWEENTM, PLURONICSTM or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

In some examples, the pharmaceutical composition described herein comprises liposomes containing the anti-PKal antibody, which can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

The anti-PKal antibody may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are known in the art, see, e.g., Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

In other examples, the pharmaceutical composition described herein can be formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(v ny alcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The pharmaceutical compositions to be used for in vivo administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration

membranes. Therapeutic antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The pharmaceutical compositions described herein can be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation.

For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, e.g. conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, e.g. water, to form a solid preformulation

composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally

effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above

containing from 0.1 to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can

comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol and cellulose acetate.

Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (e.g. TweenTM 20, 40, 60, 80 or 85) and other sorbitans (e.g. SpanTM 20, 40, 60, 80 or 85). Compositions with a surface-active agent will conveniently comprise between 0.05 and 5% surface-active agent, and can be between 0.1 and 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other

pharmaceutically acceptable vehicles, if necessary.

Suitable emulsions may be prepared using commercially available fat emulsions, such as IntralipidTM, LiposynTM, InfonutrolTM, LipofundinTM and LipiphysanTM. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g. soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing with a phospholipid (e.g. egg phospholipids, soybean phospholipids or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%. The fat emulsion can comprise fat droplets between 0.1 and 1.0 .im, particularly 0.1 and 0.5 .im, and have a pH in the range of 5.5 to 8.0.

The emulsion compositions can be those prepared by mixing an anti-PKal antibody with IntralipidTM or the components thereof (soybean oil, egg phospholipids, glycerol and water).

15 Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect.

20 Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Use of anti-PKal Antibodies for Treating Diseases/Disorders Associated with Plasma Kallikrein

30 The anti-PKal antibodies described herein would be effective in treating a disease or disorder associated the PKal. Examples of such diseases and conditions which can be treated (or prevented) by a plasma kallikrein binding protein described herein include: rheumatoid

arthritis, gout, intestinal bowel disease, oral mucositis, neuropathic pain, inflammatory pain, spinal stenosis-degenerative spine disease, arterial or venous thrombosis, post operative ileus, aortic aneurysm, osteoarthritis, vasculitis, edema, hereditary angioedema, cerebral edema, pulmonary embolism, stroke, clotting induced by ventricular assistance devices or stents, 5 head trauma or peri-tumor brain edema, sepsis, acute middle cerebral artery (MCA) ischemic event (stroke), restenosis (e.g., after angioplasty), systemic lupus erythematosus nephritis, burn injury, and DME. A plasma kallikrein binding protein described herein can also be used to promote wound healing. A plasma kallikrein binding protein described herein can also be used as an oncology treatment by mechanisms that include, but are not limited to, blocking 10 production of pro-angiogenic bradykinin.

To practice the method disclosed herein, an effective amount of the pharmaceutical composition described above can be administered to a subject (e.g., a human) in need of the treatment via a suitable route, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, 15 intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, inhalation or topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-PKal antibodies can be aerosolized using a fluorocarbon formulation and a 20 metered dose inhaler, or inhaled as a lyophilized and milled powder.

The subject to be treated by the methods described herein can be a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. A human subject who needs the treatment may be a human patient having, at risk for, or suspected of having a disease/disorder 25 associated with PKal, such as those noted above. A subject having a PKal-associated disease or disorder can be identified by routine medical examination, e.g., laboratory tests, organ functional tests, CT scans, or ultrasounds. A subject suspected of having any of such disease/disorder might show one or more symptoms of the disease/disorder. A subject at risk for the disease/disorder can be a subject having one or more of the risk factors for that 30 disease/disorder.

“An effective amount” as used herein refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the 5 individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the 10 individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

Empirical considerations, such as the half-life, generally will contribute to the 15 determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression 20 and/or amelioration and/or delay of a disease/disorder associated with PKal. Alternatively, sustained continuous release formulations of an anti-PKal may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one example, dosages for an anti-PKal antibody as described herein may be determined empirically in individuals who have been given one or more administration(s) of 25 the antibody. Individuals are given incremental dosages of the antagonist. To assess efficacy of the antagonist, an indicator of the disease/disorder can be followed.

Generally, for administration of any of the antibodies described herein, an initial candidate dosage can be about 2 mg/kg. For the purpose of the present disclosure, a typical daily dosage might range from about any of 0.1 μ g/kg to 3 μ g/kg to 30 μ g/kg to 300 μ g/kg to 30 3 mg/kg, to 30 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. For

repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a disease or disorder associated with PKal, or a symptom thereof. An exemplary dosing regimen comprises administering an initial dose of 5 about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg of the antibody, or followed by a maintenance dose of about 1 mg/kg every other week. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. For example, dosing from one-four times a week is contemplated. In some embodiments, dosing ranging from about 3 μ g/mg to about 2 mg/kg 10 (such as about 3 μ g/mg, about 10 μ g/mg, about 30 μ g/mg, about 100 μ g/mg, about 300 μ g/mg, about 1 mg/kg, and about 2 mg/kg) may be used. In some embodiments, dosing frequency is once every week, every 2 weeks, every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, or every 10 weeks; or once every month, every 15 2 months, or every 3 months, or longer. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen (including the antibody used) can vary over time.

In some embodiments, for an adult patient of normal weight, doses ranging from about 0.3 to 5.00 mg/kg may be administered. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical 20 history, as well as the properties of the individual agents (such as the half-life of the agent, and other considerations well known in the art).

For the purpose of the present disclosure, the appropriate dosage of an anti-PKal antibody will depend on the specific antibody (or compositions thereof) employed, the type and severity of the disease/disorder, whether the antibody is administered for preventive or 25 therapeutic purposes, previous therapy, the patient's clinical history and response to the antagonist, and the discretion of the attending physician. Typically the clinician will administer an anti-PKal antibody, until a dosage is reached that achieves the desired result. Administration of an anti-PKal antibody can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the 30 administration is therapeutic or prophylactic, and other factors known to skilled practitioners.

The administration of an anti-PKal antibody may be essentially continuous over a preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing a disease or disorder associated with PKal.

As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a disease/disorder associated with PKal, a symptom of the disease/disorder, or a predisposition toward the disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease/disorder.

Alleviating a disease/disorder associated with PKal includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of a disease/disorder associated with PKal means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of a disease/disorder associated with PKal includes initial onset and/or recurrence.

In some embodiments, the anti-PKal antibody described herein is administered to a subject in need of the treatment at an amount sufficient to inhibit the activity of PKal by at least 20% (e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) in vivo. In other

embodiments, the antibody is administered in an amount effective in reducing the PKal level by at least 20% (e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater).

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the pharmaceutical composition to the subject, depending upon the type of disease to be treated or the site of the disease. This composition can also be administered via other conventional routes, e.g., administered orally, parenterally, by inhalation spray, 5 topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection or infusion techniques. In addition, it can be administered to the 10 subject via injectable depot routes of administration such as using 1-, 3-, or 6-month depot injectable or biodegradable materials and methods.

Injectable compositions may contain various carriers such as vegetable oils, dimethylactamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, 15 ethanol, and polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like). For intravenous injection, water soluble antibodies can be administered by the drip method, whereby a pharmaceutical formulation containing the antibody and a physiologically acceptable excipients is infused. Physiologically acceptable excipients may include, for example, 5% dextrose, 0.9% saline, Ringer’s solution or other suitable excipients.

20 Intramuscular preparations, e.g., a sterile formulation of a suitable soluble salt form of the antibody, can be dissolved and administered in a pharmaceutical excipient such as Water-for- Injection, 0.9% saline, or 5% glucose solution.

In one embodiment, an anti-PKal antibody is administered via site-specific or targeted 25 local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the anti-PKal antibody or local delivery catheters, such as infusion catheters, an indwelling catheter, or a needle catheter, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct application. See, e.g., PCT Publication No. WO 00/53211 and U.S. Pat. No. 5,981,568.

30 Targeted delivery of therapeutic compositions containing an antisense polynucleotide,

expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol. (1993) 11:202; Chiou et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer (J. A. Wolff, ed.) (1994); Wu et al., J. Biol. Chem. (1988) 263:621; Wu et al., J. Biol. Chem. (1994) 269:542; Zenke et al., Proc. Natl. Acad. Sci. USA (1990) 87:3655; Wu et al., J. Biol. Chem. (1991) 266:338.

Therapeutic compositions containing a polynucleotide (e.g., those encoding the anti-PKal antibodies described herein) are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. In some embodiments, 10 concentration ranges of about 500 ng to about 50 mg, about 1 μ g to about 2 mg, about 5 μ g to about 500 μ g, and about 20 μ g to about 100 μ g of DNA or more can also be used during a gene therapy protocol.

The therapeutic polynucleotides and polypeptides described herein can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin 15 (see generally, Jolly, Cancer Gene Therapy (1994) 1:51; Kimura, Human Gene Therapy (1994) 5:845; Connelly, Human Gene Therapy (1995) 1:185; and Kaplitt, Nature Genetics (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters and/or enhancers. Expression of the coding sequence can be either constitutive or regulated.

20 Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Pat. Nos. 5,219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242), 25 alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). 30 Administration of DNA linked to killed adenovirus as described in Curiel, Hum. Gene Ther.

(1992) 3:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, Hum. Gene Ther. (1992) 3:147); ligand-linked DNA (see, e.g., Wu, J. Biol. Chem. (1989) 264:16985); eukaryotic cell delivery vehicles cells (see, e.g., U.S. Pat. No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Pat. No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Pat. No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP Patent No. 0524968. Additional approaches are described in Philip, Mol. Cell. Biol. (1994) 14:2411, and in Woffendin, Proc. Natl. Acad. Sci. (1994) 91:1581.

The particular dosage regimen, i.e., dose, timing and repetition, used in the method described herein will depend on the particular subject and that subject's medical history.

In some embodiments, more than one anti-PKal antibodies, or a combination of an anti-PKal antibody and another suitable therapeutic agent, may be administered to a subject in need of the treatment. The antagonist can be the same type or different from each other. The anti-PKal antibody can also be used in conjunction with other agents that serve to enhance and/or complement the effectiveness of the agents.

Treatment efficacy for a disease/disorder associated with PKal can be assessed by methods well-known in the art.

Kits For Use in Alleviating Diseases/Disorders Associated with Plasma Kallikrein

The present disclosure also provides kits for use in alleviating diseases/disorders associated with plasma kallikrein. Such kits can include one or more containers comprising an anti-PKal antibody, e.g., any of those described herein.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the anti-PKal antibody to treat, delay the onset, or alleviate a target disease

as those described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In still other embodiments, the instructions comprise a description of administering an antibody to an individual at risk of the target disease.

5 The instructions relating to the use of an anti-PKal antibody generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-
10 readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

The label or package insert indicates that the composition is used for treating, delaying the onset and/or alleviating a disease or disorder associated with PKal. Instructions may be provided for practicing any of the methods described herein.

15 The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an
20 intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PKal antibody as those described herein.

25 Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the invention provides articles of manufacture comprising contents of the kits described above.

General Techniques

30 The practice of the present invention will employ, unless otherwise indicated,

conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press;

5 Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Methods in

10 Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular Biology (F. M. Ausubel, et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology

15 (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds.,

20 Harwood Academic Publishers, 1995).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

Example 1: Identification of Critical Residues in the Catalytic Domain of Human Plasma Kallikrein Based on Crystal Structures of DX-2930-PKal Complex

The catalytic domain of human plasma kallikrein (Figure 2), fused with a His-tag, was

30 expressed in insect cells and purified initially by a nickel affinity column. The His-tag was removed from the plasma kallikrein via trypsin digestion and the free plasma kallikrein was

purified by a benzamidine affinity column, followed by a SEC column. The purified product was examined on a PAGE gel. The result indicates that the catalytic domain of human plasma kallikrein was properly expressed and purified.

DX-2930 was prepared via routine recombinant technology and purified. A recombinant Fab fragment of DX-2930 was produced via routine method and purified.

The DX-2930 Fab fragment and the catalytic domain of human plasma kallikrein were mixed at various concentrations under suitable conditions allowing formation of antibody-PKal complexes. The complexes thus formed were examined using HPLC to determine the antibody-PKal ratio in the complexes. Accordingly, the suitable concentrations of both the antibody and the PKal were identified for formation of a 1:1 complex.

The antibody-PKal complex was kept under various conditions allowing for crystallization. Diffraction analysis was performed on the crystallized complex. The crystal structures (2.1 Å and 2.4 Å) were determined based on the diffraction statistics.

According to the crystal structures, residues in the catalytic domain of human Pkal that are involved in the interaction with DX-2930 were identified. These residues are indicated (boldfaced and underlined) in Figure 2, which provides the amino acid sequence of the catalytic domain of human PKal (residues 391-638 of human PKal).

In addition, residues in DX-2930 that interact with PKal were also identified based on the crystal structure, including E1, V2, F27, T28, F29, S30, H31, R100, I101, G102, V103, P104, R105, R106, D107, G107, K108, and D111 in the heavy chain variable region, and S31, W32, Y49, K50, T53, L54, E55, S56, G57, and V58 in the light chain variable region.

These results indicate that HC CDR3 of DX-2930 is the main region that interacts with PKal (see Figure 1) and a couple of residues in the HC CDR1 and FR1 might also contribute to the interaction with PKal. In the light chain, the LC CDR2 region was found to contribute to the interaction.

Further, the results also indicate that variations at certain positions with the HC CDR3 region may be allowed. For example, position 103 requires small hydrophobic residues such as V or I. As another example, R106 may be replaced with W, and E108 may be replaced with S or D without substantially affecting the PKal binding activity. Similarly, D110 might be replaced with E.

Example 2: Affinity Maturation Results Match Structural Information Derived from Crystal Structure

The heavy chain variable region, particularly the HC CDR3 region, of antibody M0162-A04 was subject to affinity maturation. Various mutants having amino acid variations at one or more positions in the HC CDR3 region were generated and their $K_{i,app}$ values were determined following routine methods.

Briefly, PKal and a Fab at various concentrations are incubated together for 1 hour at 30 °C. A substrate peptide (cleavable by PKal) is then added to this PKal-Fab mixture. The rate of substrate peptide cleavage/proteolysis is then measured, and plotted against the concentrations of the Fab. This plot is then fit to the Morrison equation, which calculates the $K_{i,app}$ value. The results thus obtained are shown in Figure 3 and Table 2 below:

Table 2. Summary of Hv-CDR3 Affinity Maturation Results

Initial Name	Hv CDR3	$K_{i,app}$ (nM)	SEQ ID NO
M0162-A04	RTGIPRRDAFDI	2.5	1
M0199-A11	--R-----	2	2
M0201-F11	--S-----	3	3
M0202-A08	-----W----	2.8	4
M0201-A06	-----V--	3.8	5
M0202-E03	-----E-	2	6
M0199-B01	-----N	1.6	7
M0200-B01	-----S	3.6	8
M0201-H06	----V-----	0.6	9
M0202-H05	----V----V--	0.26	10
M0201-H08	----V----L-N	0.8	11
M0200-E11	----V-----N	0.4	12
M0200-H07	----V---N---N	0.4	13
M0202-F06	----V--W----	0.33	14
M0200-A10	----V----S---	0.25	15
M0202-G03	----V----S-E-	0.4	16
M0202-A12	Q---V----S-N-	0.1	17
M0202-H03	----V--W-D---	0.1	18
M0201-A07	----V----E--	0.1	19
M0202-C02	--P-V-----	0.6	20
M0202-B04	--S-V-----	0.2	21
M0202-E06	--R-V----D--	0.06	22
M0202-A01	--I-V-----	0.3	23
M0202-D09	--I-V----S--	0.2	24
M0200-D03	--I-V----S--M	0.1	25
M0202-C09	--I-V----D--	0.06	26
M0199-A08	--I-V----E--	0.06	27

X133-B02	--I-----	2.2	28
X133-D06	--I-----E--	0.33	29
X135-A01	----A-----	247.7	30
X133-G05	----S-----	1405.6	31
X133-F10	----L-----	14.7	32
X135-A03	-----E---	1.1	33

The affinity maturation results indicate that variations at certain positions within the HC CDR3 region result in high affinity/inhibitory anti-PKal antibodies as compared to the parent M0162-A04 clone. These results match with the structural information provided in 5 Example 1 above. Note that the HC CDR3 region of clone M0199-A08 is identical to that of DX-2930.

Example 3: Impact of Mutations in Plasma Kallikrein on Antibody Inhibitory Activity

The inhibitory activities of mutant X115-F02 against various PKal mutants were examined.

10 X115-F02 is an IgG that is the same as DX-2930 except that it contains a C-terminal lysine residue not present in DX-2930 and was expressed in HEK293T cells rather than CHO cells (Table 1 above). The binding specificity and affinity of X115-F02 is the same as DX-2930.

15 The wild type and four mutants of plasma kallikrein used in this study (Figure 5) are recombinant catalytic domains expressed and purified from *pichia pastoris*. Mutant 1 contains the following mutations in the S3 subsite of the active site: S478A, N481A, S506A, Y507A (numbers based on the full length prekallikrein amino acid sequence). Mutant 2 contains the following mutations in the S1' subsite of the active site: R551A, Q553A, Y555A, T558A, R560A. Mutant 4 contains the following mutations that are distal from the 20 active site: N396A, S398A, W399A. Mutant 3 was found to be inactive and therefore was not tested in the activity assay. Mutant 3 contains the following mutations in the S1' subsite of the active site: D572A, K575A, D577A.

25 The inhibitory activity of X115-F02 against the wild-type PKal and the mutants were carried out using the method described in Example 2 above and the $K_{i,app}$ values were determined. As shown in Figure 4, the mutations in Mutant 1 and 4 did not significantly affect the potency of X115-F02 inhibition of plasma kallikrein. Surprisingly, the mutations

in Mutant 2 reduced the potency approximately 65-fold. These results indicate that residues R551A, Q553A, Y555A, T558A, R560A and their adjacent residues might be important to the inhibitory activity of X115-F02 (DX-2930).

5

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or 10 similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

15

What Is Claimed Is:

1. An isolated antibody that binds human plasma kallikrein (PKal), wherein the antibody interacts with one or more of amino acid residues in the human PKal and inhibits its activity by at least 50%, wherein the amino acid residues are selected from the group consisting of V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, G438, L439, W445, Y475, K476, V477, S478, E479, G480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, G576, S578, T596, S597, W598, G599, E600, G601, C602, A603, R604, Q607, P608, G609, V610, and Y611.

10

2. The isolated antibody of claim 1, wherein the antibody binds an epitope of the PKal, the epitope comprising the segment selected from the group consisting of:

- (i) V410-C419,
- (ii) H434-L439,
- 15 (iii) Y475-G480,
- (iv) F524-K528,
- (v) Y552-Y555,
- (vi) D572-S578,
- (vii) T596-R604, and
- 20 (viii) Q607-Y611.

3. The isolated antibody of claim 1 or claim 2, wherein the antibody inhibits the activity of PKal by at least 80%.

25

4. The isolated antibody of any of claims 1-3, wherein the antibody has an apparent K_i ($K_{i,app}$) lower than about 1 nM.

5. The isolated antibody of claim 4, wherein the antibody has a $K_{i,app}$ lower than about 0.1 nM.

30

6. The isolated antibody of claim 4, wherein the antibody has a $K_{i,app}$ lower than about 0.05 nM.

7. The isolated antibody of any of claims 1-6, wherein the antibody has a binding affinity (K_D) for the PKal of less than 10^{-6} M.

8. The isolated antibody of any of claims 1-7, wherein the antibody preferentially binds the PKal as relative to a mutant of the PKal that contains one or more mutations at positions R551, Q553, Y555, T558, and R560.

10

9. An isolated antibody that binds human plasma kallikrein, wherein the antibody comprises a heavy chain variable region that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3), and wherein the HC CDR3 comprises the motif $X_{99}R_{100}X_{101}G_{102}X_{103}P_{104}R_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}$, in which:

X_{99} is R or Q,

X_{101} is T, I, R, S, or P,

X_{103} is V, I, or L,

X_{106} is R or W,

20

X_{107} is D or N,

X_{108} is A, S, D, E, or V,

X_{109} is F or L,

X_{110} is D, E, or N, and

X_{111} is I, N, M, or S.

25

10. The isolated antibody of claim 9, wherein the antibody inhibits the activity of PKal by at least 80%.

11. The isolated antibody of claim 9 or claim 10, wherein the antibody has a $K_{i,app}$ lower than about 1 nM.

12. The isolated antibody of claim 11, wherein the antibody has a $K_{i,app}$ lower than about 0.1 nM.

13. The isolated antibody of claim 12, wherein the antibody has a $K_{i,app}$ lower than 5 about 0.05 nM.

14. The isolated antibody of any of claims 9-13, wherein the antibody has a binding affinity (K_D) for the PKal of less than 10^{-6} M.

10 15. The isolated antibody of any of claims 9-13, wherein X_{99} is Q and X_{101} is I, R, S, or P.

16. The isolated antibody of any of claims 9-13, wherein X_{106} is W and X_{111} is N, M, or S.

15 17. The isolated antibody of any of claims 9-13, wherein X_{101} is I, X_{108} is E, and X_{103} is I or L.

18. The isolated antibody of any of claims 9-13, wherein X_{101} is I and X_{103} is I or 20 L.

19. The isolated antibody of any of claims 9-13, wherein X_{103} is I or L and X_{110} is D, E, or N.

25 20. The isolated antibody of any of claims 9-19, wherein the heavy chain variable region includes H_{31} in the HC CDR1.

21. The isolated antibody of any of claims 9-20, wherein the heavy chain variable region includes F_{27} , F_{29} , or both in the framework region 1 (FR1).

22. The isolated antibody of any of claims 9-20, further comprising a light chain variable region that comprises complementarity determining region 1 (LC CDR1), complementarity determining region 2 (LC CDR2), and complementarity determining region 3 (LC CDR3).

5

23. The isolated antibody of claim 22, wherein the LC CDR2 includes K₅₀, L₅₄, E₅₅, S₅₆, or a combination thereof.

24. The isolated antibody of claim 23, wherein the light chain variable region
10 further includes G₅₇ in the framework region 3 (FR3).

25. The isolated antibody of any of claims 22-24, wherein the light chain variable includes N₄₅ in the framework region 2 (FR2).

15 26. The isolated antibody of any of the preceding claims, wherein the antibody is a full-length antibody or an antigen-binding fragment thereof.

27. The isolated antibody of any of the preceding claims, wherein the antibody is a human antibody or a humanized antibody.

20

28. The isolated antibody of any of the preceding claims, wherein the antibody preferentially bind an active PKal.

25 29. A pharmaceutical composition comprising an antibody of any of the preceding claims and a pharmaceutically acceptable carrier.

30. A method for treating a disease associated with plasma kallikrein, comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 29.

30

31. The method of claim 30, wherein the subject is a human patient diagnosed with, suspected of having, or at risk for the disease.

Figure 1**Light V gene = VK1_L12 HK102/V1/L12a; J gene = JK1**

	FR1	CDR1	FR2	CDR2
559A-M0162-A04:	DIQMTQSPSTLSSAVGDRVTITC	RASQSISSWLA	WYQQKPGKAP N LIIY	K ASTLES
	DIQMTQSPSTLSSAVGDRVTITC	RASQSISSWLA	WYQQKPGKAP	LLIY AS+LES
Germline:	DIQMTQSPSTLSSAVGDRVTITC	RASQSISSWLA	WYQQKPGKAP K LIIY	D ASSLES
	FR3	CDR3	FR4	
559A-M0162-A04:	GVPSRSGSGSGTEFTLTISLQPDDEATYYC	QQYN T YWT	FGQGTVKEIK	(SEQ ID NO: 34)
	GVPSRFGSGSGSGTEFTLTISLQPDDEATYYC	QQYN+YWT	FGQGTVKEIK	(SEQ ID NO: 35)
Germline:	GVPSRSGSGSGSGTEFTLTISLQPDDEATYYC	QQYN S YWT	FGQGTVKEIK	(SEQ ID NO: 36)

Heavy V gene = VH3_3-23; J gene = JH3

	FR1	CDR1	FR2	CDR2
559A-M0162-A04:	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	HYIMM	WVRQAPGKGLEWVS	G I Y S S G G I T V YADSVKG
	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	Y M	WVRQAPGKGLEWVS	I SGG T YADSVKG
Germline:	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKGLEWVS	A I S G S G S T V YADSVKG
	FR3	CDR3	FR4	
559A-M0162-A04:	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA Y	RRTGIPRRDAFDI	WGQGTMVTVSS	(SEQ ID NO: 37)
	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA	AFDI	WGQGTMVTVSS	(SEQ ID NO: 38)
Germline:	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA K	<u>AFDI</u>	<u>WGQGTMVTVSS</u>	(SEQ ID NO: 39)

Figure 2

391	440	IVGGTNSSWG EWPWQVSSLQV <u>KLTAAQRHLCG</u> GSLIIGHQWVL TAA <u>HCFCDFGLP</u>
441	490	LQD <u>WTRIYSG</u> IILNLSDITRKD TPFSSQIKEII IHQNY <u>KVSEG</u> NH <u>DTALI</u> IRLQ
491	540	APLNNTYEFQK PISLPSKGDT STIYTNCWVT GW <u>GFSKEKGE</u> IQNILQKVNI
541	590	PLVTNEECQK R <u>YQDY</u> KITQR MVC <u>AGYKEGG</u> K <u>DACKGD</u> S <u>GG</u> PLVCKHNGMMW
591	638	RLVGI <u>TSWGE</u> <u>GCARREOPGV</u> YTKVAEYMDW IIEKTQSSDG KAQM QSPA

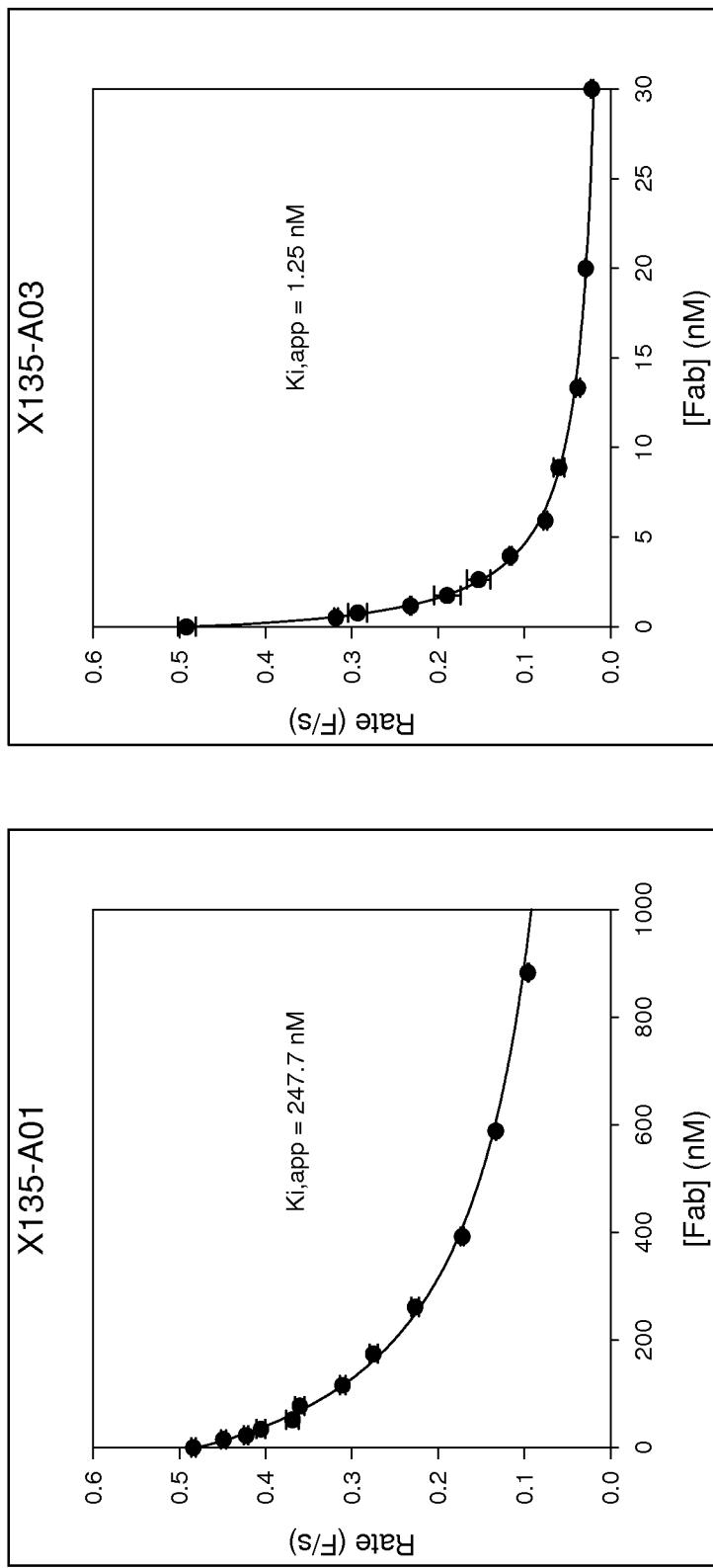
Figure 3

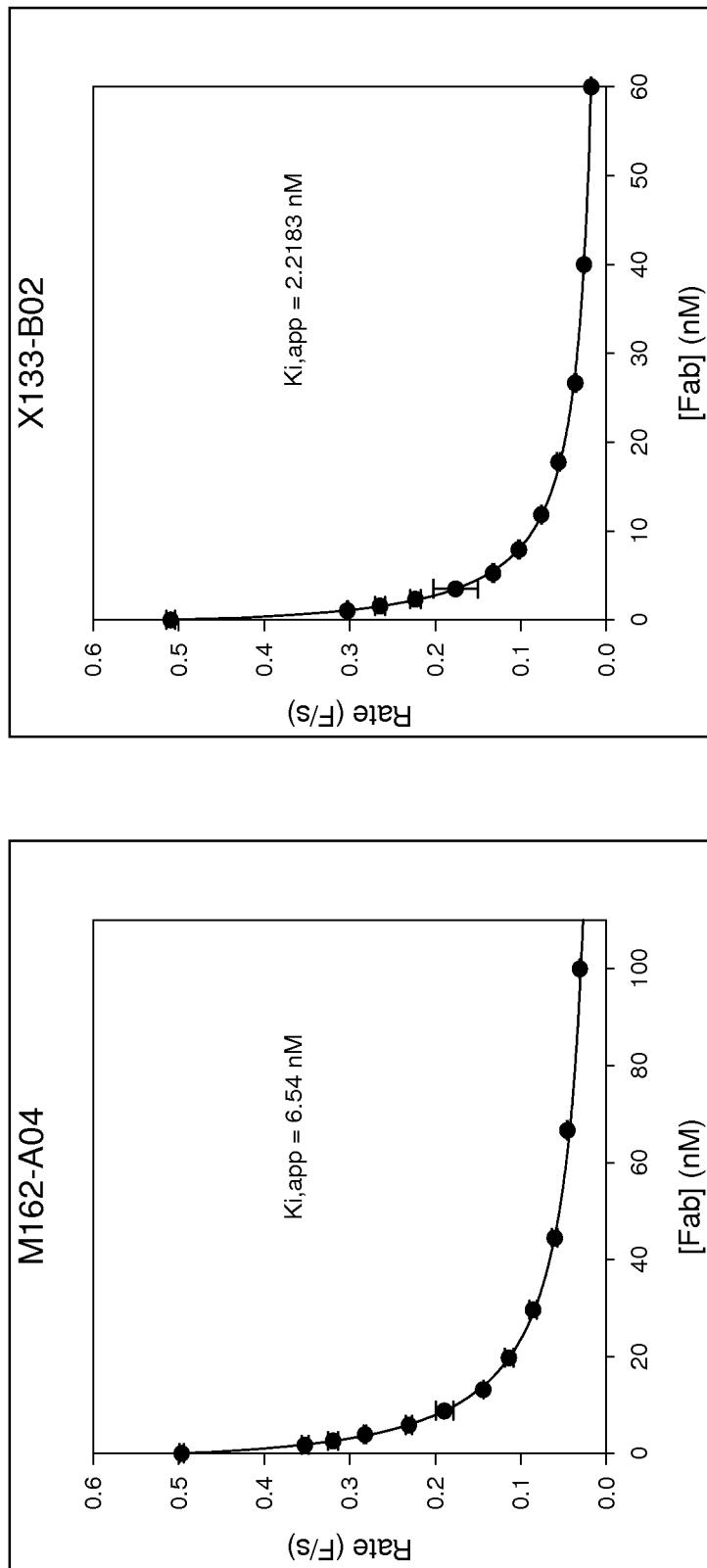
Figure 3 (Cont'd)

Figure 3 (Cont'd)

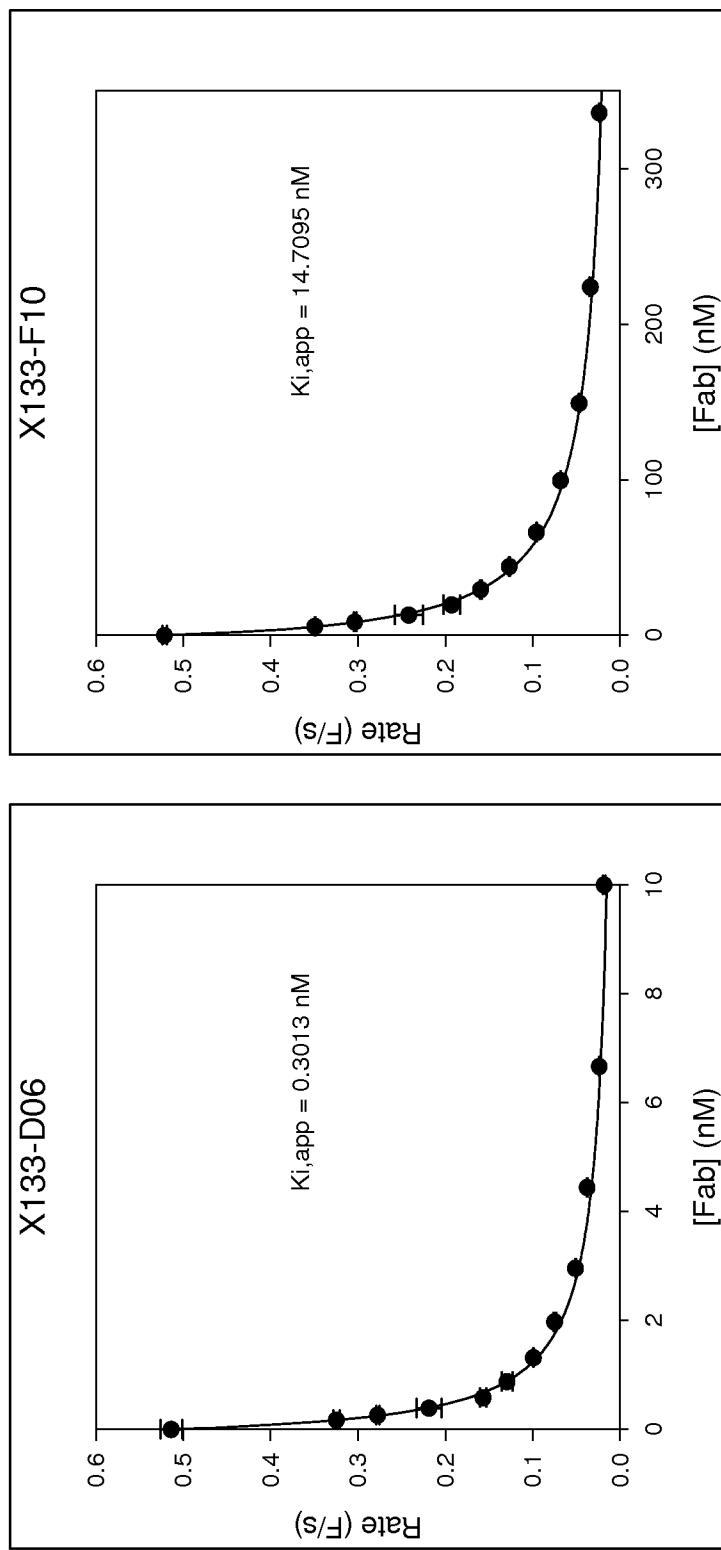


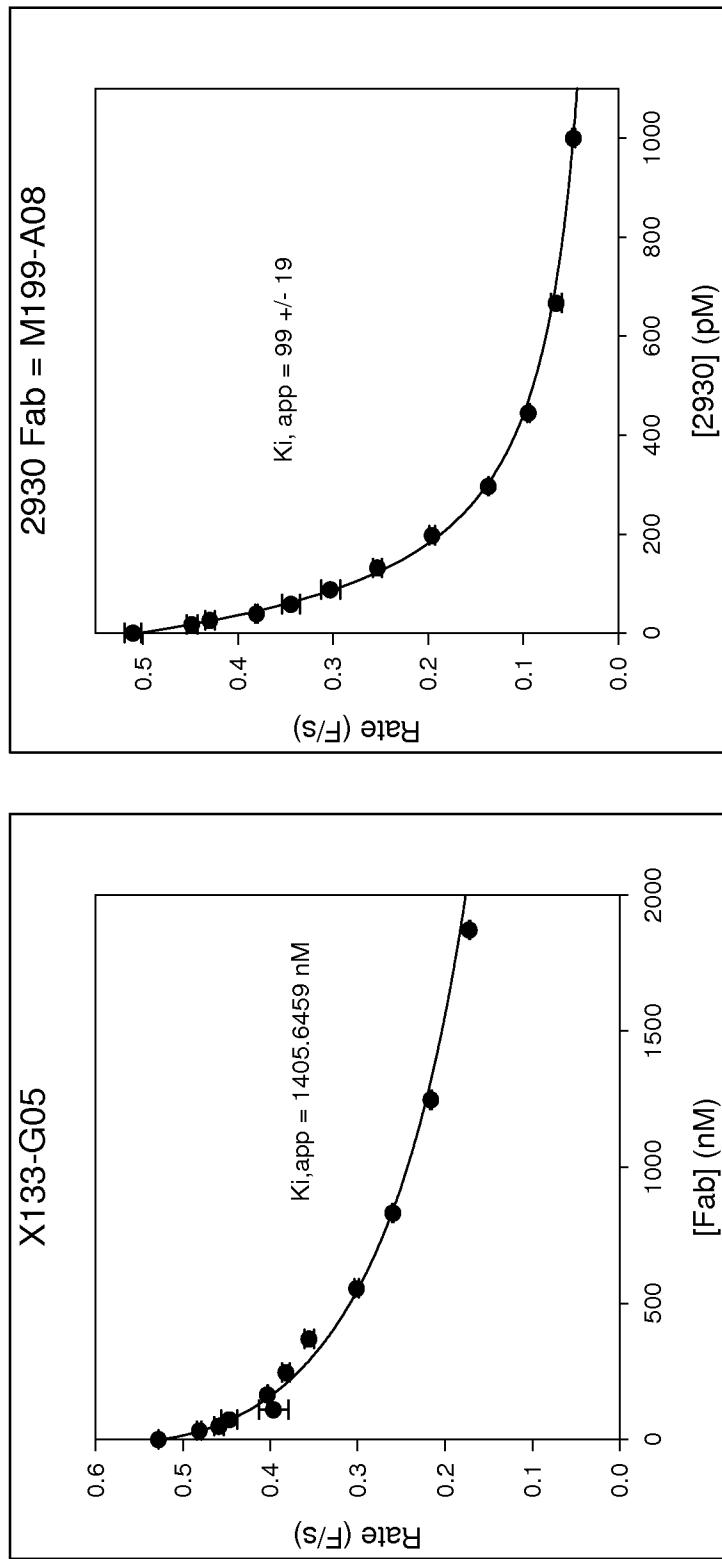
Figure 3 (Cont'd)

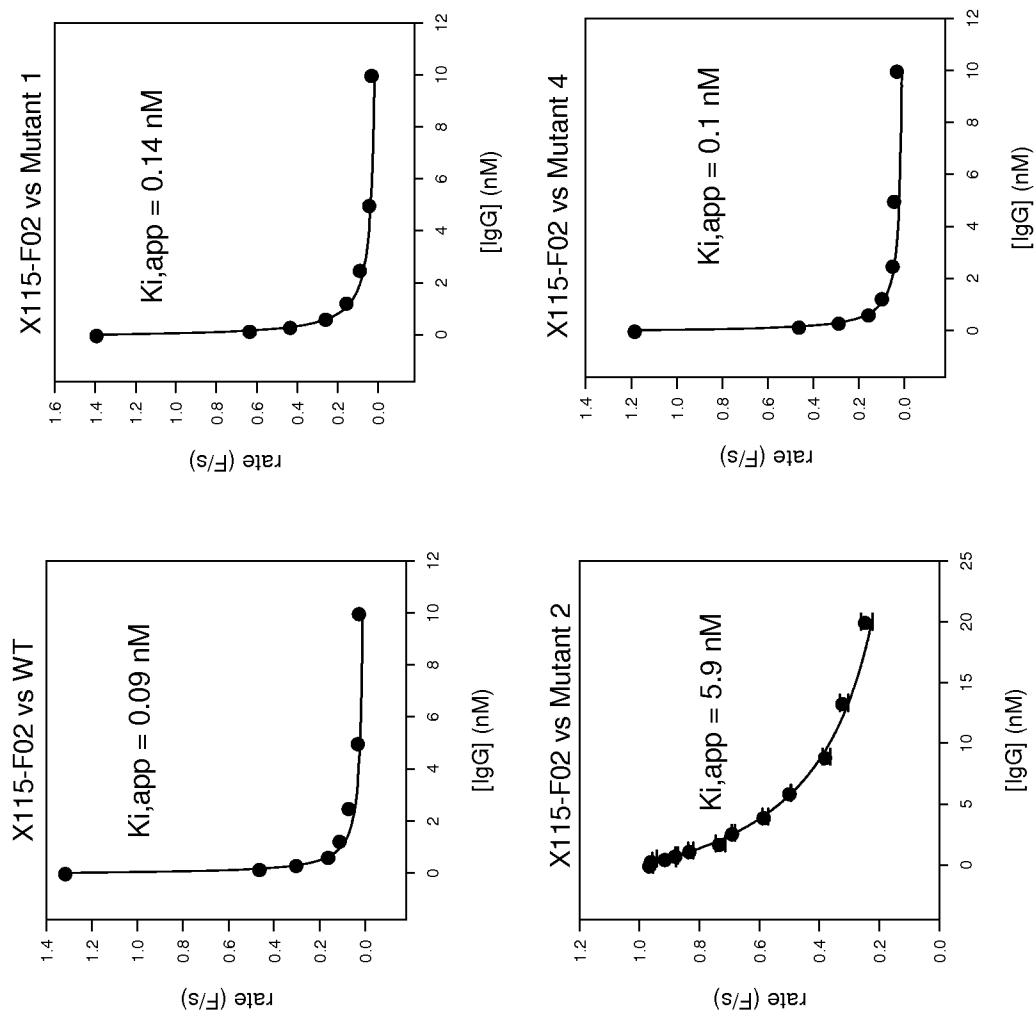
Figure 4

Figure 5

391 | IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 (k1kb1) -Mut1-forPichia IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 (k1kb1) -Mut2-forPichia IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 (k1kb1) -Mut3-forPichia IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 (k1kb1) -Mut4-forPichia IVGGTASSAG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 (k1kb1) -parentforPichia IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP

441 | LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKV**A**EG **A**HDIALIKLQ
 (k1kb1) -Mut1-forPichia LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKVSEG NHDIALIKLQ
 (k1kb1) -Mut2-forPichia LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKVSEG NHDIALIKLQ
 (k1kb1) -Mut3-forPichia LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKVSEG NHDIALIKLQ
 (k1kb1) -Mut4-forPichia LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKVSEG NHDIALIKLQ
 (k1kb1) -parentforPichia LQDVWRIYSG IINLSDITKD TPFSQIKEII IHQNYKVSEG NHDIALIKLQ

490 | 491 |
 (k1kb1) -Mut1-forPichia APLNYTEFQK PISLPA**A**GGDT STIYTNCWVT GMGF SKEKGE IONILQKVNI
 (k1kb1) -Mut2-forPichia APLNYTEFQK PISLPSKGDT STIYTNCWVT GMGF SKEKGE IONILQKVNI
 (k1kb1) -Mut3-forPichia APLNYTEFQK PISLPSKGDT STIYTNCWVT GMGF SKEKGE IONILQKVNI
 (k1kb1) -Mut4-forPichia APLNYTEFQK PISLPSKGDT STIYTNCWVT GMGF SKEKGE IONILQKVNI
 (k1kb1) -parentforPichia APLNYTEFQK PISLPSKGDT STIYTNCWVT GMGF SKEKGE IONILQKVNI

541 |
 (k1kb1) -Mut1-forPichia PLVTNEECQK RYQDYKITQR MVCAGYKEGG KDAACKGDGG PLVCKHNGMW
 (k1kb1) -Mut2-forPichia PLVTNEECQK **A**Y**A**DAK**I****A****Q**A MVCAGYKEGG KDAACKGDGG PLVCKHNGMW
 (k1kb1) -Mut3-forPichia PLVTNEECQK RYQDYKITQR MVCAGYKEGG **K****A****A****C****A****S****G** MVCAGYKEGG KDAACKGDGG PLVCKHNGMW
 (k1kb1) -Mut4-forPichia PLVTNEECQK RYQDYKITQR MVCAGYKEGG KDAACKGDGG PLVCKHNGMW
 (k1kb1) -parentforPichia PLVTNEECQK RYQDYKITQR MVCAGYKEGG KDAACKGDGG PLVCKHNGMW

590 |

Figure 5 (Cont'd)

591 | RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 41)
 (k1kb1) -Mut 1 -forPichia RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 42)
 (k1kb1) -Mut 2 -forPichia RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 43)
 (k1kb1) -Mut 3 -forPichia RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 43)
 (k1kb1) -Mut 4 -forPichia RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 44)
 (k1kb1) -parent forPichia RLVGITSWG E GCARREQPGV YTKVAEYMDW IIEKTQSSDG KAQMOSPA (SEQ ID NO: 45)

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2014/152232 A3

(43) International Publication Date
25 September 2014 (25.09.2014)

(51) International Patent Classification:
C07K 16/40 (2006.01)

(21) International Application Number:
PCT/US2014/027100

(22) International Filing Date:
14 March 2014 (14.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/791,822 15 March 2013 (15.03.2013) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

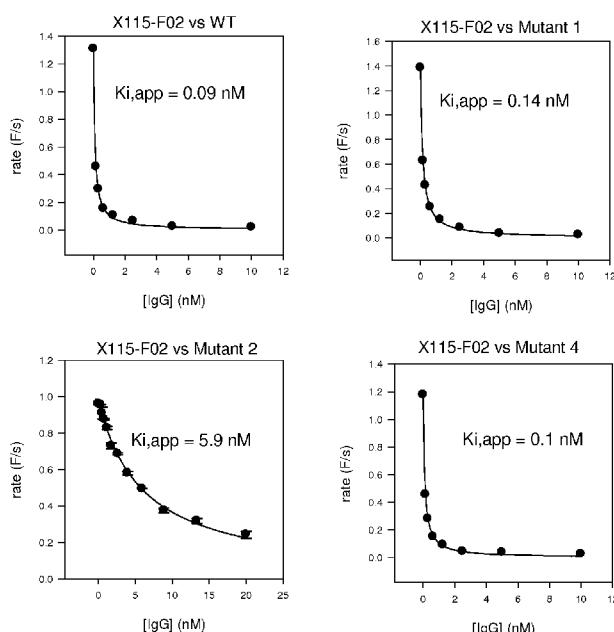
Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: ANTI-PLASMA KALLIKREIN ANTIBODIES

Figure 4



(57) Abstract: Disclosed herein are antibodies capable of binding to plasma kallikrein and inhibit its activity. Such antibodies interact with one or more critical residues in the catalytic domain of the plasma kallikrein. The antibodies may also contain specific heavy chain complementarity determining region 3 (CDRs) motifs and optionally specific residues at certain positions within both the heavy chain variable region and the light chain variable region.



- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:

13 November 2014

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/027100

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/40 (2014.01)

USPC - 424/9.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 38/00, 38/48, 39/395; C07K 16/40; C12N 9/64, 15/09 (2014.01)

USPC - 424/9.1, 158.1; 435/320.1; 514/2, 12; 530/389.3; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 38/00, C07K 16/40, 2317/92; C12N 9/6445 (2014.06)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0201756 A1 (SEXTON) 09 August 2012 (09.08.2012) entire document	1, 3, 9-13
A	US 2011/0200611 A1 (SEXTON) 18 August 2011 (18.08.2011) entire document	1, 3, 9-13
A	US 2009/0105142 A1 (MOSCICKI) 23 April 2009 (23.04.2009) entire document	1, 3, 9-13
A	TANG et al. 'Expression, crystallization, and three-dimensional structure of the catalytic domain of human plasma kallikrein.' J. Biol. Chem. 30 September 2005 (30.09.2005), Vol. 280, Pgs. 41077-41089. entire document	1, 3, 9-13

 Further documents are listed in the continuation of Box C.

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

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2014	10 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/027100

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
 on paper
 in electronic form
 - b. (time)
 in the international application as filed
 together with the international application in electronic form
 subsequently to this Authority for the purposes of search
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NO:46 was searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/027100

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 4-8, 14-31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet(s)

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 3, and 9-13 restricted to V410 of human PKa1 and amino acids residues 99 to 111 of SEQ ID NO:46.

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/027100

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3 and 9-13 are drawn to an isolated antibody that binds human plasma kallikrein (PKa1).

The first invention of Group I+ is restricted to an isolated antibody that binds human plasma kallikrein (PKa1), wherein the antibody interacts with one or more of amino acid residues in the human PKa1 and inhibits its activity by at least 50%, wherein the one or more amino acid residues are selected be V410; wherein the antibody comprises a heavy chain variable region that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3), and wherein the HC CDR3 comprises a motif, wherein the motif is selected to be amino acids residues 99 to 111 of SEQ ID NO:46. It is believed that claims 1, 3, and 9-13 read on this first named invention and thus these claims will be searched without fee to the extent that they read on V410 of human PKa1 and amino acids residues 99 to 111 of SEQ ID NO:46.

Applicant is invited to elect additional amino acid residues in the human PKa1 and/or HC CDR3 motifs with specified SEQ ID NO to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be an isolated antibody that binds human plasma kallikrein (PKa1), wherein the antibody interacts with one or more of amino acid residues in the human PKa1 and inhibits its activity by at least 50%, wherein the one or more amino acid residues are selected be L412; wherein the antibody comprises a heavy chain variable region that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3), and wherein the HC CDR3 comprises a motif, wherein the motif is selected to be amino acids residues 99 to 111 of SEQ ID NO:46. Additional amino acid residues in the human PKa1 and/or HC CDR3 motifs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element, requiring the selection of alternatives for the amino acid residues in human PKa1 "wherein the amino acid residues are selected from the group consisting of V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, G438, L439, W445, Y475, K476, V477, S478, E479, G480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, 0576, S578, T596, S597, W598, 0599, E600, 0601, C602, A603, R604, Q607, P608, 0609, V610, and Y611" and HC CDR3 motif "wherein the HC CDR3 comprises the motif X99-R100-X101-G102-X103-P104-R105-X106-X107-X108-X109-X110-X111, in which: X99 is R or Q, X101 is T, I, R, S, or P, X103 is V, I, or L, X106 is R or W, X107 is D or N, X108 is A, S, D, E, or V, X109 is F or L, X110 is D, E, or N, and X111 is I, N, M, or S".

The Groups I+ share the technical features of an isolated antibody that binds human plasma kallikrein (PKa1), wherein the antibody interacts with one or more of amino acid residues in the human PKa1 and inhibits its activity by at least 50%, wherein the amino acid residues are selected from the group consisting of V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, 0438, L439, W445, Y475, K476, V477, S478, E479, 0480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, 0576, S578, T596, S597, W598, 0599, E600, 0601, C602, A603, R604, Q607, P608, 0609, V610, and Y611; wherein the antibody comprises a heavy chain variable region that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3), and wherein the HC CDR3 comprises the motif X99-R100-X101-G102-X103-P104-R105-X106-X107-X108-X109-X110-X111, in which: X99 is R or Q, X101 is T, I, R, S, or P, X103 is V, I, or L, X106 is R or W, X107 is D or N, X108 is A, S, D, E, or V, X109 is F or L, X110 is D, E, or N, and X111 is I, N, M, or S. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 2012/0201756 A1 to Sexton discloses an isolated antibody that binds human plasma kallikrein (PKa1) (the disclosure features an isolated protein, e.g., antibody, e.g., human antibody, that binds to the active form of plasma kallikrein, e.g., human plasma kallikrein, Para. [0005]), wherein the antibody interacts with one or more of amino acid residues in the human PKa1 (the protein [antibody] binds to, e.g., positions on plasma kallikrein, Para. [0624]) and inhibits its activity by at least 50% (antibodies that inhibited pKa1 activity by over 90%, Para. [0945]), wherein the amino acid residues are selected from the group consisting of V410, L412, T413, A414, Q415, R416, L418, C419, H434, C435, F436, D437, 0438, L439, W445, Y475, K476, V477, S478, E479, 0480, D483, F524, E527, K528, Y552, D554, Y555, A564, D572, A573, C574, K575, 0576, S578, T596, S597, W598, 0599, E600, 0601, C602, A603, R604, Q607, P608, 0609, V610, and Y611 (the protein binds one or more amino acids of: Arg551, Gln553, Tyr555, Thr558, and/or Arg560 (numbering based on the human sequence)). In some embodiments, the plasma kallikrein binding protein binds one or more amino acids of: S478, N481, S525, and K526 (numbering based on the human kallikrein sequence), Para. [0627]); wherein the antibody comprises a heavy chain variable region (plasma kallikrein binding antibodies may have their HC and LC variable domain sequences, Para. [0635]) that comprises complementarity determining region 1 (HC CDR1), complementarity determining region 2 (HC CDR2), and complementarity determining region 3 (HC CDR3) (the protein is an antibody, e.g., a human antibody, having one or more, e.g., 1, 2, or 3 heavy chain CDRs, Para. [0642]), and wherein the HC CDR3 comprises the motif X99-R100-X101-G102-X103-P104-R105-X106-X107-X108-X109-X110-X111, in which: X99 is R or Q, X101 is T, I, R, S, or P, X103 is V, I, or L, X106 is R or W, X107 is D or N, X108 is A, S, D, E, or V, X109 is F or L, X110 is D, E, or N, and X111 is I, N, M, or S (antibody "M199-A08", HV-CDR3, RRIGVPRRDEFDI, Table 8, Pg. 94).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

摘要

本文公開了能夠結合血漿激肽釋放酶並抑制其活性的抗體。這樣的抗體與在血漿激肽釋放酶的催化結構域中的一個或多個關鍵殘基相互作用。所述抗體還可包含特定的重鏈互補決定區 3(CDRs)基序和可選擇地包含在重鏈可變區和輕鏈可變區內的某些位置的特定殘基。