

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
17 September 2020 (17.09.2020)(10) International Publication Number
WO 2020/186132 A8

(51) International Patent Classification:

C07K 16/40 (2006.01) A61P 9/00 (2006.01)
A61P 7/10 (2006.01)

(21) International Application Number:

PCT/US2020/022535

(22) International Filing Date:

13 March 2020 (13.03.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

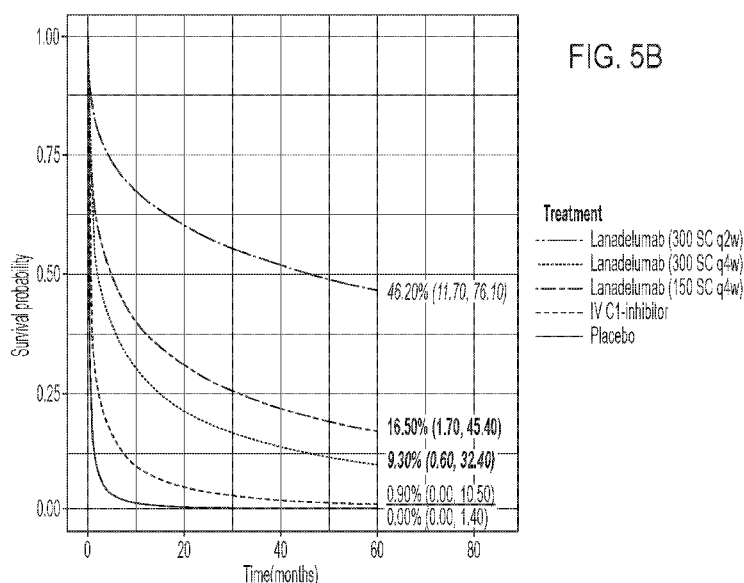
62/818,189 14 March 2019 (14.03.2019) US

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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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: PLASMA KALLIKREIN INHIBITORS AND USES THEREOF FOR TREATING HEREDITARY ANGIOEDEMA ATTACK



(57) Abstract: Provided herein are methods of treating and preventing hereditary angioedema attack in certain human patient subpopulations whose disease is well controlled in a first treatment period, which involves the use of an antibody that binds human plasma kallikrein. Such patients can be subject to a reduced dosage of the antibody and/or a prolonged dosing interval in a second treatment period.



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))*
- *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:

15 October 2020 (15.10.2020)

(15) Information about Correction:

see Notice of 15 October 2020 (15.10.2020)

PLASMA KALLIKREIN INHIBITORS AND USES THEREOF FOR TREATING HEREDITARY ANGIOEDEMA ATTACK

BACKGROUND

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, 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 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).

SUMMARY

Provided herein are regimens for treating hereditary angioedema (HAE) attack, reducing the rate of HAE attack, or blocking HAE attack using antibodies capable of binding and inhibiting human plasma kallikrein (pKal) in the active form, for example, antibodies having the same complementarity determining regions (CDRs) as DX-2930 (*a.k.a.* SHP643, lanadelumab).

In aspect, the present disclosure provides a method for treating hereditary angioedema (HAE) attack or reducing the rate of HAE attack, the method comprising: (i) administering (*e.g.*, subcutaneously) to a subject in need thereof an antibody that binds human plasma kallikrein at about 300 mg every about two weeks in a first treatment period, which is about 4-9 months (*e.g.*, 6 months); (ii) monitoring the subject for HAE attack during the first treatment period; and (iii) reducing the dosage of the antibody to about 300 mg every about 4 weeks in the subject, who is free of HAE attack in the first treatment period.

In some instances, the antibody used in the method described herein comprises the same heavy chain complementarity-determining regions (CDRs) and the same light chain CDRs as DX-2930. For example, the antibody may comprise a heavy chain immunoglobulin

variable domain (V_H) of SEQ ID NO: 3 and a light chain immunoglobulin variable domain (V_L) of SEQ ID NO: 4. Such an antibody may be a full-length antibody (*e.g.*, an IgG1 molecule). Alternatively, the antibody may be an antigen-binding fragment thereof. In one example, the antibody may comprise a heavy chain of SEQ ID NO:1 and a light chain of SEQ ID NO:2.

Any of the antibodies described herein may be formulated in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition may comprise sodium phosphate, citric acid, histidine, sodium chloride, and polysorbate 80. In some examples, the pharmaceutical composition comprises sodium phosphate at a concentration of about 30 mM, citric acid at a concentration of about 19 mM, histidine at a concentration of about 50 mM, sodium chloride is at a concentration of about 90 mM, and polysorbate 80 at about 0.01%.

The subject to be treated in any of the methods described herein may be a human patient having a low body weight, for example, less than 35 kg. In some instances, the human patient is a pediatric patient. The subject may be a human patient having, suspected of having, or at risk for HAE (*e.g.*, Type I or Type II).

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

BRIEF DESCRIPTION OF THE DRAWINGS:

Fig. 1 is a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram documenting the systemic literature review used to identify studies for an indirect treatment comparison. An asterisk (*) indicates studies describing use of interventions that lacked real-world applicability, which were excluded as inappropriate comparators and included attenuated androgens (danazol, stanozolol, methyltestosterone, fluoxymesterone, oxymetholone, and tibolone; n=17), combination therapy of SC C1-INH/recombinant human hyaluronidase (n=1), and SC C1-INH (n=2). A dagger (†) indicates inappropriate studies excluded because of differences and/or lack of clarity in study design, study population, intervention, endpoints, and/or patient characteristics, as summarized in Table 2. A double-dagger (‡) indicates the exclusion of four unnecessary non-randomized control trials, as one randomized control trial for IV C1-INH (Cinryze®) was identified as appropriate for NMA. The four nRCTs for this same intervention were excluded. C1-INH,

C1 esterase inhibitor; IV, intravenous; NMA, network meta-analysis; nRCT, non-randomized controlled trial; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; SC, subcutaneous.

Fig. 2 is a final network diagram for an indirect treatment comparison of lanadelumab (HELP Study; Banerji et al., *JAMA*. 320(20):2108-21, 2018.) and IV C1-INH (CHANGE Study; Zuraw et al., *N Engl J Med*. 363(6):513-22, 2010). The CHANGE study is a crossover study. C1-INH, C1 esterase inhibitor; IV, intravenous; q2w, every two weeks; q4w, every four weeks; SC, subcutaneous.

Fig. 3 includes attack rate ratios (95% credible interval, CrI) for all treatments compared to placebo. The rate ratios for lanadelumab vs. placebo were derived from HELP study data and are based on 26 weeks of treatment. The rate ratios for IV C1-INH vs. placebo are derived from CHANGE study data and are based on 12 weeks of treatment. C1-INH, C1 esterase inhibitor; CrI, credible interval; IV, intravenous; q2w, every two weeks; q4w, every four weeks; SC, subcutaneous.

Figs. 4A-4B include time to first attack hazard ratios (95% credible interval, CrI) for all treatments compared to placebo after day 0 (Fig. 4A) and after day 70 (Fig. 4B). The data for the IV C1-INH are from the CHANGE trial. C1-INH, C1 esterase inhibitor; CrI, credible interval; IV, intravenous; q2w, every two weeks; q4w, every four weeks; SC, subcutaneous.

Figs. 5A-5B include predicted survival curves for time to first attack after day 0 (Fig. 5A) and after day 70 (Fig. 5B). The data are labeled with predicted median (95% confidence interval) percentage of patients who were attack-free at 60 months. The data for the IV C1-INH are from the CHANGE trial. The data in Fig. 5A are as follows: lanadelumab (300 SC q2w), 26.00%, lanadelumab (300 SC q4w), 13.70%, lanadelumab (150 SC q4w), 17.60%, IV C1-inhibitor, 6.50%, and placebo, 0.60%. The data in Fig. 5B are as follows: lanadelumab (300 SC q2w), 46.20%, lanadelumab (300 SC q4w), 9.30%, lanadelumab (150 SC q4w), 16.50%, IV C1-inhibitor, 0.90%, and placebo, 0.00%. C1-INH, C1 esterase inhibitor; IV, intravenous; q2w, every two weeks; q4w, every four weeks; SC, subcutaneous.

Fig. 6 is a network of evidence for the comparators in the HELP (HELP-03) and CHANGE studies. Cinryze® is a C1-INH, C1 esterase inhibitor.

Fig. 7 is a predicted survival graph comparing time to first attack after 0 days of treatment with placebo in the HELP (HELP-03) and CHANGE studies.

Fig. 8 is a predicted survival graph comparing time to first attack after 70 days of treatment with placebo in the HELP (HELP-03) and CHANGE studies.

Fig. 9 is a predicted survival graph comparing time to first attack after 0 days of treatment with placebo or Cinryze® (C1-INH) in the CHANGE study.

Fig. 10 is a predicted survival graph comparing time to first attack after 0 days of treatment with placebo, 300 mg lanadelumab every 2 weeks (300 mg q2w), or 300 mg lanadelumab every 4 weeks (300 mg q4w) in the HELP (HELP-03) study.

Fig. 11 is a predicted survival graph comparing time to first attack after 70 days of treatment with placebo or Cinryze® (C1-INH) in the CHANGE study.

Fig. 12 is a predicted survival graph comparing time to first attack after 70 days of treatment with placebo, 300 mg lanadelumab every 2 weeks (300 mg q2w), or 300 mg lanadelumab every 4 weeks (300 mg q4w) in the HELP (HELP-03) study.

DETAILED DESCRIPTION

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are defined here. Other terms are defined as they appear in the specification.

The singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise.

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, “about” can mean within an acceptable standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to $\pm 20\%$, preferably up to $\pm 10\%$, more preferably up to $\pm 5\%$, and more preferably still up to $\pm 1\%$ of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” is implicit and in this context means within an acceptable error range for the particular value.

The term “antibody” refers to 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. An antibody may include at least one heavy (H) chain that comprises a heavy chain immunoglobulin variable domain (V_H), at least one light chain that comprises a light

chain immunoglobulin variable domain (V_L), or both. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as V_H or HV) and a light (L) chain variable region (abbreviated herein as V_L or LV). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions.

As used herein, the term “antibody” encompasses not only 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), domain antibody (dAb) fragments (de Wildt *et al.*, *Euro. J. Immunol.* (1996) 26(3): 629-639), any 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 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 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 alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Antibodies may be from any source, but primate (human and non-human primate) and primatized are preferred.

The V_H and/or V_L regions may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may omit one, two or more N- or C-terminal amino acids, internal amino acids, may include one or more insertions or additional terminal amino acids, or may include other alterations. In one embodiment, a polypeptide that includes immunoglobulin variable domain sequence can associate with another immunoglobulin variable domain sequence to form an antigen binding site, *e.g.*, a structure that preferentially interacts with plasma kallikrein.

The V_H and V_L regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (“CDRs”), interspersed with regions that are more conserved, termed “framework regions” (“FRs”). The extent of the framework region and CDRs have been defined (see, Kabat, E.A., et al. (1991) *Sequences of Proteins of*

Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

In addition to the V_H or V_L regions, the heavy chain or light chain of the antibody can further include all or part of a heavy or light chain constant region. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, *e.g.*, disulfide bonds. In IgGs, the heavy chain constant region includes three immunoglobulin domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. The light chains of the immunoglobulin may be of type kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, *e.g.*, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and/or LC CDR3. Each of the light chain (LC) and/or heavy chain (HC) CDRs can be human. HC CDR3 can be human. One or more of the framework regions can be human, *e.g.*, FR1, FR2, FR3, and/or FR4 of the HC and/or LC. For example, the Fc region can be human. In one embodiment, all the framework regions are human, *e.g.*, derived from a human somatic cell, *e.g.*, a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, *e.g.*, encoded by a germline nucleic acid. In one embodiment, the framework (FR) residues of a selected Fab can be converted to the amino-acid type of the corresponding residue in the most similar primate germline gene, especially the human germline gene. One or more of the constant regions can be human or effectively human. For example, at least 70, 75, 80, 85, 90, 92, 95, 98, or 100% of an immunoglobulin variable domain, the constant region, the constant domains (CH1, CH2, CH3, and/or CL1), or the entire antibody can be human or effectively human.

An antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the many immunoglobulin variable region genes. Full-length immunoglobulin “light chains” (about 25 KDa or about 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH-terminus. Full-length immunoglobulin “heavy chains” (about 50 KDa or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, *e.g.*, gamma (encoding about 330 amino acids). The length of human HC varies considerably because HC CDR3 varies from about 3 amino-acid residues to over 35 amino-acid residues.

The term “antigen-binding fragment” of a full length antibody refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term “antigen-binding fragment” of a full length antibody and that retain functionality include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules known as single chain Fv (scFv). See *e.g.*, U.S. Pat. Nos. 5,260,203, 4,946,778, and 4,881,175; Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883. Antibody fragments can be obtained using any appropriate technique including conventional techniques known to those with skill in the art.

The term “monospecific antibody” refers to an antibody that displays a single binding specificity and affinity for a particular target, *e.g.*, epitope. This term includes a “monoclonal antibody” or “monoclonal antibody composition,” which as used herein refers to a preparation of antibodies or fragments thereof of single molecular composition, irrespective of how the antibody was generated. Antibodies are “germlined” by reverting one or more

non-germline amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained.

The inhibition constant (K_i) provides a 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 apparent K_i ($K_{i,app}$) is obtained at different substrate concentrations by measuring the inhibitory effect of different concentrations of inhibitor (*e.g.*, inhibitory binding protein) 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) yields an estimate of the apparent K_i value. 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.

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

Equation 1

Where v = measured velocity; v_0 = velocity in the absence of inhibitor; $K_{i,app}$ = apparent inhibition constant; I = total inhibitor concentration; and E = total enzyme concentration.

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). A binding antibody may, for example, have a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M⁻¹ for a particular target molecule, *e.g.*, plasma kallikrein. Higher affinity binding of a binding 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 binding 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, 30, 40, 50, 70, 80, 90, 100, 500, 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 spectroscopy (*e.g.*, using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound and free binding

protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding 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:

$$[\text{Bound}] = N \cdot [\text{Free}] / ((1/K_A) + [\text{Free}]).$$

It is not always necessary to make an exact determination of K_A , though, since 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.

The term “binding antibody” (or “binding protein” used interchangeably herein) refers to an antibody that can interact with a target molecule. The term “target molecule” is used interchangeably with “ligand.” A “plasma kallikrein binding antibody” refers to an antibody that can interact with (*e.g.*, bind) plasma kallikrein, and includes, in particular, antibodies that preferentially or specifically interact with and/or inhibit plasma kallikrein. An antibody inhibits plasma kallikrein if it causes a decrease in the activity of plasma kallikrein as compared to the activity of plasma kallikrein in the absence of the antibody and under the same conditions.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

It is possible for one or more framework and/or CDR amino acid residues of a binding protein to include one or more mutations (for example, substitutions (*e.g.*, conservative substitutions or substitutions of non-essential amino acids), insertions, or deletions) relative to a binding protein described herein. A plasma kallikrein binding protein may have mutations (*e.g.*, substitutions (*e.g.*, conservative substitutions or substitutions of non-essential amino acids), insertions, or deletions) (*e.g.*, at least one, two, three, or four, and/or less than

15, 12, 10, 9, 8, 7, 6, 5, 4, 3, or 2 mutations) relative to a binding protein described herein, *e.g.*, mutations which do not have a substantial effect on protein function. The mutations can be present in framework regions, CDRs, and/or constant regions. In some embodiments, the mutations are present in a framework region. In some embodiments, the mutations are present in a CDR. In some embodiments, the mutations are present in a constant region. Whether or not a particular substitution will be tolerated, *i.e.*, will not adversely affect biological properties, such as binding activity, can be predicted, *e.g.*, by evaluating whether the mutation is conservative or by the method of Bowie, et al. (1990) *Science* 247:1306-1310.

An “effectively human” immunoglobulin variable region is an immunoglobulin variable region that includes a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. An “effectively human” antibody is an antibody that includes a sufficient number of human amino acid positions such that the antibody does not elicit an immunogenic response in a normal human.

An “epitope” refers to the site on a target compound that is bound by a binding protein (*e.g.*, an antibody such as a Fab or full length antibody). In the case where the target compound is a protein, the site can be entirely composed of amino acid components, entirely composed of chemical modifications of amino acids of the protein (*e.g.*, glycosyl moieties), or composed of combinations thereof. Overlapping epitopes include at least one common amino acid residue, glycosyl group, phosphate group, sulfate group, or other molecular feature.

A “humanized” immunoglobulin variable region is an immunoglobulin variable region that is modified to include a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. Descriptions of “humanized” immunoglobulins include, for example, U.S. 6,407,213 and U.S. 5,693,762.

An “isolated” antibody refers to an antibody that is removed from at least 90% of at least one component of a natural sample from which the isolated antibody can be obtained. Antibodies can be “of at least” a certain degree of purity if the species or population of species of interest is at least 5, 10, 25, 50, 75, 80, 90, 92, 95, 98, or 99% pure on a weight-weight basis.

The methods described herein involve administering multiple doses of an antibody to a human subject in need thereof. The terms “patient,” “subject” or “host” may be used interchangeably. A subject may be a subject that has undergone a prior treatment for HAE,

such as a treatment involving an antibody described herein. In some embodiments, the subject is a pediatric subject (*e.g.*, an infant, child, or adolescent subject). In some embodiments, the human subject is an adolescent less than 18 years old. In some embodiments, the human subject is an adolescent between the ages of 12 and 18 years old. In some embodiments, the subject is between the ages of 40 and less than 65 years old.

In some embodiments, the human subject is defined by gender. For example, in some embodiments, the subject is female.

In some embodiments, the human subject is defined by weight. In some embodiments, the human subject weighs less than 50 kg. In some embodiments, the human subject weighs between 50 kg and 75 kg. In some embodiments the human subject weighs between 75 kg and 100 kg. In some embodiments, the human subject weighs 100 kg or more.

In some embodiments, the human subject is defined by prior history of laryngeal attacks or absence thereof. In some embodiments, the subject has experienced at least one (*e.g.*, 1, 2, 3, 4, 5, or more) laryngeal attack (*i.e.* laryngeal HAE attack) prior to administration of the antibodies described herein. In some embodiments, the subject has not experienced a laryngeal attack prior to administration of the antibodies described herein.

The terms “prekallikrein” and “preplasma kallikrein” are used interchangeably herein and refer to the zymogen form of active plasma kallikrein, which is also known as prekallikrein.

As used herein, the term “substantially identical” (or “substantially homologous”) is used herein to refer to a first amino acid or nucleic acid sequence that contains a sufficient number of identical or equivalent (*e.g.*, with a similar side chain, for example, conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleic acid sequence such that the first and second amino acid or nucleic acid sequences have (or encode proteins having) similar activities, *e.g.*, a binding activity, a binding preference, or a biological activity. In the case of antibodies, the second antibody has the same specificity and has at least 50%, at least 25%, or at least 10% of the affinity relative to the same antigen.

Statistical significance can be determined by any art known method. Exemplary statistical tests include: the Students T-test, Mann Whitney U non-parametric test, and Wilcoxon non-parametric statistical test. Some statistically significant relationships have a P value of less than 0.05 or 0.02. Particular binding proteins may show a difference, *e.g.*, in specificity or binding that are statistically significant (*e.g.*, P value < 0.05 or 0.02). The terms “induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease” or the like, *e.g.*, which

denote distinguishable qualitative or quantitative differences between two states, may refer to a difference, *e.g.*, a statistically significant difference, between the two states.

A “therapeutically effective dosage” preferably modulates a measurable parameter, *e.g.*, plasma kallikrein activity, by a statistically significant degree or at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to modulate a measurable parameter, *e.g.*, a disease-associated parameter, can be evaluated in an animal model system predictive of efficacy in human disorders and conditions. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter *in vitro*.

The term “treating” as used herein refers to the application or administration of a composition including one or more active agents to a subject, who has HAE, a symptom of HAE, is suspected of having HAE, or a predisposition toward or risk of having HAE, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease. “Prophylactic treatment,” also known as “preventive treatment,” refers to a treatment that aims at protecting a person from, or reducing the risk for a disease to which he or she has been, or may be, exposed. In some embodiments, the treatment methods described herein aim at preventing occurrence and/or recurrence of HAE.

The term “preventing” a disease in a subject refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease is prevented, that is, administered prior to clinical manifestation of the unwanted condition (*e.g.*, disease or other unwanted state of the host animal) so that it protects the host against developing the unwanted condition. “Preventing” a disease may also be referred to as “prophylaxis” or “prophylactic treatment.”

A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Antibodies Binding to Plasma Kallikrein (pKal)

Plasma kallikrein binding antibodies (anti-pKal antibodies) for use in the methods described herein can be full-length (*e.g.*, an IgG (including an IgG1, IgG2, IgG3, IgG4), IgM, IgA (including, IgA1, IgA2), IgD, and IgE) or can include only an antigen-binding fragment

(*e.g.*, a Fab, F(ab')₂ or scFv fragment. The binding antibody can include two heavy chain immunoglobulins and two light chain immunoglobulins, or can be a single chain antibody. Plasma kallikrein binding antibodies can be recombinant proteins such as humanized, CDR grafted, chimeric, deimmunized, or *in vitro* generated antibodies, and may optionally include constant regions derived from human germline immunoglobulin sequences. In one embodiment, the plasma kallikrein binding antibody is a monoclonal antibody.

In one aspect, the disclosure features an antibody (*e.g.*, an isolated antibody) that binds to plasma kallikrein (*e.g.*, human plasma kallikrein and/or murine kallikrein) and includes at least one immunoglobulin variable region. For example, the antibody includes a heavy chain (HC) immunoglobulin variable domain sequence and/or a light chain (LC) immunoglobulin variable domain sequence. In one embodiment, the antibody binds to and inhibits plasma kallikrein, *e.g.*, human plasma kallikrein and/or murine kallikrein.

In some embodiments, the antibodies described herein have the same CDR sequences as DX-2930, *e.g.*, heavy chain CDR sequences set forth as SEQ ID NOs: 5-7 and light chain CDR sequences set forth as SEQ ID NOs: 8-10. In some embodiments, the antibody comprises the same CDR sequences as DX-2930 and a LC immunoglobulin variable domain sequence that is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein (*e.g.*, overall or in framework regions). In some embodiments, the antibody comprises the same CDR sequences as DX-2930 and an HC immunoglobulin variable domain sequence that is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein (*e.g.*, overall or in framework regions). In some embodiments, the antibody comprises the same CDR sequences as DX-2930 and LC sequence that is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC sequence described herein (*e.g.*, overall or in framework regions). In some embodiments, the antibody comprises the same CDR sequences as DX-2930 and HC sequence that is at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC sequence described herein (*e.g.*, overall or in framework regions).

The plasma kallikrein binding protein may be an isolated antibody (*e.g.*, at least 70, 80, 90, 95, or 99% free of other proteins). In some embodiments, the plasma kallikrein binding antibody, or composition thereof, is isolated from antibody cleavage fragments (*e.g.*, DX-2930) that are inactive or partially active (*e.g.*, bind plasma kallikrein with a $K_{i,app}$ of 5000 nM or greater) compared to the plasma kallikrein binding antibody. For example, the plasma kallikrein binding antibody is at least 70% free of such antibody cleavage fragments;

in other embodiments the binding antibody is at least 80%, at least 90%, at least 95%, at least 99% or even 100% free from antibody cleavage fragments that are inactive or partially active.

The plasma kallikrein binding antibody may additionally inhibit plasma kallikrein, *e.g.*, human plasma kallikrein.

In some embodiments, the plasma kallikrein binding antibody does not bind prekallikrein (*e.g.*, human prekallikrein and/or murine prekallikrein), but binds to the active form of plasma kallikrein (*e.g.*, human plasma kallikrein and/or murine kallikrein).

In certain embodiments, the antibody binds at or near the active site of the catalytic domain of plasma kallikrein, or a fragment thereof, or binds an epitope that overlaps with the active site of plasma kallikrein.

The antibody can bind to plasma kallikrein, *e.g.*, human plasma kallikrein, with a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} . In one embodiment, the antibody binds to human plasma kallikrein with a K_{off} slower than 1×10^{-3} , 5×10^{-4} s^{-1} , or 1×10^{-4} s^{-1} . In one embodiment, the antibody binds to human plasma kallikrein with a K_{on} faster than 1×10^2 , 1×10^3 , or 5×10^3 $M^{-1}s^{-1}$. In one embodiment, the antibody binds to plasma kallikrein, but does not bind to tissue kallikrein and/or plasma prekallikrein (*e.g.*, the antibody binds to tissue kallikrein and/or plasma prekallikrein less effectively (*e.g.*, 5-, 10-, 50-, 100-, or 1000-fold less or not at all, *e.g.*, as compared to a negative control) than it binds to plasma kallikrein).

In one embodiment, the antibody inhibits human plasma kallikrein activity, *e.g.*, with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} M. The antibody can have, for example, an IC_{50} of less than 100 nM, 10 nM, 1, 0.5, or 0.2 nM. For example, the antibody may modulate plasma kallikrein activity, as well as the production of Factor XIIa (*e.g.*, from Factor XII) and/or bradykinin (*e.g.*, from high-molecular-weight kininogen (HMWK)). The antibody may inhibit plasma kallikrein activity, and/or the production of Factor XIIa (*e.g.*, from Factor XII) and/or bradykinin (*e.g.*, from high-molecular-weight kininogen (HMWK)). The affinity of the antibody for human plasma kallikrein can be characterized by a K_D of less than 100 nM, less than 10 nM, less than 5 nM, less than 1 nM, less than 0.5 nM. In one embodiment, the antibody inhibits plasma kallikrein, but does not inhibit tissue kallikrein (*e.g.*, the antibody inhibits tissue kallikrein less effectively (*e.g.*, 5-, 10-, 50-, 100-, or 1000-fold less or not at all, *e.g.*, as compared to a negative control) than it inhibits plasma kallikrein).

In some embodiments, the antibody has an apparent inhibition constant ($K_{i,app}$) of less than 1000, 500, 100, 5, 1, 0.5 or 0.2 nM.

Plasma kallikrein binding antibodies may have their HC and LC variable domain sequences included in a single polypeptide (*e.g.*, scFv), or on different polypeptides (*e.g.*, IgG or Fab).

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain. In another, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the antibody is an IgG, *e.g.*, IgG1, IgG2, IgG3, or IgG4. The antibody can be a soluble Fab. In other implementations the antibody includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA + VH::CH1, HSA::LC + VH::CH1, or other appropriate construction.

In one embodiment, the antibody is a human or humanized antibody or is non-immunogenic in a human. For example, the antibody includes one or more human antibody framework regions, *e.g.*, all human framework regions, or framework regions at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to human framework regions. In one embodiment, the antibody includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the antibody is a primate or primatized antibody or is non-immunogenic in a human. For example, the antibody includes one or more primate antibody framework regions, *e.g.*, all primate framework regions, or framework regions at least 85, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% identical to primate framework regions. In one embodiment, the antibody includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibbons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers. In some embodiments, the affinity of the primate antibody for human plasma kallikrein is characterized by a K_D of less than 1000, 500, 100, 10, 5, 1, 0.5 nM, *e.g.*, less than 10 nM, less than 1 nM, or less than 0.5 nM.

In certain embodiments, the antibody includes no sequences from mice or rabbits (*e.g.*, is not a murine or rabbit antibody).

In some embodiments, the antibody used in the methods described herein may be DX-2930 as described herein or a functional variant thereof.

In one example, a functional variant of DX-2930 comprises the same complementary determining regions (CDRs) as DX-2930. In another example, the functional variants of DX-2930 may contain one or more mutations (e.g., conservative substitutions) in the FRs of either the V_H or the V_L as compared to those in the V_H and V_L of DX-2930. Preferably, such mutations do not occur at residues which are predicted to interact with one or more of the CDRs, which can be determined by routine technology. In other embodiments, the functional variants described herein contain one or more mutations (e.g., 1, 2, or 3) within one or more of the CDR regions of DX-2930. Preferably, such functional variants retain the same regions/residues responsible for antigen-binding as the parent. In yet other embodiments, a functional variant of DX-2930 may comprise a V_H chain that comprises an amino acid sequence at least 85% (e.g., 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to that of the V_H of DX-2930 and/or a V_L chain that has an amino acid sequence at least 85% (e.g., 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to that of the V_L of DX-2930. These variants are capable of binding to the active form of plasma kallikrein and preferably do not bind to prekallikrein.

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.

In some embodiments, the antibody used in the methods and compositions described herein may be the DX-2930 antibody. The heavy and light chain full and variable sequences for DX-2930 are provided below, with signal sequences in italics. The CDRs are boldfaced and underlined.

DX-2930 Heavy Chain Amino Acid Sequence (451 amino acids, 49439.02 Da)

*MGWSCILFLVATATGAHSEVQLLES*GGGLVQPGGSLRLSCAASGFTFS**HYIMMWVRQ**
 APGKGLEWVS**GIYSSGGITVYADSVKGR**FTISRDN SKNTLYLQMNSLR AEDTAVYY
 CAY**RRIGVPRRDEFDI**WGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV
 KDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH

KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE
ALHNHYTQKSLSLSPG (SEQ ID NO: 1)

DX-2930 Light Chain Amino Acid Sequence (213 amino acids, 23419.08 Da)

MGWSCILFLVATATGAHSDIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKP
GKAPKLLIYKASTLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNTYWTF
GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC (SEQ ID NO: 2)

DX-2930 Heavy Chain Variable Domain Amino Acid Sequence

EVQLLES GGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGG
ITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIW
GQGTMTVTSS (SEQ ID NO: 3)

DX-2930 Light Chain Variable Domain Amino Acid Sequence

DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESG
VPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNTYWTFGQGTKVEIK (SEQ ID NO:
4)

Table 1A. CDRs for DX-2930.

CDR	Amino acid sequence
Heavy chain CDR1	HYIMM (SEQ ID NO: 5)
Heavy chain CDR2	GIYSSGGITVYADSVKG (SEQ ID NO: 6)
Heavy chain CDR3	RRIGVPRRDEFDI (SEQ ID NO: 7)
Light chain CDR1	RASQSISSWLA (SEQ ID NO: 8)
Light chain CDR2	KASTLES (SEQ ID NO: 9)
Light chain CDR3	QQYNTYWT (SEQ ID NO: 10)

Antibody Preparation

An antibody as described herein (*e.g.*, DX-2930) 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 and Greenfield, (2013) *Antibodies: A Laboratory Manual*, Second edition, Cold Spring Harbor Laboratory Press.

The sequence encoding the antibody of interest, *e.g.*, DX-2930, 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.

Some antibodies, *e.g.*, Fabs, can be produced in bacterial cells, *e.g.*, *E. coli* cells (see *e.g.*, Nadkarni, A. et al., 2007 *Protein Expr Purif* 52(1):219-29). For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be transferred into a bacterial cell that cannot suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the periplasm and/or media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (*e.g.*, scFv's) are expressed in a yeast cell such as *Pichia* (see, *e.g.*, Powers et al., 2001, *J. Immunol. Methods*. 251:123-35; Schoonooghe S. et al., 2009 *BMC Biotechnol.* 9:70; Abdel-Salam, HA. et al., 2001 *Appl Microbiol Biotechnol* 56(1-2):157-64; Takahashi K. et al., 2000 *Biosci Biotechnol Biochem* 64(10):2138-44; Edqvist, J. et al., 1991 *J Biotechnol* 20(3):291-300), *Hansenula*, or *Saccharomyces*. One of skill in the art can optimize antibody production in yeast by optimizing, for example, oxygen conditions (see *e.g.*, Baumann K., et al. 2010 *BMC Syst. Biol.* 4:141), osmolarity (see *e.g.*, Dragosits, M. et al., 2010 *BMC Genomics* 11:207), temperature (see *e.g.*, Dragosits, M. et al., 2009 *J Proteome Res.* 8(3):1380-92), fermentation conditions (see *e.g.*, Ning, D. et al. 2005 *J. Biochem. and Mol. Biol.* 38(3): 294-299), strain of yeast (see *e.g.*, Kozyr, AV et al. 2004 *Mol Biol (Mosk)* 38(6):1067-75; Horwitz, AH. et al., 1988 *Proc Natl Acad Sci USA* 85(22):8678-82; Bowdish, K. et al. 1991 *J Biol Chem* 266(18):11901-8), overexpression of proteins to enhance antibody production (see *e.g.*, Gasser, B. et al., 2006 *Biotechnol. Bioeng.* 94(2):353-61), level of acidity of the culture (see *e.g.*, Kobayashi H., et al., 1997 *FEMS Microbiol Lett* 152(2):235-42), concentrations of substrates and/or ions (see *e.g.*, Ko JH. et al., 2006 *Appl Biochem Biotechnol* 60(1):41-8). In addition, yeast systems can be used to produce antibodies with an extended half-life (see *e.g.*, Smith, BJ. et al. 2001 *Bioconjug Chem* 12(5):750-756).

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in

Urlaub and Chasin, 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in Kaufman and Sharp, 1982, *Mol. Biol.* 159:601-621), lymphocytic cell lines, *e.g.*, NS0 myeloma cells and SP2 cells, COS cells, HEK293T cells (*J. Immunol. Methods* (2004) 289(1-2):65-80), and a cell from a transgenic animal, *e.g.*, a transgenic mammal. For example, the cell is a mammary epithelial cell.

In some embodiments, plasma kallikrein binding antibodies are produced in a plant or cell-free based system (see *e.g.*, Galeffi, P., et al., 2006 *J Transl Med* 4:39).

In addition to the nucleic acid sequence encoding the diversified immunoglobulin domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr*⁻ host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into *dhfr*⁻ CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (*e.g.*, derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

For antibodies that include an Fc domain, the antibody production system may produce antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecules is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcγ receptors and complement C1q (Burton and Woof, 1992, *Adv. Immunol.* 51:1-84; Jefferis et al., 1998, *Immunol. Rev.* 163:59-76). In one embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other eukaryotic post-translational modifications.

Antibodies can also be produced by a transgenic animal. For example, U.S. Pat. No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

Pharmaceutical Compositions

An antibody as described herein (*e.g.*, DX-2930) can be present in a composition, *e.g.*, a pharmaceutically acceptable composition or pharmaceutical composition. The antibody as described herein (*e.g.*, DX-2930) can be formulated together with a pharmaceutically acceptable carrier. In some embodiments, 150 mg or 300 mg of DX-2930 antibody are present in a composition optionally with a pharmaceutically acceptable carrier, *e.g.*, a pharmaceutically acceptable composition or pharmaceutical composition.

A pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for subcutaneous, intravenous, intramuscular, parenteral, spinal, or epidermal administration (*e.g.*, by injection or infusion), although carriers suitable for inhalation and intranasal administration are also contemplated.

The pharmaceutically acceptable carrier in the pharmaceutical composition described herein may include one or more of a buffering agent, an amino acid, and a tonicity modifier. Any suitable buffering agent or combination of buffering agents may be used in the pharmaceutical composition described herein to maintain or aid in maintaining an appropriate pH of the composition. Non-limiting examples of buffering agents include sodium

phosphate, potassium phosphate, citric acid, sodium succinate, histidine, Tris, and sodium acetate. In some embodiments, the buffering agents may be at a concentration of about 5-100 mM, 5-50 mM, 10-50 mM, 15-50 mM, or about 15-40 mM. For example, the one or more buffering agents may be at a concentration of about 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 35 mM, 36 mM, 37 mM, 38 mM, 39 mM, or about 40 mM. In some examples, the pharmaceutically acceptable carrier comprises sodium phosphate and citric acid, which may be at a concentration of about 30 mM and about 19 mM, respectively.

In some embodiments, the pharmaceutically acceptable carrier includes one or more amino acids, which may decrease aggregation of the antibody and/or increase stability of the antibody during storage prior to administration. Exemplary amino acids for use in making the pharmaceutical compositions described herein include, but are not limited to, alanine, arginine, asparagine, aspartic acid, glycine, histidine, lysine, proline, or serine. In some examples, the concentration of the amino acid in the pharmaceutical composition may be about 5-100 mM, 10-90 mM, 20-80 mM, 30-70 mM, 40-60 mM, or about 45-55 mM. In some examples, the concentration of the amino acid (*e.g.*, histidine) may be about 40 mM, 41 mM, 42 mM, 43 mM, 44 mM, 45 mM, 46 mM, 47 mM, 48 mM, 49 mM, 50 mM, 51 mM, 52 mM, 53 mM, 54 mM, 55 mM, 56 mM, 57 mM, 58 mM, 59 mM, or about 60 mM. In one example, the pharmaceutical composition contains histidine at a concentration of about 50 mM.

Any suitable tonicity modifier may be used for preparing the pharmaceutical compositions described herein. In some embodiments, the tonicity modifier is a salt or an amino acid. Examples of suitable salts include, without limitation, sodium chloride, sodium succinate, sodium sulfate, potassium chloride, magnesium chloride, magnesium sulfate, and calcium chloride. In some embodiments, the tonicity modifier in the pharmaceutical composition may be at a concentration of about 10-150 mM, 50-150 mM, 50-100 mM, 75-100 mM, or about 85-95 mM. In some embodiments, the tonicity modifier may be at a concentration of about 80 mM, 81 mM, 82 mM, 83 mM, 84 mM, 85 mM, 86 mM, 87 mM, 88 mM, 89 mM, 90 mM, 91 mM, 92 mM, 93 mM, 94 mM, 95 mM, 96 mM, 97 mM, 98 mM, 99 mM, or about 100 mM. In one example, the tonicity modifier may be sodium chloride, which may be at a concentration of about 90 mM.

The pharmaceutically acceptable carrier in the pharmaceutical compositions described herein may further comprise one or more pharmaceutically acceptable excipients. In general, pharmaceutically acceptable excipients are pharmacologically inactive substances. Non-

limiting examples of excipients include lactose, glycerol, xylitol, sorbitol, mannitol, maltose, inositol, trehalose, glucose, bovine serum albumin (BSA), dextran, polyvinyl acetate (PVA), hydroxypropyl methylcellulose (HPMC), polyethyleneimine (PEI), gelatin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), polyethylene glycol (PEG), ethylene glycol, glycerol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), polyoxyethylene sorbitan monolaurate (Tween-20), polyoxyethylene sorbitan monooleate (Tween-80), sodium dodecyl sulphate (SDS), polysorbate, polyoxyethylene copolymer, potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate, trimethylamine N-oxide, betaine, zinc ions, copper ions, calcium ions, manganese ions, magnesium ions, CHAPS, sucrose monolaurate and 2-O-beta-mannoglycerate. In some embodiments, the pharmaceutically acceptable carrier comprises an excipient between about 0.001%-0.1%, 0.001%-0.05%, 0.005-0.1%, 0.005%-0.05%, 0.008%-0.05%, 0.008%-0.03% or about 0.009%-0.02%. In some embodiments, the excipient is at about 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, or about 0.1%. In some embodiments, the excipient is polyoxyethylene sorbitan monooleate (Tween-80). In one example, the pharmaceutically acceptable carrier contains 0.01% Tween-80.

In some examples, the pharmaceutical composition described herein comprises the anti-pKal antibody as also described herein (*e.g.*, DX-2930), and one or more of sodium phosphate (*e.g.*, sodium phosphate dibasic dihydrate), citric acid (*e.g.*, citric acid monohydrate), histidine (*e.g.*, L-histidine), sodium chloride, and Polysorbate 80. For example, the pharmaceutical composition may comprise the antibody, sodium phosphate, citric acid, histidine, sodium chloride, and Polysorbate 80. In some examples, the antibody is formulated in about 30 mM sodium phosphate, about 19 mM citric acid, about 50 mM histidine, about 90 mM sodium chloride, and about 0.01% Polysorbate 80. The concentration of the antibody (*e.g.*, DX-2930) in the composition can be about 150 mg/mL or 300 mg/mL. In one example, the composition comprises or consists of about 150 mg DX-2930 per 1 mL solution, about 30 mM sodium phosphate dibasic dihydrate, about 19 mM (*e.g.*, 19.6 mM) citric acid monohydrate, about 50 mM L-histidine, about 90 mM sodium chloride, and about 0.01% Polysorbate 80. In another example, the composition comprises or consists of about 300 mg DX-2930 per 1 mL solution, about 30 mM sodium phosphate dibasic dihydrate, about 19 mM (*e.g.*, 19.6 mM) citric acid monohydrate, about 50 mM L-histidine, about 90 mM sodium chloride, and about 0.01% Polysorbate 80.

A pharmaceutically acceptable salt is a salt that retains the desired biological activity of the compound and does not impart any undesired toxicological effects (see, *e.g.*, Berge, S.M., et al., 1977, *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous, and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids, and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium, and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine, and the like.

The compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form can depend on the intended mode of administration and therapeutic application. Many compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. An exemplary mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In one embodiment, the plasma kallikrein binding protein is administered by intravenous infusion or injection. In another embodiment, the plasma kallikrein binding protein is administered by intramuscular injection. In another embodiment, the plasma kallikrein binding protein is administered by subcutaneous injection. In another preferred embodiment, the plasma kallikrein binding protein is administered by intraperitoneal injection.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. In some embodiments, the antibody is administered subcutaneously.

The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the binding protein in the required amount in an

appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

An antibody as described herein (*e.g.*, DX-2930) can be administered by a variety of methods, including intravenous injection, subcutaneous injection, or infusion. For example, for some therapeutic applications, the antibody can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or 7 to 25 mg/m². The route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are available. See, *e.g.*, Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., 1978, Marcel Dekker, Inc., New York.

Pharmaceutical compositions can be administered with medical devices. For example, in one embodiment, a pharmaceutical composition disclosed herein can be administered with a device, *e.g.*, a needleless hypodermic injection device, a pump, or implant.

In certain embodiments, an antibody as described herein (*e.g.*, DX-2930) can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds disclosed herein cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties that are

selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, V.V. Ranade, 1989, *J. Clin. Pharmacol.* 29:685).

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody as described herein (*e.g.*, DX-2930) is about 150 mg or 300 mg. As will be understood by one of ordinary skill in the art, a therapeutically or prophylactically effective amount of an antibody may be lower for a pediatric subject than for an adult subject. In some embodiments, the effective amount that is administered to a pediatric subject is a fixed dose or a weight based dose. In some embodiments, effective amount that is less than about 150 mg or 300 mg is administered to a pediatric subject. In some embodiments, a therapeutically or prophylactically effective amount of an antibody is administered every two weeks or every four weeks for a first treatment period. In some embodiments, the antibody may be administered to the subject for a second treatment period. In some embodiments, the therapeutically or prophylactically effective amount of the antibody in the first treatment period is different than the therapeutically or prophylactically effective amount of the antibody in the second treatment period. In some embodiments, the therapeutically or prophylactically effective amount of the antibody in the first treatment period is 150 mg and the therapeutically or prophylactically effective amount of the antibody in the second treatment period is 300 mg. In some embodiments, the therapeutically or prophylactically effective amount of the antibody in the first treatment period is the same as the therapeutically or prophylactically effective amount of the antibody in the second treatment

period. In one example, therapeutically or prophylactically effective amount of the antibody in the first treatment period and the second treatment period is 300 mg.

In some embodiments, an exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody as described herein (*e.g.*, DX-2930) is about 300 mg. In some embodiments, a therapeutically or prophylactically effective amount of an antibody is administered in a single dose. If the subject experiences a HAE attack, the antibody may be further administered to the subject in multiple doses, such in doses of about 300 mg administered every two weeks.

Kits

An antibody as described herein (*e.g.*, DX-2930) can be provided in a kit, *e.g.*, as a component of a kit. For example, the kit includes (a) a DX-2930 antibody, *e.g.*, a composition (*e.g.*, a pharmaceutical composition) that includes the antibody, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to a method described herein and/or the use of an antibody as described herein (*e.g.*, DX-2930), *e.g.*, for a method described herein. In some embodiments, the kit comprises one or more doses of DX-2930. In some embodiments, the one or more doses are 150 mg or 300 mg.

The informational material of the kit is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to using the antibody to treat, prevent, or diagnosis of disorders and conditions, *e.g.*, a plasma kallikrein associated disease or condition.

In one embodiment, the informational material can include instructions to administer an antibody as described herein (*e.g.*, DX-2930) in a suitable manner to perform the methods described herein, *e.g.*, in a suitable dose, dosage form, mode of administration or dosing schedule (*e.g.*, a dose, dosage form, dosing schedule or mode of administration described herein). In another embodiment, the informational material can include instructions to administer an antibody as described herein (*e.g.*, DX-2930) to a suitable subject, *e.g.*, a human, *e.g.*, a human having, or at risk for, a plasma kallikrein associated disease or condition. For example, the material can include instructions to administer an antibody as described herein (*e.g.*, DX-2930) to a patient with a disorder or condition described herein, *e.g.*, a plasma kallikrein associated disease, *e.g.*, according to a dosing schedule described

herein. The informational material of the kits is not limited in its form. In many cases, the informational material, *e.g.*, instructions, is provided in print but may also be in other formats, such as computer readable material.

An antibody as described herein (*e.g.*, DX-2930) can be provided in any form, *e.g.*, liquid, dried or lyophilized form. It is preferred that an antibody be substantially pure and/or sterile. When an antibody is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When an antibody is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, *e.g.*, sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing an antibody as described herein (*e.g.*, DX-2930). In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in association with the container. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (*e.g.*, a pack) of individual containers, each containing one or more unit dosage forms (*e.g.*, a dosage form described herein) of an antibody as described herein (*e.g.*, DX-2930). For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of an antibody as described herein (*e.g.*, DX-2930). The containers of the kits can be air tight, waterproof (*e.g.*, impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, *e.g.*, a syringe, or any such delivery device. In one embodiment, the device is an implantable device that dispenses metered doses of the antibody. The disclosure also features a method of providing a kit, *e.g.*, by combining components described herein.

Treatment

In some aspects, the disclosure provides the use of an antibody as described herein (*e.g.*, DX-2930) in treating HAE. In particular, the present disclosure provides a treatment regimen that allows for reducing the dosage of DX-2930 and/or extending the dosing schedule (*e.g.*, from once every two weeks to once every four weeks) for patients whose

disease is well controlled (*e.g.*, free of attack in a period of time such as 4-9 months) and/or for patients with low body weight (*e.g.*, pediatric patients).

(i) Hereditary angioedema

Hereditary angioedema (HAE) is also known as “Quincke edema,” C1 esterase inhibitor deficiency, C1 inhibitor deficiency, and hereditary angioneurotic edema (HANE). HAE is characterized by unpredictable, recurrent attacks of severe subcutaneous or submucosal swelling (angioedema), which can affect, *e.g.*, the limbs, face, genitals, gastrointestinal tract, and airway (Zuraw, 2008). Symptoms of HAE include, *e.g.*, swelling in the arms, legs, lips, eyes, tongue, and/or throat; airway blockage that can involve throat (larynx) swelling, sudden hoarseness and/or cause death from asphyxiation (Bork et al., 2012; Bork et al., 2000). Approximately 50% of all HAE patients will experience a laryngeal attack in their lifetime, and there is no way to predict which patients are at risk of a laryngeal attack (Bork et al., 2003; Bork et al., 2006). HAE symptoms also include repeat episodes of abdominal cramping without obvious cause; and/or swelling of the intestines, which can be severe and can lead to abdominal cramping, vomiting, dehydration, diarrhea, pain, shock, and/or intestinal symptoms resembling abdominal emergencies, which may lead to unnecessary surgery (Zuraw, 2008). Swelling may last up to five or more days. About one-third of individuals with this HAE develop a non-itchy rash called erythema marginatum during an attack. Most patients suffer multiple attacks per year.

HAE is an orphan disorder, the exact prevalence of which is unknown, but current estimates range from 1 per 10,000 to 1 per 150,000 persons, with many authors agreeing that 1 per 50,000 is likely the closest estimate (Bygum, 2009; Goring et al., 1998; Lei et al., 2011; Nordenfelt et al., 2014; Roche et al., 2005).

Plasma kallikrein plays a critical role in the pathogenesis of HAE attacks (Davis, 2006; Kaplan and Joseph, 2010). In normal physiology, C1-INH regulates the activity of plasma kallikrein as well as a variety of other proteases, such as C1r, C1s, factor XIa, and factor XIIa. Plasma kallikrein regulates the release of bradykinin from high molecular weight kininogen (HMWK). Due to a deficiency of C1-INH in HAE, uncontrolled plasma kallikrein activity occurs and leads to the excessive generation of bradykinin. Bradykinin is a vasodilator which is thought to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain (Craig et al., 2012; Zuraw et al., 2013).

Swelling of the airway can be life threatening and causes death in some patients. Mortality rates are estimated at 15-33%. HAE leads to about 15,000-30,000 emergency department visits per year.

Trauma or stress, *e.g.*, dental procedures, sickness (*e.g.*, viral illnesses such as colds and the flu), menstruation, and surgery can trigger an attack of angioedema. To prevent acute attacks of HAE, patients can attempt to avoid specific stimuli that have previously caused attacks. However, in many cases, an attack occurs without a known trigger. Typically, HAE symptoms first appear in childhood and worsen during puberty. On average, untreated individuals have an attack every 1 to 2 weeks, and most episodes last for about 3 to 4 days (ghr.nlm.nih.gov/condition/hereditary-angioedema). The frequency and duration of attacks vary greatly among people with hereditary angioedema, even among people in the same family.

There are three types of HAE, known as types I, II, and III, all of which can be treated by the methods described herein. It is estimated that HAE affects 1 in 50,000 people, that type I accounts for about 85 percent of cases, type II accounts for about 15 percent of cases, and type III is very rare. Type III is the most newly described form and was originally thought to occur only in women, but families with affected males have been identified.

HAE is inherited in an autosomal dominant pattern, such that an affected person can inherit the mutation from one affected parent. New mutations in the gene can also occur, and thus HAE can also occur in people with no history of the disorder in their family. It is estimated that 20-25% of cases result from a new spontaneous mutation.

Mutations in the SERPING1 gene cause hereditary angioedema type I and type II. The SERPING1 gene provides instructions for making the C1 inhibitor protein, which is important for controlling inflammation. C1 inhibitor blocks the activity of certain proteins that promote inflammation. Mutations that cause hereditary angioedema type I lead to reduced levels of C1 inhibitor in the blood. In contrast, mutations that cause type II result in the production of a C1 inhibitor that functions abnormally. Approximately 85% of patients have Type I HAE, characterized by very low production of functionally normal C1-INH protein, while the remaining approximately 15% of patients have Type II HAE and produce normal or elevated levels of a functionally impaired C1-INH (Zuraw, 2008). Without the proper levels of functional C1 inhibitor, excessive amounts of bradykinin are generated from high molecular weight kininogen (HMWK), and there is increased vascular leakage mediated by bradykinin binding to the B2 receptor (B2-R) on the surface of endothelial cells (Zuraw, 2008). Bradykinin promotes inflammation by increasing the leakage of fluid through the

walls of blood vessels into body tissues. Excessive accumulation of fluids in body tissues causes the episodes of swelling seen in individuals with hereditary angioedema type I and type II.

Mutations in the F12 gene are associated with some cases of hereditary angioedema type III. The F12 gene provides instructions for making coagulation factor XII. In addition to playing a critical role in blood clotting (coagulation), factor XII is also an important stimulator of inflammation and is involved in the production of bradykinin. Certain mutations in the F12 gene result in the production of factor XII with increased activity. As a result, more bradykinin is generated and blood vessel walls become more leaky, which leads to episodes of swelling. The cause of other cases of hereditary angioedema type III remains unknown. Mutations in one or more as-yet unidentified genes may be responsible for the disorder in these cases.

HAE can present similarly to other forms of angioedema resulting from allergies or other medical conditions, but it differs significantly in cause and treatment. When hereditary angioedema is misdiagnosed as an allergy, it is most commonly treated with antihistamines, steroids, and/or epinephrine, which are typically ineffective in HAE, although epinephrine can be used for life-threatening reactions. Misdiagnoses have also resulted in unnecessary exploratory surgery for patients with abdominal swelling, and in some HAE patients abdominal pain has been incorrectly diagnosed as psychosomatic.

Like adults, children with HAE can suffer from recurrent and debilitating attacks. Symptoms may present very early in childhood, and upper airway angioedema has been reported in HAE patients as young as the age of 3 (Bork et al., 2003). In one case study of 49 pediatric HAE patients, 23 had suffered at least one episode of airway angioedema by the age of 18 (Farkas, 2010). An important unmet medical need exists among children with HAE, especially adolescents, since the disease commonly worsens after puberty (Bennett and Craig, 2015; Zuraw, 2008).

C1 inhibitor therapies, as well as other therapies for HAE, are described in Kaplan, A.P., *J Allergy Clin Immunol*, 2010, 126(5):918-925.

Acute treatment of HAE attacks is provided to halt progression of the edema as quickly as possible. C1 inhibitor concentrate from donor blood, which is administered intravenously, is one acute treatment; however, this treatment is not available in many countries. In emergency situations where C1 inhibitor concentrate is not available, fresh frozen plasma (FFP) can be used as an alternative, as it also contains C1 inhibitor.

Purified C1 inhibitor, derived from human blood, has been used in Europe since 1979. Several C1 inhibitor treatments are now available in the U.S. and two C1 inhibitor products are now available in Canada. Berinert P (CSL Behring), which is pasteurized, was approved by the F.D.A. in 2009 for acute attacks. Cinryze (ViroPharma), which is nanofiltered, was approved by the F.D.A. in 2008 for prophylaxis. Rhucin (Pharming) is a recombinant C1 inhibitor under development that does not carry the risk of infectious disease transmission due to human blood-borne pathogens.

Treatment of an acute HAE attack also can include medications for pain relief and/or IV fluids.

Other treatment modalities can stimulate the synthesis of C1 inhibitor, or reduce C1 inhibitor consumption. Androgen medications, such as danazol, can reduce the frequency and severity of attacks by stimulating production of C1 inhibitor.

Helicobacter pylori can trigger abdominal attacks. Antibiotics to treat *H. pylori* will decrease abdominal attacks.

Newer treatments attack the contact cascade. Ecallantide (KALBITOR[®], DX-88, Dyax) inhibits plasma kallikrein and has been approved in the U.S.. Icatibant (FIRAZYR[®], Shire) inhibits the bradykinin B2 receptor, and has been approved in Europe and the U.S.

Diagnosis of HAE can rely on, *e.g.*, family history and/or blood tests. Laboratory findings associated with HAE types I, II, and III are described, *e.g.*, in Kaplan, A.P., *J Allergy Clin Immunol*, 2010, 126(5):918-925. In type I HAE, the level of C1 inhibitor is decreased, as is the level of C4, whereas C1q level is normal. In type II HAE, the level of C1 inhibitor is normal or increased; however, C1 inhibitor function is abnormal. C4 level is decreased and C1q level is normal. In type III, the levels of C1 inhibitor, C4, and C1q can all be normal.

Symptoms of HAE can be assessed, for example, using questionnaires, *e.g.*, questionnaires that are completed by patients, clinicians, or family members. Such questionnaires are known in the art and include, for example, visual analog scales. See, *e.g.*, McMillan, C.V. et al. *Patient*. 2012;5(2):113-26. In some embodiments, the subject has HAE type I or HAE type II. HAE type I or HAE type II may be diagnosed using any method known in the art, such as by clinical history consistent with HAE (*e.g.*, subcutaneous or mucosal, nonpruritic swelling episodes) or diagnostic testing (*e.g.*, C1-INH functional testing and C4 level assessment).

(ii) Reducing Dosage of DX-2930 in HAE treatment

The disclosure provides methods of treating (*e.g.*, ameliorating, stabilizing, or eliminating one or more symptoms) of hereditary angioedema (HAE) by administering to an HAE patient with an anti-pKal antibody such as DX-2930 at a first dosing schedule, for example 300 mg every two weeks, for a first treatment period (*e.g.*, 4-9 weeks). Occurrence of HAE attack is monitored in the patient subject to the first period of treatment following standard medical practice. When the patient is free of HAE attack in the first treatment period, dosage of the antibody can be reduced and/or dosing interval of the antibody can be prolonged, for example, reduced to 300 mg every four weeks or 300 mg every six weeks.

In some embodiments, the human patient may have a low body weight. As used herein, a low body weight, when applied to an adult, refers to the body weight of the adult that is significantly lower than the average body weight of adults with matched physical features, such as height, age, gender, etc. For example, an adult patient having a low body weight may have a body weight that is at least 20% (*e.g.*, 30%, 40%, 50%, or above) lower than the average of body weight of adults with matched physical features as noted above. In some instances, the human patient is an adult HAE patient having a body weight lower than 40 kg (*e.g.*, lower than 35 kg, lower than 30 kg, lower than 25 kg, etc.). In other instances, the human patient having a low body weight may be a pediatric patient (*e.g.*, younger than 15 yrs). Such a pediatric patient may have a body weight less than 30 kg (*e.g.*, lower than 25 kg, lower than 20 kg, lower than 15 kg, or lower than 10 kg, etc.).

In some embodiments, the subject may be defined by gender. For example, in some embodiments, the subject is female. In other embodiments, the subject is male.

In some embodiments, the human subject is defined by prior history of laryngeal attacks or absence thereof. In some embodiments, the subject has experienced at least one (*e.g.*, 1, 2, 3, 4, 5, or more) laryngeal attack (*i.e.* laryngeal HAE attack) prior to administration of the antibodies described herein. In some embodiments, the subject has not experienced a laryngeal attack prior to administration of the antibodies described herein.

Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, *e.g.*, prevent onset, or prevent deterioration of a disease or condition.

Methods of administering DX-2930 antibodies are also described in "Pharmaceutical Compositions." Suitable dosages of the antibody used can depend on the age and weight of the subject and the particular drug used. The antibody can be used as competitive agents to

inhibit, reduce an undesirable interaction, *e.g.*, between plasma kallikrein and its substrate (*e.g.*, Factor XII or HMWK). The dose of the antibody can be the amount sufficient to block 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 99%, or 99.9% of the activity of plasma kallikrein in the patient, especially at the site of disease. In some embodiments, 150 mg or 300 mg of the antibody is administered every two weeks or every four weeks. In some embodiments, the antibody is administered to the subject in a first treatment period comprising administration of 150 mg or 300 mg of the antibody every two weeks or every four weeks. In some embodiments, the antibody is administered to the subject in a second treatment period following the first treatment period. In some embodiments, 300 mg of the antibody is administered in a single dose. If the subject experiences an HAE attack after the single dose, the antibody may be administered at 300 mg every two weeks in the first treatment period.

In one embodiment, the antibodies are used to inhibit an activity (*e.g.*, inhibit at least one activity of plasma kallikrein, *e.g.*, reduce Factor XIIa and/or bradykinin production) of plasma kallikrein, *e.g.*, *in vivo*. The binding proteins can be used by themselves or conjugated to an agent, *e.g.*, a cytotoxic drug, cytotoxin enzyme, or radioisotope.

The antibodies can be used directly *in vivo* to eliminate antigen-expressing cells via natural complement-dependent cytotoxicity (CDC) or antibody dependent cellular cytotoxicity (ADCC). The antibodies described herein can include complement binding effector domain, such as the Fc portions from IgG1, -2, or -3 or corresponding portions of IgM which bind complement. In one embodiment, a population of target cells is *ex vivo* treated with an antibody described herein and appropriate effector cells. The treatment can be supplemented by the addition of complement or serum containing complement. Further, phagocytosis of target cells coated with an antibody described herein can be improved by binding of complement proteins. In another embodiment target, cells coated with the antibody which includes a complement binding effector domain are lysed by complement.

Methods of administering DX-2930 antibodies are described in "Pharmaceutical Compositions." Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The antibodies can be used as competitive agents to inhibit or reduce an undesirable interaction, *e.g.*, between a natural or pathological agent and the plasma kallikrein.

A therapeutically effective amount of an antibody as described herein, can be administered to a subject having, suspected of having, or at risk for HAE, thereby treating

(*e.g.*, ameliorating or improving a symptom or feature of a disorder, slowing, stabilizing and/or halting disease progression) the disorder.

The antibody described herein can be administered in a therapeutically effective amount. A therapeutically effective amount of an antibody is the amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, *e.g.*, curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment.

Dosage regimens can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In other examples, a bolus may be administered followed by several doses over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In other examples, a dose may be divided into several doses and be administered over time. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Any of the subjects described herein may have undergone prior treatment of HAE, such as a prophylactic or therapeutic treatment of HAE. Aspects of the present disclosure also provide methods of administering an antibody as described herein (*e.g.*, DX-2930) to a subject that has received one or more prior treatment for HAE. In some embodiments, the prior treatment of HAE is a treatment that involves an antibody described herein (*e.g.*, DX-2930). In some embodiments, the subject was previously administered multiple doses of DX-2930 every two weeks or every four weeks. In some embodiments, the subject was previously administered DX-2930 at 150 mg every two weeks. In some embodiments, the subject was previously administered DX-2930 at 300 mg every two weeks. In some embodiments, the subject was previously administered DX-2930 at 300 mg every four weeks. In some embodiments, the multiple doses of the antibody of the prior treatment are administered at least two times, at least three times, at least four times, at least five times, at least six times, at least seven times, at least eight times, at least nine times, at least ten times, at least eleven times, at least twelve time, at least thirteen times.

In some embodiments, the subject has received one or more prior treatment for HAE, which may involve any of the therapeutic agent for HAE known in the art. Exemplary anti-HAE agents include, but are not limited to, C1-inhibitors (*e.g.*, Cinryze[®], Berinert[®], or Ruconest[®]), plasma kallikrein inhibitors (*e.g.*, Kalbitor[®]), bradykinin receptor inhibitors (*e.g.*, Firazyr[®]), attenuated androgens (*e.g.*, danazol), and anti-fibrinolytics (*e.g.*, traexamic acid). In some examples, a subject may undergo a tapering period before receiving the anti-pKal antibody treatment as described herein. A tapering period refers to a period, prior to the anti-pKal antibody treatment, during which a subject who is on an anti-HAE treatment (*e.g.*, C1-INH, oral androgen, and/or oral anti-fibrinolytics) gradually reduces the dosage, frequency, or both of the anti-HAE agent such that the subject can gradually transit from the prior HAE treatment to the anti-pKal antibody treatment as described herein. In some embodiments, the tapering involving a gradual or step-wise method of reducing the dosage of the prior treatment and/or the frequency with which the prior treatment is administered. The tapering period may last 2-4 weeks and can vary based on factors of an individual patient. In some examples, the prior treatment terminates before the anti-pKal antibody treatment starts. In other examples, the prior treatment may terminate within a suitable timeframe (*e.g.*, 2 weeks, 3 weeks, or 4 weeks) after the subject is given his or her first dose of the anti-pKal antibody.

Alternatively, a subject who is on a prior HAE treatment may be transitioned to the anti-pKal antibody treatment as described herein directly without the tapering period.

In other embodiments, the subject is free of any prior treatment of HAE before the first treatment, first treatment period, and/or the follow-on single and multiple dose treatments as described herein (the second treatment period). In some embodiments, the subject is free of any treatment other than with the antibodies described herein during the first treatment period and/or during the second treatment period. In some embodiments, the subject is free of any prior treatment of HAE for at least two weeks (*e.g.*, at least two, three, four, five weeks or more) before the first treatment or first treatment period, during the first treatment or first treatment period, and/or during the second treatment period. In some embodiments, the subject is free of long-term prophylaxis for HAE (*e.g.*, C1 inhibitor, attenuated androgens, anti-fibrinolytics) for at least the two weeks prior to the first treatment or first treatment period, during the first treatment period, and/or during the second treatment period. In some embodiments, the subject is free of an HAE treatment involving an angiotensin-converting enzyme (ACE) inhibitor for at least the four weeks prior to the first treatment or first treatment period, during the first treatment period, and/or during the second treatment period. In some embodiments, the subject is free of an estrogen-containing

medication for at least the four weeks prior to the first treatment or first treatment period, during the first treatment period, and/or during the second treatment period. In some embodiments, the subject is free of androgens (*e.g.* stanozolol, danazol, oxandrolone, methyltestosterone, testosterone) for at least the two weeks prior to the first treatment or first treatment period, during the first treatment period and/or during the second treatment period.

Any of the methods described herein may further comprise monitoring the patient for side effects (*e.g.*, elevation of creatine phosphatase levels) and/or inhibition levels of pKal by the antibody (*e.g.*, serum or plasma concentration of the antibody or the pKal activity level) before and after the treatment or during the course of treatment. If one or more adverse effect is observed, the dose of the antibody might be reduced or the treatment might be terminated. If the inhibition level is below a minimum therapeutic level, further doses of the antibody might be administered to the patient. Patients may also be evaluated for the generation of antibody against the administered antibody; activity of C1-inhibitor, C4, and/or C1q; quality of life; incidence of any HAE attacks, health-related quality of life, anxiety and/or depression (*e.g.*, Hospital Anxiety and Depression Scale (HADS)), work productivity (*e.g.*, Work Productivity and Activity Impairment Questionnaire (WPAI)), preference of the subcutaneous administration of the antibody (*e.g.*, D-2930) relative to other injectibles, quality of life (*e.g.*, angioedema-quality of life (AE-QOL), EuroQoL Group 5-dimension report).

In some embodiments, the plasma or serum concentration of the antibody (*e.g.*, DX-2930) may be measured during the course of the treatment (*e.g.*, after the initial dosage) for assessing the efficacy of the treatment. If the plasma or serum concentration of the antibody is lower than about 80 nM, a follow-up dosage may be needed, which may be the same or higher than the initial dosage. The plasma or serum concentration of the antibody may be measured by determining the protein level of the antibody in a plasma or serum sample obtained from the subject, *e.g.*, by an immune assay or MS assay. The plasma or serum concentration of the antibody may also be measured by determining the inhibitory level of pKal in a plasma or serum sample obtained from a subject treated with the antibody. Such assays may include the synthetic substrate assay or the Western blot assay for measuring cleaved kininogen as described herein.

Alternatively or in addition, the plasma or serum level of creatine kinase and/or one or more coagulation parameters (*e.g.*, activated partial thromboplastin time (aPTT), prothrombin time (PT), bleeding events) can be monitored during the course of the treatment. If the plasma or serum level of creatine kinase is found to elevate during the treatment, the dosage

of the antibody may be reduced or the treatment may be terminated. Similarly, if one or more coagulation parameters are found to be significantly affected during the treatment, the dosage of the antibody may be modified or the treatment may be terminated.

In some embodiments, an optimal dosage (*e.g.*, optimal prophylactic dosage or optimal therapeutic dosage) of the antibody (*e.g.*, DX-2930) may be determined as follows. The antibody is given to a subject in need of the treatment at an initial dose. The plasma concentration of the antibody in the subject is measured. If the plasma concentration is lower than 80 nM, the dose of the antibody is increased in a subsequent administration. A dosage of the antibody that maintains the antibody plasma concentration above about 80 nM can be chosen as the optimal dosage for the subject. The creatine phosphokinase level of the subject can be monitored during the course of treatment and the optimal dosage for that subject can be further adjusted based on the creatine phosphokinase level, *e.g.*, the dosage of the antibody might be reduced if elevation of creatine phosphokinase is observed during treatment.

(iii) Combination Therapies

An antibody as described herein (*e.g.*, DX-2930) can be administered in combination with one or more of the other therapies for treating a disease or condition associated with plasma kallikrein activity, *e.g.*, a disease or condition described herein. For example, an antibody as described herein (*e.g.*, DX-2930) can be used therapeutically or prophylactically (*e.g.*, before, during, or after the course of treatment) with another anti-plasma kallikrein Fab or IgG (*e.g.*, another Fab or IgG described herein), another plasma kallikrein inhibitor, a peptide inhibitor, small molecule inhibitor, or surgery. Examples of plasma kallikrein inhibitors that can be used in combination therapy with a plasma kallikrein binding antibodies described herein include plasma kallikrein inhibitors described in, *e.g.*, WO 95/21601 or WO 2003/103475.

One or more plasma kallikrein inhibitors can be used in combination with an antibody as described herein (*e.g.*, DX-2930). For example, the combination can result in a lower dose of the inhibitor being needed, such that side effects are reduced.

An antibody as described herein (*e.g.*, DX-2930) can be administered in combination with one or more current therapies for treating HAE. For example, DX-2930 antibody can be co-used with a second anti-HAE therapeutic agent such as ecallantide, a C1 esterase inhibitor (*e.g.*, CINRYZETM), aprotinin (TRASYLOL[®]), and/or a bradykinin B2 receptor inhibitor (*e.g.*, icatibant (FIRAZYR[®])).

The term “combination” refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlaps in time. The agents or therapies can be administered at the same time (*e.g.*, as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of a plasma kallikrein binding antibody described herein can also be used to reduce the dosage of another therapy, *e.g.*, to reduce the side effects associated with another agent that is being administered. Accordingly, a combination can include administering a second agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of the plasma kallikrein binding antibody. In some embodiments, a subject can be given a C1-inhibitor as a loading IV dose or SC dose simultaneously with the first dose of an anti-pKal antibody (*e.g.*, DX-2930) as described herein. The subject can then continue with the anti-pKal antibody treatment (without further doses of the C1-inhibitor).

A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of a plasma kallikrein associated disease treatment.

(iv) Assays for assessing a treatment regimen

Also within the scope of the present disclosure are assay methods for assessing efficacy of any of the treatment methods described herein. In some embodiments, the plasma or serum concentration of one or more biomarkers (*e.g.*, 2-chain HMWK) associated with HAE may be measured prior to and/or during the course of the treatment (*e.g.*, after the initial dosage) for assessing the efficacy of the treatment. In some embodiments, the plasma or serum concentration (level) of one or more biomarkers associated with HAE obtained at a time point after administration of a dosage is compared to the concentration of the biomarker in a sample obtained at an earlier time point after administration of a dosage or prior to administration of the initial dosage. In some embodiments, the biomarker is 2-HMWK.

The level of the biomarker may be measured by detecting the biomarker in a plasma or serum sample obtained from the subject, *e.g.*, by an immunoassay, such as Western blot assay or ELISA, using an antibody that specifically detects the biomarker. In some embodiments, the level of 2-HMWK in a plasma or serum sample obtained from the subject

is assessed by an immunoassay. Antibodies for use in immunoassays for the detection of 2-HWMK are known in the art and selection of such an antibody for use in the methods described herein will be evident to one of ordinary skill in the art.

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.

EXAMPLES

Example 1: Indirect Treatment Comparison of DX-2930 and Intravenous C1 Esterase Inhibitor Treatments for Long-term Prophylaxis of Hereditary Angioedema Attacks

Study Overview

A systematic literature review was undertaken to identify and summarize the existing evidence for efficacy and safety of long-term prophylactic treatment of patients with type I and type II hereditary angioedema (HAE) for an indirect treatment comparison (ITC). Additionally, data from the HELP study (Banerji et al., *JAMA* 320(20):2108-21, 2018) were analyzed to establish the optimal parametric survival models for use in an ITC with the evidence identified by the systematic literature review. All analyses were conducted using R (R Core Team, 2018, www.R-project.org).

The HELP study assessed the efficacy and safety of subcutaneous lanadelumab (DX-2930) in preventing acute angioedema attacks in patients with type I and type II HAE in which three lanadelumab dosing regimens (300 mg every two weeks [q2w], 300 mg every four weeks [q4w], and 150 mg q4w) were investigated and compared with placebo (Banerji et al., *JAMA* 320(20):2108-21, 2018).

Materials and Methods

A systematic literature search through June 2017 was conducted using MEDLINE, Embase, MEDLINE In-Process, and the Cochrane Library database. A search of conference proceedings was conducted for the years 2016 and 2017 and included EAACI; American College of Allergy, Asthma and Immunology; World Allergy Congress; and European Society for Immunodeficiency. An additional search of key health technology assessment agencies included the National Institute for Health and Care Excellence (NICE), Canadian

Agency for Drugs and Technologies in Health Common Drug Review, Scottish Medicines Consortium, and All Wales Medicines Strategy Group. The search focused on studies for long-term prophylaxis of angioedema attacks in patients with type I or type II HAE who were ≥ 12 years of age based on the criteria described in Table 1B.

Table 1B. Key Inclusion and Exclusion Criteria for Literature Search

Category	Inclusion Criteria	Exclusion Criteria
Population	• Patient with type I and type II HAE • Any race • Age ≥ 12 years	• Healthy volunteers • Pediatric population (age < 12 years) • Type III HAE • Disease other than HAE
Interventions	• Prophylactic treatments (short- or long-term; mono- and/or combination therapy): - Berinert[®] - Cinryze[®] (formerly Ceter) - Lanadelumab (DX-2930) - Danazol - Stanozolol - Oxandrolone - Methyl testosterone - Testosterone	• Nonpharmacologic treatments - Fresh frozen plasma, solvent detergent plasma, antifibrinolytic agents • Acute treatments - Icatibant (Firazyr[®]), ecallantide (Kalbitor[®]), recombinant C1 esterase inhibitor (Ruconest[®]) • Surgery • Studies assessing interventions not in the list
Comparators	No restrictions	
Outcomes	No restrictions	
Study design	• RCTs irrespective of blinding status • Non-RCTs • Observational studies • Single-arm studies • Cohort studies (prospective and retrospective) • Long-term follow-up studies • Systematic reviews and meta-analyses of RCTs/non-RCTs^a	• Case reports, case series • Pharmacokinetic and economic studies • Preclinical studies • Reviews, letters, and comment articles
Language	No restrictions ^b	

HAE, hereditary angioedema; RCT, randomized controlled trial.

^aSystematic reviews and meta-analyses of RCTs and non-RCTs were included and flagged for bibliography screening to determine if literature searches missed any potentially relevant studies.

^bNon-English language publications were included and explored if no sufficient evidence was identified from publications written in English.

The development and reporting of this analysis followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., *PLoS Med.*

6(7):e1000097, 2009). Randomized controlled trials were considered the highest standard of evidence for ITC, and data from nonrandomized studies were also considered if no randomized controlled trial data were available.

Abstracts and full-text articles were evaluated for inclusion by two independent reviewers, and data extraction from the selected literature was performed by one reviewer and independently validated by a second reviewer; any uncertainties were resolved by a third reviewer. A descriptive assessment of the included randomized controlled trials was performed by two independent reviewers using comprehensive assessment criteria based on the recommendations in the NICE manufacturer's submission template (Single technology appraisal (STA): user guide for company evidence submission template. UK: *National Institute for Health and Care Excellence*; www.nice.org.uk/process/pmg24/chapter/instructions-for-companies#quality-assessment-of-the-relevant-randomised-controlled-trials, 2015).

Parametric Survival Models

Kaplan-Meier curves were derived for the time to first HAE attack (defined as all investigator-confirmed attacks) after day 0 and day 70 for each lanadelumab dosing regimen and placebo using patient-level data from the HELP study (Banerji et al., *JAMA* 320(20):2108-21, 2018). A univariate Cox proportional hazards model with treatment as the only covariate was fit to estimate the relative treatment effect (*i.e.*, HR) for each lanadelumab dose compared with placebo.

To extrapolate long-term survival curves to be used in the NMA, standard parametric survival models (Exponential, Weibull, log-normal, log-logistic, Gompertz, and generalized gamma survival models) (Latimer, Decision Support Unit, National Institute for Health and Clinical Excellence; nicedsu.org.uk/wp-content/uploads/2016/03/NICE-DSU-TSD-Survival-analysis.updated-March-2013.v2.pdf, 2011) were fit to the Kaplan-Meier data. If the standard models did not fit to the observed data, a more flexible proportional hazards spline model was considered, which is an extension of the standard Weibull model that models the log cumulative hazard as a natural cubic spline function of log time (Royston et al., *Stat Med.* 21(15):2175-97, 2002). Methods employed to assess the fit of the model were clinical plausibility of extrapolations, goodness-of-fit measures, and visual inspection by overlaying fitted survival curves with observed survival data.

Attack Rate and Time to First HAE Attack

Outcomes of interest for this ITC were event (HAE attack) rate and time to event (first HAE attack). Attack rate was defined as the number of attacks experienced in a 28-day cycle, with relative treatment effects estimated as rate ratios (RRs). Time to first attack was defined as the time a patient with HAE had their first attack after day 0 (date of first administered dose of prophylactic therapy) or day 70 (approximate day at which steady state plasma lanadelumab concentration is reached). For time to first attack, relative treatment effects were estimated as hazard ratios (HRs).

A Bayesian NMA, relying on Markov chain Monte Carlo methods, was developed to evaluate the outcomes of attack rate and time to first attack. Relative efficacy was estimated using a treatment effect model to allow synthesis of direct and indirect evidence in one analysis while accounting for correlation arising from multi-arm trials (Dias et al., Decision Support Unit, National Institute for Health and Clinical Excellence; nicedsu.org.uk/wp-content/uploads/2017/05/TSD2-General-meta-analysis-corrected-2Sep2016v2.pdf, 2011). Some studies in the evidence network did not report time to first attack. However, because the proportion of patients who had not experienced an HAE attack was reported for every study, an extension of the NMA method was used to synthesize the count data and time-to-event data in the same analysis (Woods et al., *BMC Med Res Methodol.* 10:54, 2010). Treatment effects were compared using credible intervals (CrIs); for cases in which the CrI for treatment versus placebo did not include the value 1, the results were considered statistically significant. Both HELP and CHANGE were randomized, double-blind trials in patients with confirmed HAE (Banerji et al., *JAMA.* 320(20):2108-21, 2018; Zuraw et al., *N Engl J Med.* 363(6):513-22, 2010). Fixed- and random-effects models were considered for the NMA, and the choice of model was based on an assessment of study design, inclusion criteria, and patient characteristics.

Predicted Survival Curves

Predicted survival curves were derived for each treatment arm separately for time to first attack after day 0 and day 70 by combining the HRs from the ITC and the spline survival curve fitted to the placebo data from the HELP study. These curves were used to estimate the proportion of attack-free patients and 95% confidence intervals (CIs) at 60 months after the start of prophylactic treatment (day 0 or day 70) for each comparator in the ITC.

Results

Systematic Literature Review

A systematic literature review was undertaken. A summary of the records identified is presented in Fig. 1. The search identified 1299 records, of which 52 (22 records from seven randomized controlled trials [RCTs] and 30 records from 23 nonrandomized controlled trials [nRCTs]) met the eligibility criteria and were selected for detailed feasibility assessment for possible inclusion in the ITC with lanadelumab results from the HELP study. Twenty-two records were excluded because they were secondary publications for which a primary publication had already been identified among the 52 records that met the eligibility criteria. Of the remaining records in the feasibility assessment, 20 lacked real-world applicability and were therefore excluded as inappropriate comparators. Included in these were two RCTs (Gelfand et al., *N Engl J Med.* 295(26):1444-8, 1976; Sheffer et al., *Ann Int Med.* 86(3):306-8, 1977) and 15 nRCTs (Füst et al., *Eur J Clin Invest.* 41(3):256-62, 2011; Agostoni et al., *Medicine (Baltimore)*. 71(4):206-15, 1992; Bork et al., *Ann Allergy Asthma Immunol.* 100(2):153-61, 2008; Caminoa et al., *Allergy.* 68(suppl 97):61, 2013; Cicardi et al., *J Allergy Clin Immunol.* 99(2):194-6, 1997; Davis et al., *Johns Hopkins Med J.* 135(6):391-8, 1974; Farkas et al., *J Oral Maxillofac Surg.* 57(4):404-8, 1999; Kreuz et al., *Transfusion.* 49(9):1987-95, 2009; Obtulowicz et al., *Int Rev Allergol Clin Immunol.* 3(3):163-6, 1997; Ott et al., *Clin Endocrinol (Oxf).* 66(2):180-4, 2007; Psarros et al., *Int Arch Allergy Immunol.* 164(4):326-32, 2014; Rosen et al., *Birth Defects: Orig Artic Ser.* 16(1):499-507; 1980; Steiner et al., *Orphanet J Rare Dis.* 11:43; 2016; Winnewisser et al., *J Int Med.* 241(1):39-46, 1997; Zotter et al., *Orphanet J Rare Dis.* 9:205, 2014) describing the use of attenuated androgens and one RCT (Riedl et al., *Allergy Asthma Proc.* 37(6):489-500, 2016) describing the use of a combination therapy of SC C1-INH/recombinant human hyaluronidase in patients with HAE, since these interventions are not recommended by the WAO/EAACI guidelines for HAE management as long-term prophylaxis (Maurer et al., *Allergy.* 73(8):1575-96, 2018); two RCTs (Longhurst et al., *N Engl J Med.* 376(12):1131-40, 2017; Zuraw et al., *Allergy.* 70(10):1319-28, 2015) describing the use of SC C1-INH were excluded because this product is not commercially available outside the United States and Canada (CSL Behring. Global product list; www.cslbehring.com/products/global-products-list; retrieved 2019 Feb. 20), limiting the real-world applicability of any comparison.

One record reported results from a phase 3 study of an IV C1-INH (Zuraw et al., *N Engl J Med.* 363(6):513-22, 2010) and was deemed appropriate for ITC with lanadelumab

results from the HELP study. Because this RCT was appropriate for ITC, and because nRCTs were only considered if no RCTs for a given intervention could be identified, the four nRCTs for this intervention (Bernstein et al., *J Allergy Clin Immunol Pract.* 2(1):77-84 2014; Aygören-Pürsün et al., *J Allergy Clin Immunol.* 137(2):AB251, 2016; Rasmussen et al., *Ann Allergy Asthma Immunol.* 116(5):476-7, 2016; Zuraw et al., *Am J Med.* 125(9):938.e1-.e7, 2012) were subsequently excluded. The remaining five studies (Banerji et al., *N Engl J Med.* 376(8):717-28, 2017; Bork et al., *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 112(1):58-64, 2011; Bouillet et al., *Allergy.* 72(Suppl 103):593, 2017; Farkas et al., *Allergy.* 67(12):1586-93, 2012; Levi et al., *J Allergy Clin Immunol.* 117(4):904-8, 2006) were excluded because of differences and/or lack of clarity in study design, study population, intervention, endpoints, and/or patient characteristics (Table 2). The network diagram for the two studies included in the ITC is shown in Fig. 2. The design for each study is summarized in Table 3, and baseline demographic characteristics and results for primary outcomes for both studies are presented in Table 4.

Table 2. Summary of Feasibility Assessment of Select Records Identified by Systematic Literature Review for Potential Inclusion in the Indirect Treatment Comparison^a

Study	Study Design	Patients, n	Treatment, Dose, Regimen	Key Criteria for Inclusion/Exclusion From Indirect Treatment Comparison
Banerji 2017 [64]	Randomized, double-blind, placebo-controlled clinical trial	11	Lanadelumab 400 mg q2w SC	Excluded based on study design and endpoints: • Phase 2 dose-escalation study • Outcomes focused on PK/PD • Attack rate measured as adverse event up to 120 days after final dose
		5	Lanadelumab 300 mg q2w SC	
		4	Lanadelumab 100 mg q2w SC	
		4	Lanadelumab 30 mg q2w SC	
		13	Placebo	
Levi 2006 [68]	Nonrandomized clinical trial	43	IV C1-INH (Cetor [®] 1000 units every 5–7 days)	Excluded based on intervention and lack of endpoint data: • Attack rate is reported only graphically, no values are available
Bork 2011 [65]	Retrospective, observational study	33	IV C1-INH (Berinert [®] 500 U; single dose)	Excluded based on study population: • Investigated as short-term prophylaxis for prevention of an attack following a surgical procedure
		18	IV C1-INH (Berinert [®] 1000 U; single dose)	

Study	Study Design	Patients, n	Treatment, Dose, Regimen	Key Criteria for Inclusion/Exclusion From Indirect Treatment Comparison
Bouillet 2017 [66]	Retrospective, observational study	132	IV C1-INH (Berinert®) ^b	Excluded based on study population: • Used only as an acute treatment after an attack
Farkas 2012 [67]	Retrospective, observational study	87	IV C1-INH (Berinert® 500 U; single dose)	Excluded based on study population: • Investigated as short-term prophylaxis for prevention of an attack following a dental, diagnostic, or surgical procedure
		38	Danazol (oral; 2.5–10 mg/kg per day for 5 days)	
		9	Tranexamic acid (oral; 20–40 mg/kg per day for 5 days)	

C1-INH, C1 esterase inhibitor; IV, intravenous; PD, pharmacodynamics; PK, pharmacokinetics; q2w, every 2 weeks; SC, subcutaneous.

^aDoes not include assessment of the feasibility of records that were included in the ITC (n=1) or excluded as secondary publications (n=22), inappropriate comparators (n=20), or unnecessary nRCTs (n=4).

^bDetailed dosing information was not reported.

Table 3. Trial Design of the HELP and CHANGE Studies Used for Indirect Treatment Comparison

Characteristic	HELP Study ^b	CHANGE Study ^a
Study design	Double-blind, placebo-controlled	Double-blind, placebo-controlled
Trial type	Parallel	Crossover ^a
Disease state inclusion criteria	- Confirmed HAE diagnosis - C1-INH functional level <40% or 40%–50% if C4 level below normal range ≥1 of the following: - Age ≤30 years at reported onset of first angioedema symptoms - Family history consistent with HAE types I or II - C1q level within normal range - Experience baseline rate of ≥1 investigator-confirmed HAE attack per four weeks during run-in period	- Confirmed HAE diagnosis - Low antigenic or functional C1-INH level or known HAE-causing mutation in <i>C1-INH</i> gene - Low C4 level - Normal C1q level ≥2 attacks per month (for prophylaxis study)
Primary endpoint	Number of investigator-confirmed attacks (day 0–182)	Number of attacks during each treatment period (normalized to number of days participated)
Administration	Subcutaneous	Intravenous
Washout period	≥2 weeks	None
Treatment period	26 weeks	12 weeks ^a

C1-INH, C1 esterase inhibitor; HAE, hereditary angioedema.

^aThe CHANGE study included two parts: part A, which evaluated IV C1-INH use in acute attacks, and part B, which evaluated its use in prophylaxis. Only part B is summarized here and was a crossover study that consisted of two 12-week treatment periods in which patients were randomly assigned to receive either IV C1-INH or placebo during the first treatment period and then crossed

over, in a second treatment period, to the treatment that was not received during the first treatment period (Zuraw et al., *N Engl J Med.* 363(6):513-22, 2010).

^bBanerji et al., *JAMA* 320(20):2108-21, 2018

Table 4. Demographic Characteristics and Primary Outcomes From the HELP and CHANGE Studies Used for Indirect Treatment Comparison

Characteristic	HELP Study ^b				CHANGE Study ^a	
	Lanadelu mab 300 mg q2w (n=27)	Lanadelu mab 300 mg q4w (n=29)	Lanadelu mab 150 mg q4w (n=28)	Placebo (n=41)	IV C1-INH (n=11)	Placebo (n=11)
Mean (SD) age, y	40.3 (13.3)	39.5 (12.8)	43.4 (14.9)	40.1 (16.8)	41.7 (19.3)	34.5 (14.8)
Female patients, n (%)	15 (55.6)	19 (65.5)	20 (71.4)	34 (82.9)	9 (81.8)	11 (100)
Type II HAE, n (%)	4 (14.8)	2 (6.9)	3 (10.7)	3 (7.3)	2 (18.2)	2 (18.2)
Patients with ≥ 2 attacks/month prior to study entry, n (%)	20 (74.1)	20 (69.0)	18 (64.3)	29 (70.7)	11 (100)	11 (100)
Primary endpoint						
Rate of investigator-confirmed HAE attacks per 4 wk (day 0–182), LS mean (95% CI)	0.26 (0.15, 0.46)	0.53 (0.36, 0.77)	0.48 (0.31, 0.74)	1.97 (1.64, 2.36)	NA	NA
Change vs placebo, % (95% CI)	–86.9 (–92.8, –76.2)*	–73.3 (–82.4, –59.5)*	–75.6 (–84.7, –61.2)*	NA	NA	NA
Rate of investigator-confirmed HAE attacks per 4 wk (day 70–182), LS mean (95% CI)	0.16 (0.07, 0.35)	0.37 (0.22, 0.60)	0.42 (0.26, 0.68)	1.88 (1.54, 2.30)	NA	NA
Change vs placebo, % (95% CI)	–91.5 (–96.1, –81.1)*	–80.6 (–88.5, –67.3)*	–77.6 (–86.7, –62.3)*	NA	NA	NA
HAE attack rate per 12 wk, mean ^a	NA	NA	NA	NA	6.26	12.73
Change vs placebo, mean (95% CI)	NA	NA	NA	NA	–6.47 (–4.21, –8.73)*	NA

C1-INH, C1 esterase inhibitor; CI, confidence interval; HAE, hereditary angioedema; IV, intravenous; LS, least squares; NA, not applicable; q2w, every two weeks; q4w, every four weeks; SD, standard deviation.

^aAn attack was defined as a discrete episode during which the subject progressed from no angioedema to symptoms of angioedema; attacks that progressed from one site to another, or that began to regress and then became worse before complete resolution, were considered to be a single attack (Zuraw et al., *N Engl J Med.* 363(6):513-22, 2010).

^bBanerji et al., *JAMA* 320(20):2108-21, 2018

* $P < 0.001$ vs placebo.

Time to First Attack (HELP Study)

As demonstrated in the HELP study, time to first HAE attack was increased relative to placebo after each time point for all three lanadelumab dosing regimens, corresponding to reduced risks of experiencing a first attack after day 0 and day 70 from the start of treatment. After day 0, the median number of days to first attack was 59 (95% CI: 28–not estimable [NE]) for patients receiving lanadelumab 300 mg q2w; 28 (95% CI: 10–101) for lanadelumab 300 mg q4w; and 26 (95% CI: 11–NE) for lanadelumab 150 mg q4w, compared with 8 (95% CI: 6–18) for patients receiving placebo. After day 70, more than 50% of patients receiving lanadelumab 300 mg q2w and 150 mg q4w had no attacks through the end of the 6-month treatment period; thus, the median number of days to first attack was NE. The median time to first attack after day 70 in patients receiving lanadelumab 300 mg q4w was 61 days (95% CI: 25–NE) compared with 12 days (95% CI: 6–16) for patients receiving placebo.

As observed in the HELP study, 2.4% of patients receiving placebo were attack-free from day 0 to the end of the treatment period compared with 44%, 31%, and 39% of patients receiving lanadelumab 300 mg q2w, 300 mg q4w, and lanadelumab 150 mg q4w, respectively (Table 5). Similar or greater percentages of attack-free patients were observed with lanadelumab treatment from day 70 to the end of the treatment period. Proportions of attack-free lanadelumab-treated patients were also derived from the Kaplan-Meier estimates of time to first attack after treatment to confirm the validity of the Kaplan-Meier survival analysis. These estimated percentages of attack-free patients were generally consistent with the observed results from each day 0 and day 70 to the end of the 6-month treatment period (Table 5).

Table 5. Observed and Estimated Percentages of Attack-Free Patients in the HELP Study After Day 0 and Day 70 to End of Treatment

	Lanadelumab 300 mg q2w (n=27)	Lanadelumab 300 mg q4w (n=29)	Lanadelumab 150 mg q4w (n=28)	Placebo (n=41)
Attack-free patients, n (%)				
After Day 0 to End of Treatment				
Observed, n (%)	12 (44.4)	9 (31.0)	11 (39.3)	1 (2.4)
Estimated, n (%) [95% CI] ^a	27 (44.4 [29.2, 67.8])	29 (31.0 [18.0, 53.4])	28 (39.3 [24.8, 62.3])	41 (2.4 [0.4, 16.9])
After Day 70 to End of Treatment				
Observed, n (%) ^b	20 (76.9)	13 (44.8)	15 (53.6)	1 (2.7)

Estimated, n (% [95% CI]) ^{a,b}	26 (76.9 [62.3, 94.9])	29 (43.0 [28.0, 65.8])	28 (53.6 [37.9, 75.6])	37 (2.7 [0.4, 18.7])
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CI, confidence interval; q2w, every two weeks; q4w, every four weeks.

^aKaplan-Meier estimates of time to first attack after treatment were used to derive the proportions of patients who remained attack-free.

^bDoes not include patients who discontinued prior to day 70: lanadelumab 300 mg q2w, n=26; lanadelumab 300 mg q4w, n=29; lanadelumab 150 mg q4w, n=28; placebo, n=37.

Model Selection for Time to First Attack Endpoints

Various parametric survival models were applied to data for time to first attack after days 0 and 70. Of the six standard parametric models, the Akaike information criterion (AIC) values indicated that the Gompertz model fit the Kaplan-Meier data best for the duration of the study (*i.e.*, after day 0 and day 70). The Gompertz model predicted that patients who were attack-free from day 0 through the end of the study would remain attack-free indefinitely after the study ended. Conservatively based on the possibility of pharmacological drug tolerance and loss of response in which treatment effectiveness may lessen with prolonged exposure (Roda et al., *Clin Transl Gastroenterol.* 7:e135, 2016; Salvà Lacombe et al., *Drugs.* 51(4):552-70, 1996), however, the Gompertz model was considered clinically implausible.

Thus, because none of the standard distributions for parametric survival analysis fit the observed data well, the more flexible parametric model of a proportional hazards spline was fit to the data. A spline model with one internal knot (k=1) was found to fit the data best, and this was confirmed based on visual comparison with the Kaplan-Meier data and clinical plausibility of a reduction over time in the relative effect of each lanadelumab dose versus placebo. The addition of further internal knots (k=2–5) to the spline model did not lead to corresponding improvement in model fit.

Indirect Treatment Comparison via Network Meta-analysis

A fixed-effects model was chosen for the NMA because no systematic differences were identified between the patient populations in the HELP study and CHANGE trial; both patient populations were similar in age, sex, and percentage of patients with type I or type II HAE (Tables 3 and 4, above). It was therefore assumed that each trial was estimating the same treatment effect and any variation between studies was due only to sampling variation.

Attack Rate

RRs showed a significant reduction in attack rate for patients who received lanadelumab (all dosing regimens) or IV C1-INH compared with placebo (Fig. 3).

Reductions in attack rate (per 4-week cycle) were 87%, 73%, and 76% for the lanadelumab 300 mg q2w, 300 mg q4w, and 150 mg q4w treatment arms, respectively (corresponding to respective median RRs [95% CrI] of 0.13 [0.07–0.24], 0.27 [0.18–0.40], and 0.24 [0.15–0.39]) and 51% for IV C1-INH (corresponding to median RR [95% CrI] of 0.49 [0.40–0.60]) compared with placebo.

Time to First Attack

When comparing treatments with placebo, a significantly lower risk of first attack after day 0 was observed for all lanadelumab dosing regimens (median HR [95% CrI]: 300 mg q2w, 0.27 [0.13–0.55]; 300 mg q4w, 0.39 [0.21–0.74]; 150 mg q4w, 0.34 [0.17–0.66]), corresponding to a 61%–73% reduction in risk of first attacks after day 0 (Fig. 4A). Results were similar for risk of first attacks after day 70 (median HR [95% CrI]: 300 mg q2w, 0.09 [0.04–0.22]; 300 mg q4w, 0.27 [0.14–0.53]; 150 mg q4w, 0.20 [0.10–0.41]), with corresponding reductions of 73%–91% (Fig. 4B). In contrast, although a reduction in the risk of first attack was observed with IV C1-INH treatment compared with placebo, these reductions were not statistically significant (median HR [95% CrI]: day 0, 0.54 [0.23–1.19]; day 70, 0.53 [0.23–1.19]).

Predicted Survival Curves for 60-month Duration

Predicted survival curves for 60-month duration based on time to first attack were derived for each treatment after day 0 and day 70 (Figs. 5A-5B) by combining the HRs from the ITC and the spline survival curve fit to the HELP study placebo data. The highest predicted proportions of attack-free patients were observed with lanadelumab 300 mg q2w over both time points. When data for time to first attack after day 0 were extrapolated out to 60 months, the predicted percentages of attack-free patients were 26%, 6.5%, and 0.6% for treatment with lanadelumab 300 mg q2w, IV C1-INH, and placebo, respectively. When time to first attack data were extrapolated out to 60 months, the predicted percentages of attack-free patients were 46%, 0.9%, and 0% after day 70 for lanadelumab 300 mg q2w, IV C1-INH, and placebo, respectively.

A proportional hazards spline model with one internal knot ($k=1$) was found to best fit observed time to first attack data from the HELP study. These data demonstrated that a key benefit for receiving lanadelumab compared with placebo for prophylaxis of HAE attacks was the increase in duration of attack-free period. The highest predicted proportions of attack-free patients were observed with lanadelumab 300 mg q2w over all time points,

whereas the lowest predicted proportions of patients who were attack-free were observed with IV C1-INH and placebo. Based on the approximately 14-day half-life of lanadelumab (range, 13.8–15.0 days), time to steady-state concentrations of lanadelumab has been estimated to be approximately 70 days. Thus, after day 70, when lanadelumab had reached steady-state concentration, more than 50% of patients in the lanadelumab 300-mg q2w treatment arm did not have an attack during the rest of the 6-month study period, and the median number of days to first attack after day 70 in the 300-mg q4w arm was 61 compared with 12 in the placebo arm. Based on the predicted survival curves, the proportion of attack-free patients after the first six months of treatment with lanadelumab 300 mg q2w (45% and 72%) increased as time to first attack was assessed later (after day 0 and day 70, respectively); a similar pattern was observed with the lanadelumab 300 mg q4w (31% and 37%, respectively) and 150 mg q4w (36% and 47%, respectively) treatment arms, but patients receiving C1-INH showed an opposite pattern of decreases (20% and 13%, respectively) with subsequent assessment. These findings remained generally consistent upon extrapolation of these data out to 60 months.

Accordingly, HAE patients on lanadelumab treatment for a period (*e.g.*, 6 months) and showed free of HAE attack may receive a reduced dosage of lanadelumab, for example, from 300 mg/2 weeks to 300 mg/4 weeks.

Example 2: Indirect Treatment Comparison of DX-2930 and Intravenous C1 Esterase Inhibitor Treatments Using Individual Patient Data from the HELP-03 and CHANGE Studies

Objectives

The objective was to perform an indirect treatment comparison (ITC) of lanadelumab (300 mg subcutaneous every 2 weeks (300 mg SC q2w), 300 mg subcutaneous every four weeks (300 mg SC q 2w)) and Cinryze® (C1-INH) 1000 units intravenous (1000 U IV) using individual patient data (IPD) from the HELP-03 (HELP) and CHANGE studies. Using IPD in lieu of aggregated data is advantageous for several reasons. First, IPD provides more information, which reduces heterogeneity across networks and resolves potential issues of inconsistency. Further, IPD facilitates estimation of subgroup effects, aids convergence, and overall, yields more precise estimates. Additionally, IPD allows within-study associations to be distinguished from across-study associations. Utilizing IPD, even only from a few studies,

can also reduce ecological bias, which is often problematic when the evidence is sparse and the sample sizes are small.

Outcomes of interest

Both the HELP and CHANGE studies cited the number of HAE attacks per 28 days. A prior study by BresMed reported the time to first HAE attack following treatment at day 0 and 70 in the HELP study. This outcome measure was estimated using IPD reported in the CHANGE study. Only the HELP study disclosed the proportion of patients who experience $\geq 50\%$ reduction in the number of attacks. While the CHANGE study did not report this outcome measure, it was not considered relevant for the ITC.

Conclusion

Overall, the similarity assumption was not deemed to hold between the HELP and CHANGE studies with respect to the baseline HAE attack rate per 28 days. This was confirmed by expert clinician opinion. Other covariates of interest (*e.g.*, age, gender, and weight) also differed between the studies, but these were considered to have a lesser impact on the results. Therefore, it was determined that the base case analysis would account for HAE attack rate per 28 days at baseline, while regression models in sensitivity analyses would include other key covariates which might potentially modify treatment effects. Any differences between the base case and sensitivity analysis findings might indicate that the results of the base case were not robust and may possibly be driven by the study differences. In this case, a second sensitivity analysis is planned to evaluate whether an assessment of attacks per patient or investigators influences the results of the attack rates per 28 days. To account for this, the analysis will include only patient-reported HAE attacks in both studies.

Common comparator

As shown in the network of evidence in Fig. 6, the common comparator for Cinryze[®] (C1-INH) and lanadelumab in the HELP and CHANGE studies was placebo. The HELP study compared three different lanadelumab dose regimens to placebo, whereas the CHANGE study was a cross-over study comparing Cinryze[®] to placebo. Excluding the lanadelumab 150 mg dose (not approved for this indication) reduced the number of comparisons and circumvented the issue of multiplicity (*e.g.*, multiple hypothesis testing). If multiple hypotheses are tested simultaneously, the alpha error increases, and therefore the significance level requires adjustment (*e.g.*, Bonferroni). Identifying statistically significant

differences at the significance level $<5\%$ may be difficult. Therefore, it is advised that multiple hypothesis testing be avoided.

Methods

Statistical approaches to evidence synthesis can be characterized primarily as frequentist or Bayesian methods. Frequentist methods, such as adjusted indirect comparisons (*e.g.*, Bucher method, matched-adjusted indirect comparison [MAIC], and simulated treatment comparison [STC]), allow the indirect comparison of two interventions in a single step. The term network meta-analysis (NMA) refers to the simultaneous comparison of a larger network of interventions. In a Bayesian method, minimally informative priors are usually assigned treatment effects.

For the purposes of this ITC, a frequentist approach was preferred over a Bayesian approach due to the limited evidence base ($N=2$ studies). Currently, various frequentist approaches for including IPD in statistical models for evidence synthesis exist. These methods differ mainly on the basis of inclusion or exclusion of a common comparator arm (“anchor”).

As a second step, the Bucher method is often used to combine the relative treatment effects in anchored comparisons for obtaining the final ITC results. Only two interventions may be compared at once. If multiple comparisons are of interest, multiple analyses must be conducted. The corresponding point estimates are expected to be very similar to those obtained through a Bayesian analysis. However, the 95% confidence intervals are usually not as wide as the credible intervals of a Bayesian analysis.

Following an extensive feasibility assessment, the present ITC was performed using a frequentist-based anchored approach. Importantly, this approach preserved randomization. In the base case analysis, regression models included the HAE attack rate per 28 days at baseline. Other covariates of interest (*e.g.*, age, gender, and weight) were added in sensitivity analyses to investigate the overall impact of these variables on the results. A second sensitivity analysis evaluated the impact of patient-reported HAE attacks not confirmed by investigators on the base case results.

Poisson regression - HAE attack rate per 28 days

Poisson regression analyses were performed to estimate rate ratios (RRs) for the HAE attack rates per 28 days. Poisson regression requires that the following assumptions are met:

(1) observations are independent, (2) the counts follow a Poisson distribution, and (3) the conditional mean and variance of the model are identical.

Given that the outcome of interest (HAE attack rate per 28 days) is expressed as count data, Poisson regression was deemed appropriate, and the number of attacks per treatment period was considered in the models. Following Banjeri et al., the models included an offset variable in order to adjust for differences in follow-up time. The offset was defined as the logarithm of the number of days a patient was observed during the treatment period.

Measures of treatment effects (model inputs) were expressed as the estimated RR of attacks per 28 days. For the lanadelumab regimens (300 mg q2w and 300 mg q4w), this measure was represented as the ratio of the rate of attacks per 28 days during the respective treatment relative to that with placebo. For Cinryze® (C1-INH), the RR indicated the ratio of the rate of attacks per 28 days during treatment with Cinryze® relative to that with placebo. The estimated RRs generated by the Poisson models were then input into the ITC.

Non-parametric estimation and Cox regression

For the outcome “time to first attack” a Cox regression analysis was conducted to estimate the hazard ratio (HR) of first attack with each of the lanadelumab regimens (300 mg q2w and 300 mg q4w) relative to placebo, and the HR of a first attack with Cinryze® relative to placebo. As described, the estimated HRs were then input into the ITC (via the Bucher method) comparing the lanadelumab dose regimens and Cinryze®. While Cox regression is semi-parametric and therefore, does not specify requirements regarding the form of the baseline hazard, the following assumptions must be met: (1) the proportional hazard, (2) linear relationship between covariates, and (3) independent observations.

Kaplan-Meier curves were derived via non-parametric estimation to model the outcome “time to first attack after 0 and 70 days”. Patients were censored at the date of their last data capture. The graphs showed that the hazard functions were proportional over time and therefore, statistical measures to account for non-proportional hazards were not required.

In the base case, the model was adjusted for the number of HAE attacks per 28 days at baseline. For the sensitivity analyses, the model also included relevant covariates (age, gender, and weight). Due to the small sample size in the CHANGE study, no formal variable selection was conducted. Estimates obtained from these adjusted models were compared to unadjusted estimates from a model excluding the covariates.

Since the CHANGE study was a cross-over study, individuals who were observed twice (under placebo and Cinryze® treatment) were not independent. As described, mixed

models, including fixed factors for treatment, period, and sequence, and a random effect for study subjects, were employed to account for repeated measurements in the study.

Results

1. HAE attack rate per 28 days

Table 6 shows that the respective baseline attack rates for placebo and Cinryze[®] treatment were identical. The CHANGE cross-over study did not include a run-in period. The attack rate per 28 days was higher with placebo during the treatment period compared to the baseline attack rate with Cinryze[®] treatment. The mean number of attacks per treatment period was considerably lower for Cinryze[®] than for placebo. Treatment duration was comparable between the two interventions. The time to first attack after 0 and 70 days of treatment was considerably shorter with placebo than with Cinryze[®] treatment.

Table 6. Descriptive Statistics on HAE Attacks in the CHANGE Study.

Placebo Treatment					
Variable	Number (N)	Mean	Standard Deviation	Minimum	Maximum
Baseline rate	22	3.80	1.95	1.84	8.00
Rate per 28 days during treatment period	22	4.24	1.55	1.98	6.83
Number of attacks per treatment period	22	12.73	4.80	6.00	22.00
Duration of treatment period (days)	22	84.05	5.96	67.00	96.00
Time to first attack after 0 days of treatment	22	4.77	7.85	0.00	32.00
Time to first attack after 70 days of treatment	21	3.48	3.41	0.00	14.00
Cinryze[®] Treatment					
Baseline rate	22	3.80	1.95	1.84	8.00
Rate per 28 days during treatment period	22	2.09	1.85	0.00	5.88
Number of attacks per treatment period	22	6.14	5.43	0.00	17.00

Duration of treatment period (days)	22	80.05	10.42	34.00	86.00
Time to first attack after 0 days of treatment	22	20.68	28.16	0.00	82.00
Time to first attack after 70 days of treatment	21	7.10	4.30	0.00	14.00

Table 7. Descriptive Statistics on HAE Attacks in the HELP Study.

Placebo Treatment					
Variable	Number (N)	Mean	Standard Deviation	Minimum	Maximum
Baseline rate	41	4.02	3.26	0.97	14.67
Rate per 28 days during treatment period	41	2.45	2.08	0.00	8.31
Number of attacks per treatment period	41	13.95	12.01	0.00	54.00
Duration of treatment period (days)	41	168.39	45.37	13.00	197.00
Time to first attack after 0 days of treatment	41	21.41	34.45	2.00	183.00
Time to first attack after 70 days of treatment	37	21.43	28.68	1.00	113.00
Lanadelumab 300 mg q4w					
Baseline rate	29	3.71	2.51	0.97	10.50
Rate per 28 days during treatment period	29	0.60	0.80	0.00	2.91
Number of attacks per treatment period	29	3.62	4.80	0.00	19.00
Duration of treatment period (days)	29	178.17	21.68	73.00	188.00
Time to first attack after 0 days of treatment	29	74.90	77.96	1.00	185.00
Time to first attack after 70 days of treatment	29	62.62	46.18	2.00	115.00

70 days of treatment					
Lanadelumab 300 mg q2w					
Baseline rate	27	3.52	2.33	0.97	9.00
Rate per 28 days during treatment period	27	0.31	0.50	0.00	1.85
Number of attacks per treatment period	27	1.70	2.83	0.00	12.00
Duration of treatment period (days)	27	177.74	28.56	35.00	186.00
Time to first attack after 0 days of treatment	27	97.04	80.53	1.00	186.00
Time to first attack after 70 days of treatment	26	92.42	40.04	6.00	116.00

As shown in Table 7, both lanadelumab 300 mg q2w and 300 mg q4w reduced the mean number of attacks per treatment period compared to placebo. The mean baseline attack rates were comparable across the three study arms. Treatment duration was shortest in the placebo arm, while the time to first attack was longer with lanadelumab treatment (300 mg q2w and 300 mg q4w) compared to placebo.

HAE attack rate per 28 days – Poisson regression

Further, the estimated mean HAE attack rate per 28 days was 1.99 (95% CI: 1.56; 2.52) with Cinryze[®] treatment and 4.03 (95% CI: 3.31; 4.91) with placebo. The corresponding RR (0.49) indicated that patients treated with Cinryze[®] had 0.49 times the rate of HAE attacks compared to those treated with placebo. This difference was statistically significant (since the corresponding 95% CI does not include 1). The resulting percent change for the mean attack rate was -51% for patients treated with Cinryze[®] relative to placebo.

Table 8. Base Case Results in the CHANGE Study.

			95% CI	
	Treatment	Estimate	Lower	Upper
Model-based treatment	C1-INH	1.99	1.56	2.52

period HAE attack rate (attacks/28 days)	Placebo	4.03	3.31	4.91
Rate ratio		0.4926	0.3950	0.6142
% change in mean attack rate		-51%	-60%	-39%

As shown in Table 9, the estimated mean HAE attack rate per 28 days was 0.26 (95% CI: 0.15; 0.45) with lanadelumab 300 mg q2w, 0.54 (95% CI: 0.37, 0.77) with lanadelumab q2w, and 2.0 (95% CI: 1.69; 2.38) with placebo treatment. The corresponding RRs indicated that patients treated with lanadelumab 300 mg q2w and lanadelumab q4w had 0.13 and 0.27 times the rate of HAE attacks, respectively, compared to patients treated with placebo. These estimates were statistically significant as evidenced by exclusion of the value “1” in the corresponding 95% CIs. The resulting percent change in mean attack rate was -87% for patients treated with lanadelumab 300 mg q2w and -73% for those treated with lanadelumab 300 mg q2w relative to placebo.

Table 9. Base Case Results in the HELP Study.

			95% CI	
	Treatment	Estimate	Lower	Upper
Model-based treatment period HAE attack rate (attacks/28 days)	Lanadelumab 300 mg q2w	0.26	0.15	0.45
	Lanadelumab 300 mg q4w	0.54	0.37	0.77
	Placebo	2.00	1.69	2.38
Rate ratio	Lanadelumab 300 mg q2w vs. placebo	0.1309	0.0742	0.2309
	Lanadelumab 300 mg q4w vs. placebo	0.2674	0.1803	0.3965
% change in mean attack rate	Lanadelumab 300 mg q2w vs. placebo	-87%	-93%	-77%
	Lanadelumab	-73%	-82%	-60%

	300 mg q4w vs. placebo			
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Indirect comparison using the Bucher method (Table 10) showed that patients treated with lanadelumab 300 mg q2w and lanadelumab 300 mg q4w had 0.27 times and 0.54 times the rate of HAE attacks, respectively, compared to those treated with Cinryze®. These results were statistically significant as evidenced by exclusion of the value “1” in the corresponding 95% CIs. The resulting percent changes in the mean attack rate relative to Cinryze® were -73% and -46% for patients treated with lanadelumab 300 mg q2w and lanadelumab 300 mg q4w, respectively. The point estimates and credible/confidence intervals generated in this ITC of IPD appeared consistent with the findings in Bayesian NMA.

Table 10. Indirect Comparison of Lanadelumab and Cinryze® using the Bucher Method (Base Case).

		ITC			Bayesian NMA
Treatment 1	Treatment 2	RR	95% CI Lower	95% CI Upper	RR
Lanadelumab 300 mg q2w	Cinryze®	0.2657	0.1451	0.4864	0.27 (0.14; 0.51)
% change in mean attack rate -73% (CI: -85%; -51%)					
Lanadelumab 300 mg q4w	Cinryze®	0.5429	0.3478	0.8473	0.54 (0.34; 0.86)
% change in mean attack rate -46% (CI: -65%, -15%)					

Sensitivity analysis accounting for patient-reported HAE attacks in the HELP study

As shown in Table 11, the estimated mean HAE attack rate per 28 days was 0.27 (CI: 0.16; 0.46) with lanadelumab 300 mg q2w, 0.55 (CI: 0.38; 0.78) with lanadelumab 300 mg q4w, and 2.02 (95% CI: 1.71; 2.39) with placebo. The corresponding RRs indicated that patients treated with lanadelumab 300 mg q2w and those treated with lanadelumab 300 mg q4w had 0.13 and 0.27 times the rate of HAE attacks, respectively, compared to those treated with placebo. These estimates were statistically significant since the corresponding 95% CI does not include 1. The percent changes in mean attack rate relative to placebo were -87% and -73% for patients treated with lanadelumab 300 mg q2w and for those treated with lanadelumab 300 mg q4w, respectively.

Table 11. Results for Patient-reported HAE attacks (HELP study).

			95% CI	
	Treatment	RR	Lower	Upper
Model-based treatment period HAE attack rate (attacks/28 days)	Lanadelumab 300 mg q2w	0.27	0.16	0.46
	Lanadelumab 300 mg q4w	0.55	0.38	0.78
	Placebo	2.02	1.71	2.39
Rate ratio	Lanadelumab 300 mg q2w vs. placebo	0.1327	0.0758	0.2324
	Lanadelumab 300 mg q4w vs. placebo	0.2704	0.1832	0.3992
% change in mean attack rate	Lanadelumab 300 mg q2w vs. placebo	-87%	-92%	-77%
	Lanadelumab 300 mg q4w vs. placebo	-73%	-82%	-60%

In the base case, indirect comparison via the Bucher method showed that patients treated with lanadelumab 300 mg q2w had 0.27 times the rate of HAE attacks compared to those treated with Cinryze[®] (Table 12), while those treated with lanadelumab 300 mg q4w experienced 0.55 times the rate of HAE attacks compared to those treated with Cinryze[®]. These estimates were statistically significant since the corresponding 95% CI does not include 1. The percent changes in the mean attack rate relative to Cinryze[®] were -73% and -45% for treatment with lanadelumab 300 mg q2w and lanadelumab 300 mg q4w, respectively. A comparable sensitivity analysis was not conducted in the Bayesian NMA.

Table 12. Indirect Comparison Using the Bucher method (Patient-reported HAE Attacks from HELP Study).

		ITC			Bayesian NMA
Treatment 1	Treatment 2	RR	95% CI Lower	95% CI Upper	
Lanadelumab 300 mg q2w	Cinryze®	0.2694	0.1482	0.4896	N/A
% change in mean attack rate -73% (95% CI: -85%; -51%)					
Lanadelumab 300 mg q4w	Cinryze®	0.5490	0.3532	0.8535	NA
% change in mean attack rate -45% (95% CI -65%; -15%)					

Sensitivity analysis accounting for all covariates (normalized baseline attack rate, age, gender, weight)

As shown in Table 13, the estimated mean HAE attack rate per 28 days was 2.21 (95% CI: 1.43; 3.41) with Cinryze® and 4.37 (95% CI: 2.89; 6.60) with placebo. The corresponding RR of 0.51 indicated that patients treated with Cinryze® had 0.51 times the mean rate of HAE attacks compared to those treated with placebo. This result was statistically significant since the corresponding 95% CI does not include 1. The resulting percent change in the mean attack rate with Cinryze® treatment relative to placebo was -49%.

Table 13. Results for HAE Attacks Accounting for All Covariates of Interest (CHANGE Study).

			95% CI	
	Treatment	Estimate	Lower	Upper
Model-based treatment period HAE attack rate (attacks per 28 days)	Cinryze®	2.21	1.43	3.41
	Placebo	4.37	2.89	6.60
Rate ratio		0.5053	0.4032	0.6332
% change in mean attack rate		-49%	-60%	-37%

As shown in Table 14, the estimated mean HAE attack rate per 28 days was 0.27 (95% CI; 0.16; 0.46) for lanadelumab 300 mg q2w, 0.54 (95% CI; 0.37; 0.78) for lanadelumab 300 mg q4w, and 2.00 (95% CI; 1.63; 2.45) for placebo. The corresponding RRs showed that patients treated with lanadelumab 300 mg q2w and those treated with lanadelumab 300 mg q4w had 0.14 and 0.27 times the rate of HAE attacks, respectively, compared to patients who received placebo. These results were statistically significant since the corresponding 95% CI does not include 1. The resulting percent changes in the mean attack rate relative to placebo were -86% and -73% for lanadelumab 300 mg q2w and lanadelumab 300 mg q4w, respectively.

Table 14. Results for HAE Attacks Accounting for All Covariates of Interest (HELP study).

			95% CI	
	Treatment	Estimate	Lower	Upper
Model-based treatment period HAE attack rate (attacks/28 days)	Lanadelumab 300 mg q2w	0.27	0.16	0.48
	Lanadelumab 300 mg q4w	0.54	0.37	0.78
	Placebo	2.00	1.63	2.45
Rate ratio	Lanadelumab 300 mg q2w vs. placebo	0.1371	0.0763	0.2465
	Lanadelumab 300 mg q4w vs. placebo	0.2693	0.1808	0.4011
% change in mean attack rate	Lanadelumab 300 mg q2w vs. placebo	-86%	-92%	-75%
	Lanadelumab 300 mg q4w vs. placebo	-73%	-82%	-60%

For the base case, indirect comparison via the Bucher method showed that patients treated with lanadelumab 300 mg q2w and lanadelumab 300 mg q4w experienced 0.27 times and 0.53 times the rate of HAE attacks, respectively, compared to those treated with Cinryze® (Table 15). These results were statistically significant as evidenced by exclusion of the value

“1” in the corresponding 95% CIs. The resulting percentage change in mean attack rate relative to Cinryze® was -73% and -47% for patients treated with lanadelumab 300 mg q2w and lanadelumab 300 mg q4w, respectively. No Bayesian NMA results were available for this sensitivity analysis.

Table 15. Indirect Comparison of Lanadelumab in Different Dosing Regimens and Cinryze® Using the Bucher Method (All Covariates of Interest).

		ITC			Bayesian NMA
Treatment 1	Treatment 2	RR	95% CI Lower	95% CI Upper	
Lanadelumab 300 mg q2w	Cinryze®	0.2714	0.1456	0.5061	N/A
% change in mean attack rate -73% (95% CI: -85%; -49%)					
Lanadelumab 300 mg q4w	Cinryze®	0.5329	0.3396	0.8634	NA
% change in mean attack rate -47% (95% CI -66%; -15%)					

This section summarizes the results of the ITC of lanadelumab (300 mg q2w and 300 mg q4w) and Cinryze®. The base case and two sensitivity analyses demonstrated statistically significant differences in the HAE attack rates per 28 days between lanadelumab (300 mg q2w and 300 mg q4w) and Cinryze®.

Two independent Poisson regression models generated RR estimates identifying the relative treatment effects of lanadelumab (300 mg q2w and 300 mg q4w) vs. placebo and Cinryze® vs. placebo. The base case accounted for the normalized HAE attack rate per 28 days at baseline, while a mixed model was used to account for the cross-over design of the CHANGE study. Using the Bucher method, the corresponding results were applied as inputs into the ITC.

The first sensitivity analysis included all HAE attacks reported in the HELP study, regardless of whether or not the attacks had been confirmed by an investigator. Regression models for a second sensitivity analysis initially included age, gender, and weight. The two sensitivity analyses yielded results consistent with the base case and did not change the conclusions. These findings were also confirmed by the lack of statistically significant effects for the covariates (apart from normalized baseline attack rate) on the results.

2. Comparability of Placebo Effects in CHANGE and HELP studies

The output of the non-parametric estimation showed that the mean time (days) to the first attack after 0 days of placebo was considerably shorter in the CHANGE study than in the HELP study (4.773 days [SE 1.673] vs. 19.634 days [SE 4.212]). In Fig. 7, the probability of “no first attack” after 0 days of treatment is plotted on the y-axis while the observation time horizon is plotted on the x-axis. Placebo treatment in the CHANGE and HELP studies was not equally effective; patients in the CHANGE study experienced first attacks considerably earlier than those in the HELP study, and the corresponding observation time horizon was much shorter.

The output of the non-parametric estimation showed that the mean time (days) to the first attack after 70 days of placebo was considerably shorter in the CHANGE study than in the HELP study (3.143 days [SE 0.563] vs. 21.378 days [SE 4.687]). In Fig. 8, the probability of “no first attack” after 70 days of treatment is plotted on the y-axis, while the observation time horizon is plotted on the x-axis. Placebo treatment in the CHANGE and HELP studies was not equally effective; patients in the CHANGE study experienced first attacks considerably earlier than those in the HELP study, and the corresponding observation time horizon was much shorter.

This section summarizes the findings of the analysis comparing the placebo effects between the CHANGE and HELP studies. Placebo effects with respect to the time to first attack after 0 days of treatment and time to first attack after 70 days of treatment were not comparable between the two studies. This was assessed via the Kaplan-Meier graphs, various statistical tests (log-rank, Wilcoxon, likelihood ratio tests), and Cox regression models, which included the normalized attack at baseline, age, gender, and weight as covariates and accounted for differences in study design. There was no placebo effect in the CHANGE study, where individuals experienced fewer attacks at baseline than during placebo treatment. A placebo effect was observed in the HELP study.

In a randomized controlled trial, patients are randomly assigned to treatment arms to ensure comparability of relevant covariates between the groups. An ITC is conducted in the event that head-to-head treatment comparisons are lacking. The similarity assumption between studies must hold to ensure that results of the ITC are valid. Therefore, covariates of interest must be comparable between the corresponding study arms. This is usually ascertained during the process of feasibility assessment.

Placebo effects are often not comparable between the studies included in an ITC. However, evaluating the implications of these differences is not straightforward. With

respect to the present ITC, differences between placebo effects might have favored either Cinryze® or lanadelumab. The absence of a placebo effect in the CHANGE study implied that patients during C1-INH treatment may have only benefited from the effect of the active treatment without additional placebo effects. This would be expected to occur even if Cinryze® were not efficacious. In contrast, a placebo effect was observed in the HELP study. Therefore, patients treated with lanadelumab may have benefited from the efficacy of active treatment as well as the additional placebo effect. This difference may have favored lanadelumab over Cinryze® in the present analysis.

In a linear regression on the change from baseline for the HAE attack rate per 28 days, the outcome measure of interest is the rate difference. The rate difference in change from baseline in comparison to placebo was higher in the CHANGE study than in the HELP study since no placebo effect was observed in the former, leading to ITC results favoring Cinryze®.

In Poisson regression, however, the relative treatment effect is defined as the RR at follow-up, and the baseline attack rate is included as a covariate. Compared to linear regression, the analysis uses a different measure of outcome. As a result, the differences regarding the placebo effect between the two studies had little impact on the analysis, and demonstrating a statistically significant difference favoring lanadelumab over Cinryze® was feasible.

As for time to first attack after 0 and 70 days of treatment, attacks occurred more frequently and considerably earlier during placebo treatment in the CHANGE study than in the HELP study. As discussed, assessing the implications of these findings is not straightforward.

3. Time to first attack – non-parametric estimation and Cox regression

In the non-parametric estimation, the mean time to first attack (in days) after 0 days of placebo treatment was considerably shorter in the CHANGE study than in the HELP study (4.773 days [SE 1.673] vs. 19.634 days [SE 4.212]).

As shown in Fig. 9, the probability of “no first attack” after 0 days of treatment is plotted on the y-axis, while the observation time horizon is plotted on the x-axis. Placebo was less effective than Cinryze® treatment in the CHANGE study. Patients who received placebo experienced their first attacks considerably earlier than during Cinryze® treatment. Given that the two curves do not intersect, the proportional hazards assumptions may be

deemed to hold. That confirms that Cox regression is a valid method for estimating hazard ratios (HRs) for the ITC.

As shown in Fig. 10, the probability of “no first attack” after 0 days of treatment is plotted on the y-axis, while the observation time horizon is plotted on the x-axis. In the HELP study, placebo was less effective than the two lanadelumab dose regimens. Patients receiving placebo experienced first attacks considerably earlier than those during lanadelumab treatment. Given that none of the curves intersect, the proportional hazards assumption may be deemed to hold. This confirms that Cox regression is a valid method for estimating HRs for the ITC.

The different analytical methods used may contribute to discrepant findings. The present ITC employed a frequentist Cox regression model, which accounted for repeated measurements (including fixed effects for treatment period and sequence and random effects for study subjects), and the baselined HAE attack rate was normalized. In the prior NMA, which employed a Bayesian approach, these measures were not taken and only the results of random effects models used for comparisons were reported.

In comparing lanadelumab 300 mg q2w and lanadelumab 300 mg q4w to placebo, the Cox regression generated HRs of 0.27 (95% CI: 0.15; 0.50) and 0.38 (95% CI: 0.22; 0.67), respectively (Table 16). The Bayesian NMA produced comparable HRs of 0.27 with corresponding 95% credible interval (CrI) of (0.13; 0.55) and 0.39 with 95% CrI of (0.21; 0.74), respectively.

Table 16. Base Case Results from HELP Study.

Contrast	HR	Standard Error	Confidence Limits		Bayesian NMA (FE): HR and 95% credible interval (CrI)
Lanadelumab 300 mg q2w vs placebo	0.2689	0.0843	0.1454	0.4972	0.27 (0.13; 0.55)
Lanadelumab 300 mg q4w vs placebo	0.3837	0.1079	0.2211	0.6657	0.39 (0.21; 0.74)

Table 17. Base Case Results from CHANGE Study.

Contrast	HR	Standard Error	Confidence Limits		Bayesian NMA : HR (RE, FE not reported)
Cinryze® vs. placebo	0.3669	0.1287	0.1845	0.7298	0.7298

Indirect comparison via the Bucher method showed that individuals treated with lanadelumab 300 mg q2w had 0.73 times the hazard of a first HAE attack after 0 days of treatment compared to individuals treated with Cinryze® (Table 18). Treatment with lanadelumab 300 mg q4w increased the hazard of an attack by 1.05 times compared to Cinryze®. This finding was not statistically significant as evidenced by inclusion of the value “1” in the corresponding 95% CIs. The point estimates generated in the ITC using IPD differed from the results of the Bayesian NMA, primarily due to the different methodologies applied (as previously described). As the interval bounds appeared similar, the conclusions did not change.

Table 18. Indirect Comparison of Lanadelumab (300 mg q2w and 300 mg q4w) and Cinryze® using the Bucher Method (Base Case).

			ITC		Bayesian NMA
Treatment 1	Treatment 2	HR	95% CI Lower	95% CI Upper	HR (95% CrI)
Lanadelumab 300 mg q2w	Cinryze®	0.7327	0.2913	1.8429	0.51 (0.22; 1.3)
Lanadelumab 300 mg q4w	Cinryze®	1.0456	0.4332	2.5239	0.73 (0.26; 2.09)

In comparing Cinryze® and placebo, the Cox regression generated a HR of 0.37 (95% CI; 0.18; 0.76) (Table 19). These results were consistent with the base case.

Table 19. Base results in the CHANGE Study.

Contrast	HR	SE	95% CI Lower	95% CI Upper
Cinryze® vs.	0.3738	0.1349	0.1843	0.7582

placebo				
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In comparing lanadelumab 300 mg q2w and lanadelumab 300 mg q4w to placebo, the Cox regression generated HRs of 0.27 (95% CI: 0.14; 0.51) and 0.37 (95% CI: 0.21; 0.66), respectively (Table 20). These estimates are consistent with previous findings. Including additional covariates did not affect the primary findings.

Table 20. Base Case Results in the HELP Study.

Contrast	HR	SE	95% CI Lower	95% CI Upper
Lanadelumab 300 mg q2w vs. placebo	0.2675	0.0890	0.1394	0.5133
Lanadelumab 300 mg q4w vs. placebo	0.3733	0.1071	0.2127	0.6552

Indirect comparison via the Bucher method estimated that patients treated with lanadelumab 300 mg q2w had 0.72 times the hazard of a first HAE attack after 0 days of treatment compared to those treated with Cinryze[®] treatment (Table 21). Lanadelumab 300 mg q4w treatment resulted with nearly the same hazard of HAE attacks when compared to Cinryze[®]. This finding was not statistically significant as evidence by inclusion of the value “1” in the corresponding 95% CIs. The results were consistent with the base case.

Table 21. Indirect Comparison of Lanadelumab and Cinryze[®] Using the Bucher Method (Base Case).

Treatment 1	Treatment 2	ITC		
		HR	95% CI Lower	95% CI Upper
Lanadelumab 300 mg q2w	Cinryze [®]	0.7157	0.2735	1.8727
Lanadelumab 300 mg q4w	Cinryze [®]	0.9988	0.4045	2.4659

In the non-parametric estimation, the mean time to first attack (in days) after 70 days of placebo treatment was considerably shorter in the CHANGE study than in the HELP study (3.1429 days [SE 0.5625] vs. 21.378 days [SE 4.687]).

The probability of “no first attack” after 70 days of treatment is plotted on the y-axis of the graph in Fig. 11, while the observation time horizon is plotted on the x-axis. In the CHANGE study, placebo was less effective than Cinryze[®] treatment. Patients treated with placebo experienced their first attack considerably earlier than during treatment with Cinryze[®]. Given that the two curves do not intersect, it can be concluded that the Cox regression is a valid method for estimating HRs for the ITC.

As shown in Fig. 12, the probability of “no first attack” after 70 days of treatment is plotted on the y-axis of and the observation time horizon is plotted on the x-axis. Placebo treatment was less effective than lanadelumab treatment (300 mg q2w and 300 mg q4w) in the HELP study. Patients treated with placebo experienced first attacks considerably earlier than those treated with lanadelumab. Given that none of the curves intersect, it can be concluded that the Cox is a valid method for estimating HRs for the ITC.

In comparing Cinryze[®] and placebo, the Cox regression generated a HR of 0.34 (95% CI: 0.16; 0.72). In the Bayesian NMA, the corresponding HR was 0.53 (0.00; 318.88) (Table 22).

Table 22. Base Case Results in the CHANGE Study.

Contrast	HR	Standard Error	Confidence Limits		Bayesian NMA (FE): HR and 95% credible interval (CrI)
Cinryze [®] vs. placebo	0.3421	0.1287	0.1636	0.7153	0.53 (0.00; 318.88)

In comparing lanadelumab 300 mg q2w and lanadelumab 300 mg q4w to placebo, the Cox regression yielded HRs of 0.07 (95% CI; 0.03; 0.16) and 0.21 (95% CI; 0.11; 0.40), respectively (Table 23). In the Bayesian NMA, the corresponding HRs were 0.09 (95% CI; 0.04; 0.22) and 0.27 (95% CI; 0.14; 0.53), respectively.

Table 23. Base Case Results in the HELP Study.

Contrast	HR	SE	95% CI Lower	95% CI Upper	Bayesian NMA (FE): HR
Lanadelumab 300 mg q2w vs. placebo	0.0651	0.0306	0.0259	0.1638	0.09 (0.04; 0.22)
Lanadelumab 300 mg q4w vs. placebo	0.2116	0.0686	0.1121	0.3996	0.27 (0.14; 0.53)

Indirect comparison via the Bucher method showed that patients treated with lanadelumab 300 mg q2w had 0.19 times the hazard of a first HAE attack after 70 days of treatment compared to those treated with Cinryze[®] (Table 24). These results were statistically significant as evidenced by exclusion of the value “1” in the corresponding 95% CIs (95% CI: 0.0584; 0.6200). The hazard of HAE attacks during treatment with lanadelumab 300 mg q4w was 0.62 times the hazard of attacks during Cinryze[®] treatment. These results comparing treatment with lanadelumab 300 mg q4w to treatment with Cinryze[®] were not statistically significant, as evidenced by the inclusion of “1” in the corresponding 95% CIs (95% CI: 0.2336, 1.6381).. The point estimates generated in the ITC using IPD differed from the results of the Bayesian NMA. As the interval bounds appeared similar, the conclusions did not change.

Table 24. Indirect Comparison of Lanadelumab and Cinryze[®] via the Bucher Method (Base Case).

Treatment 1	Treatment 2	ITC			Bayesian NMA: HR
		HR	95% CI Lower	95% CI Upper	
Lanadelumab 300 mg q2w	Cinryze [®]	0.1903	0.0584	0.6200	0.17 (0.05; 0.57)
Lanadelumab 300 mg q4w	Cinryze [®]	0.6186	0.2336	1.6381	0.51 (0.18; 1.49)

In comparing lanadelumab 300 mg q2w and lanadelumab 300 mg q4w to placebo, the Cox regression yielded HRs of 0.05 (95% CI; 0.02; 0.14) and 0.19 (95% CI; 0.10; 0.38), respectively (Table 25). The addition of the covariate inclusion did not have an impact.

Table 25. Base Case Results in the HELP Study.

Comparison	HR	SE	95% CI Lower	95% CI Upper
Lanadelumab 300 mg q2w vs. placebo	0.0516	0.0266	0.0188	0.1417
Lanadelumab 300 mg q4w vs. placebo	0.1930	0.0658	0.0990	0.3764

In comparing Cinryze[®] and placebo, the Cox regression generated a HR of 0.32 (95% CI: 0.14; 0.71) (Table 26). These results were consistent with the base case findings.

Table 26. Base Case Results in the CHANGE Study.

Contrast	HR	SE	95% CI Lower	95% Upper
HR Cinryze [®] vs. placebo	0.3193	0.1295	0.1441	0.7072

Indirect comparison via the Bucher method showed that patients treated with lanadelumab 300 mg q2w had 0.15 times the hazard of a first HAE attack after 70 days of treatment compared to those treated with Cinryze[®] (Table 27). These results were statistically significant as evidenced by exclusion of the value “1” in the corresponding 95% CIs (95% CI: 0.0431; 0.5286). Treatment with lanadelumab 300 mg q4w resulted in 0.56 times the hazard of HAE attacks when compared with Cinryze[®]. These results comparing treatment with lanadelumab 300 mg q4w to treatment with Cinryze[®] were not statistically significant, as evidenced by the inclusion of “1” in the corresponding 95% CIs (95% CI: 0.2086, 1.5262). .

Table 27. Indirect Comparison of Lanadelumab and Cinryze® via the Bucher Method (Base Case).

Treatment 1	Treatment 2	ITC		
		HR	95% CI Lower	95% CI Upper
Lanadelumab 300 mg q2w	Cinryze®	0.1508	0.0431	0.5286
Lanadelumab 300 mg q4w	Cinryze®	0.5643	0.2086	1.5262

Conclusion

Consistent with the results of the Bayesian NMA using aggregate data, the present regression-based frequentist ITC demonstrated that lanadelumab 300 mg q2w and lanadelumab 300 mg q4w were statistically more effective than Cinryze® at reducing the rate of HAE attacks per 28 days (RR 0.27, 95% CI [0.15; 0.49] and RR 0.54, 95% CI [0.35; 0.85], respectively). A sensitivity analysis, which included patient-reported HAE attacks in the HELP study, confirmed these findings and yielded RR point estimates and 95% CIs in line with the primary results. A second sensitivity analysis including the covariates age, gender, and weight produced similar findings and further supported the outcomes of the base case analysis.

With respect to the time to first attack after 0 days of treatment and the time to first attack after 70 days of treatment, the present ITC drew conclusions similar to those noted in the Bayesian NMA. Lanadelumab 300 mg q2w and lanadelumab 300 mg q4w were not statistically more effective compared to Cinryze® in prolonging the time to a first attack after 0 days of treatment (HR 0.73, 95% CI [0.29; 1.84] and HR 1.05, 95% CI [0.43, 2.52], respectively). Lanadelumab 300 mg q2w and lanadelumab 300 mg q4w, however, appeared to exhibit greater efficacy in increasing the time to a first attack after 70 days of treatment compared to Cinryze® (HR 0.19, 95% CI [0.06; 0.62] and HR 0.62, 95% CI [0.23; 1.64], respectively). Only the difference between lanadelumab 300 mg q2w and Cinryze® was statistically significant. In the sensitivity analyses for the time to first attack after 0 and 70 days of treatment, models including age, gender, and weight yielded lower HR point estimates; however, the findings did not change any of the base case conclusions.

Although the conclusions of the present ITC were consistent with those of the Bayesian NMA, the corresponding point estimates and uncertainty intervals differed. This

was presumably due to the lack of accounting for repeated measurements in the Bayesian NMA (with respect to the CHANGE cross-over study), as well as differences in data input types.

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 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.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of examples only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials,

kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also

allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

What Is Claimed Is:

1. A method for treating hereditary angioedema (HAE) attack or reducing the rate of HAE attack, the method comprising:

administering to a subject in need thereof an antibody that binds human plasma kallikrein at about 300 mg every about two weeks in a first treatment period, which is about 4-9 months;

monitoring the subject for HAE attack during the first treatment period; and

reducing the dosage of the antibody to about 300 mg every about 4 weeks in the subject, who is free of HAE attack in the first treatment period;
wherein the antibody comprises the same heavy chain complementarity-determining regions (CDRs) and the same light chain CDRs as DX-2930.

2. The method of claim 1, wherein the first treatment period is about 6 months.

3. The method of claim 1 or claim 2, wherein the patient has a low body weight.

4. The method of claim 3, wherein the patient has a body weight of less than 35 kg.

5. The method of any one of claims 1-4, wherein the patient is a pediatric patient.

6. The method of any one of claims 1-3, wherein the antibody comprises a heavy chain immunoglobulin variable domain (V_H) of SEQ ID NO: 3 and a light chain immunoglobulin variable domain (V_L) of SEQ ID NO: 4.

7. The method of any one of claims 1-6, wherein the antibody is a full length antibody or an antigen-binding fragment thereof.

8. The method of any one of claims 1-7, wherein the antibody is an IgG1 molecule.

9. The method of claim 8, wherein the antibody comprises a heavy chain of SEQ ID NO:1 and a light chain of SEQ ID NO:2.

10. The method of any one of claims 1-9, wherein the antibody is formulated in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

11. The method of claim 10, wherein the pharmaceutical composition comprises sodium phosphate, citric acid, histidine, sodium chloride, and polysorbate 80.

12. The method of claim 11, wherein the sodium phosphate is at a concentration of about 30 mM, the citric acid is at a concentration of about 19 mM, the histidine is at a concentration of about 50 mM, the sodium chloride is at a concentration of about 90 mM, and the polysorbate 80 is at about 0.01%.

13. The method of any one of claims 1-12, wherein the antibody is administered subcutaneously.

14. The method of any one of claims 1-13, wherein the subject is a human patient having, suspected of having, or at risk for HAE.

15. The method of claim 14, wherein the subject has HAE type I or type II.

1/14

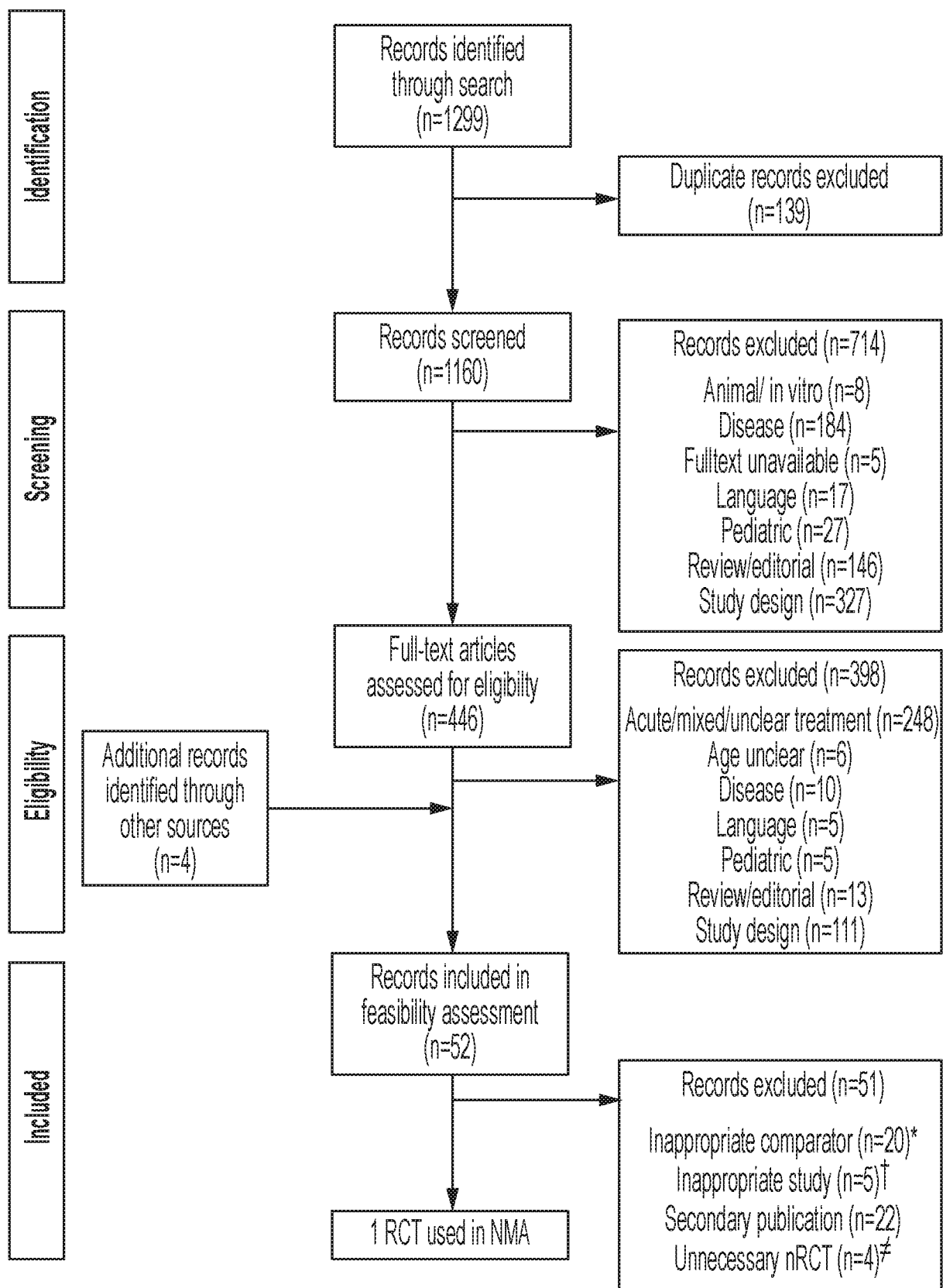


FIG. 1

2/14

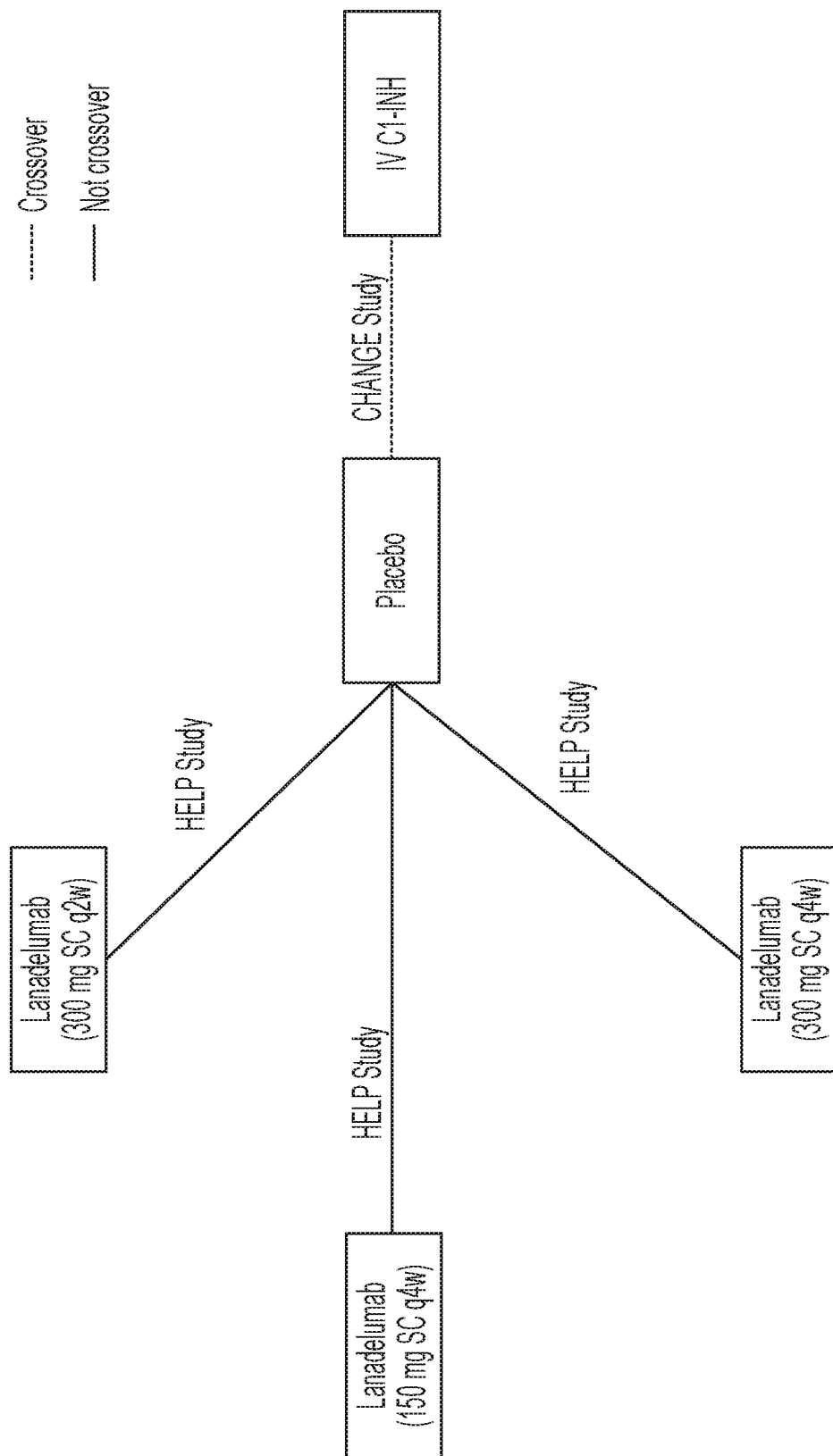


FIG. 2

3/14

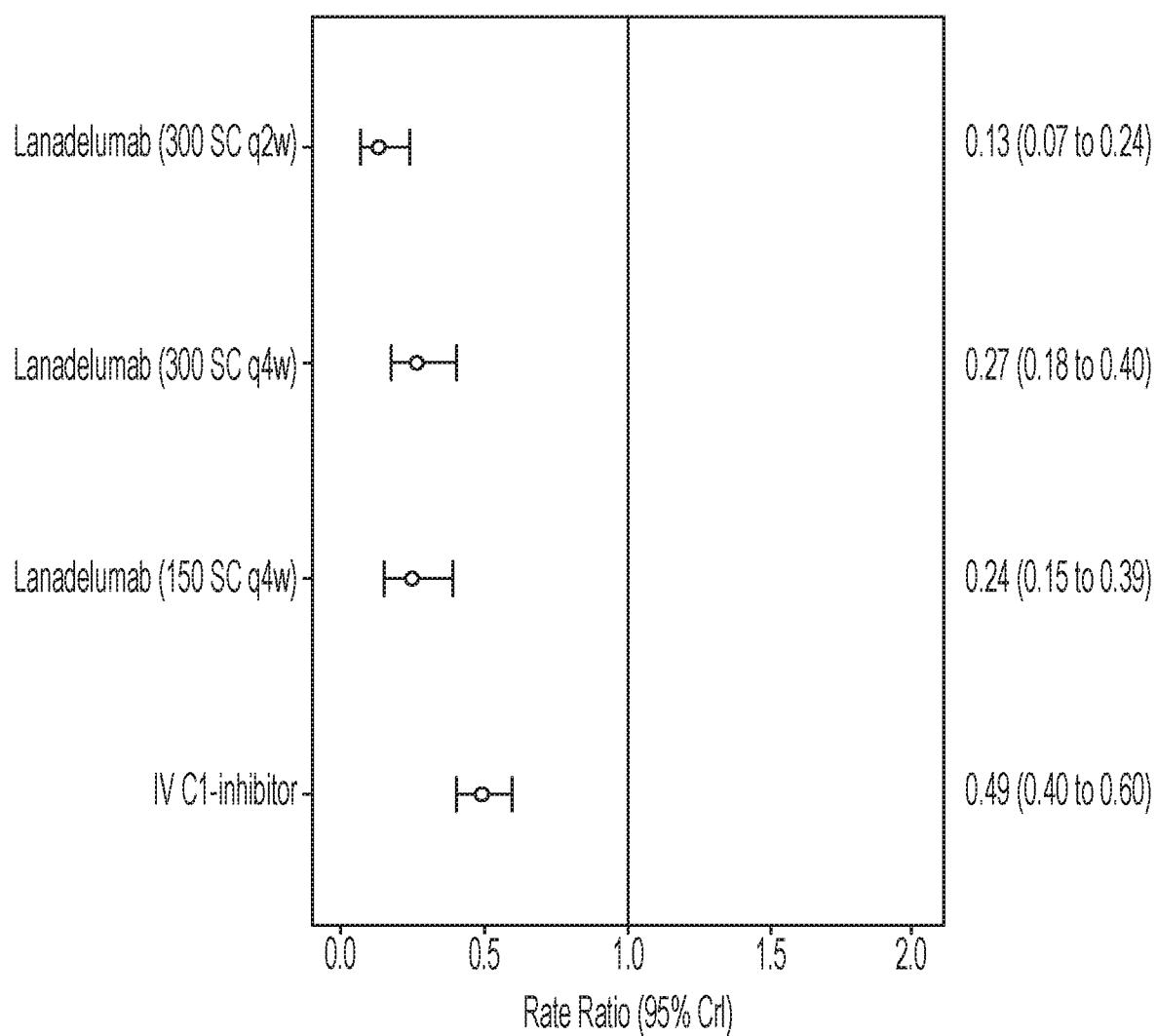


FIG. 3

5/14

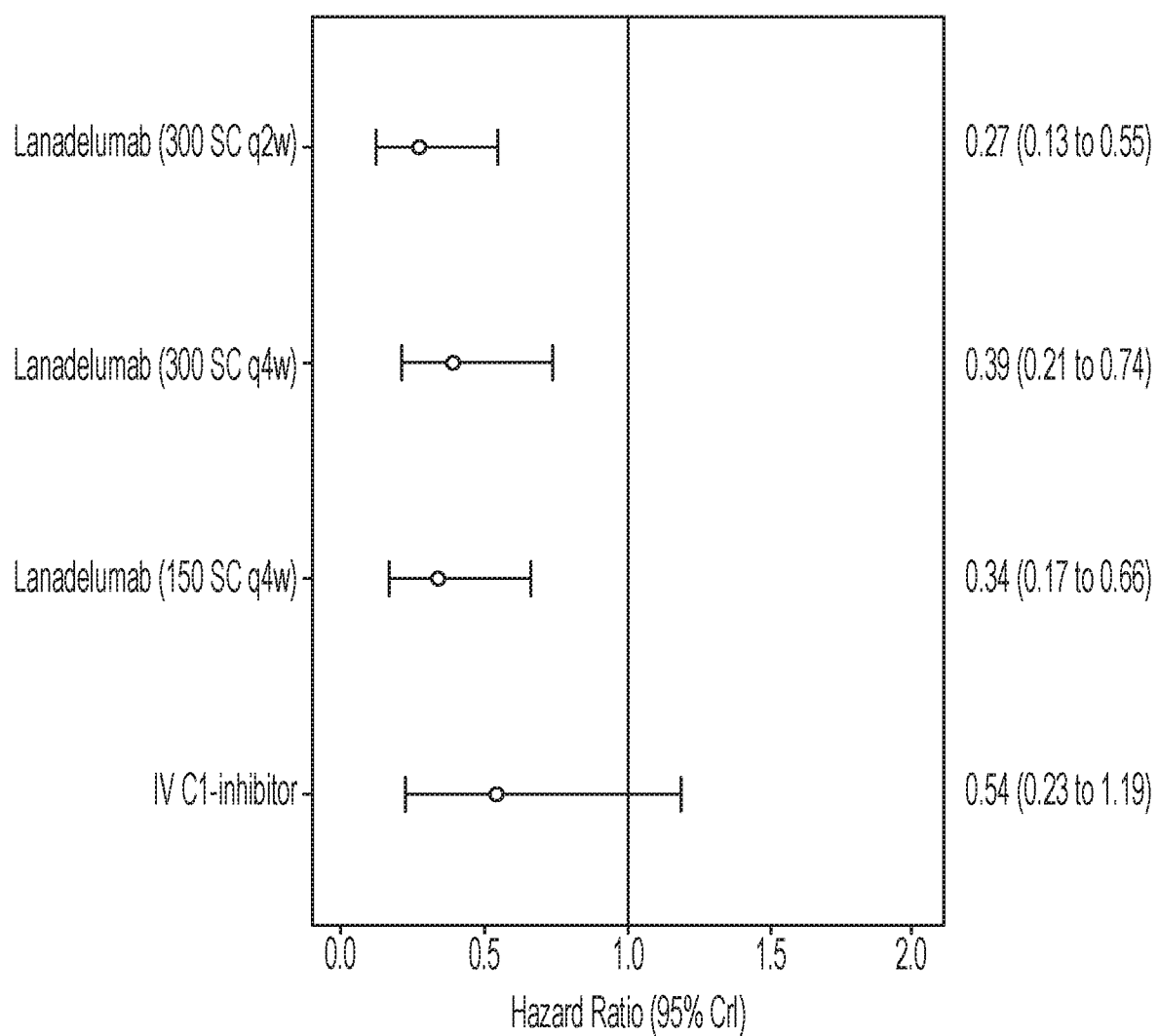


FIG. 4A

4/14

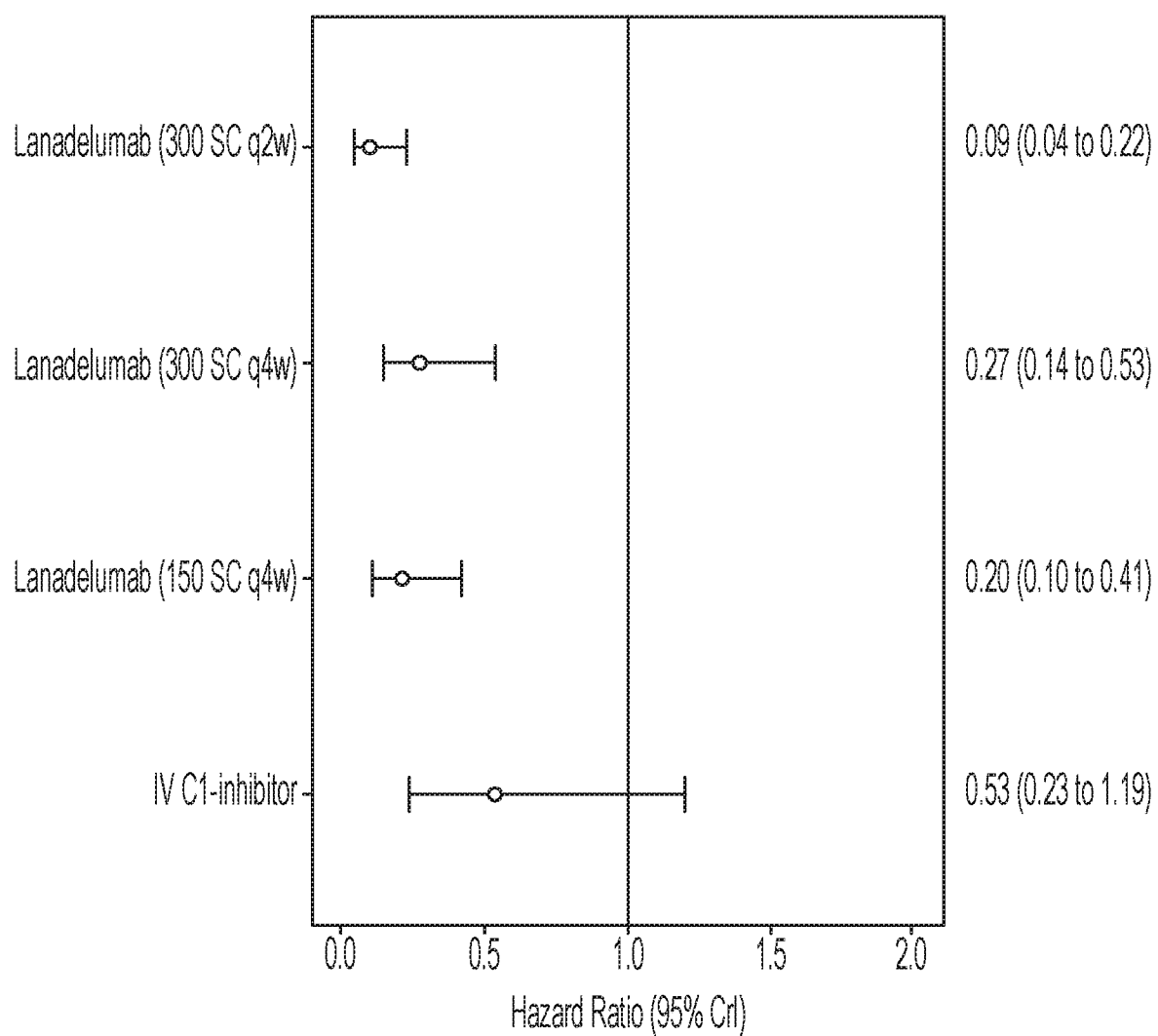


FIG. 4B

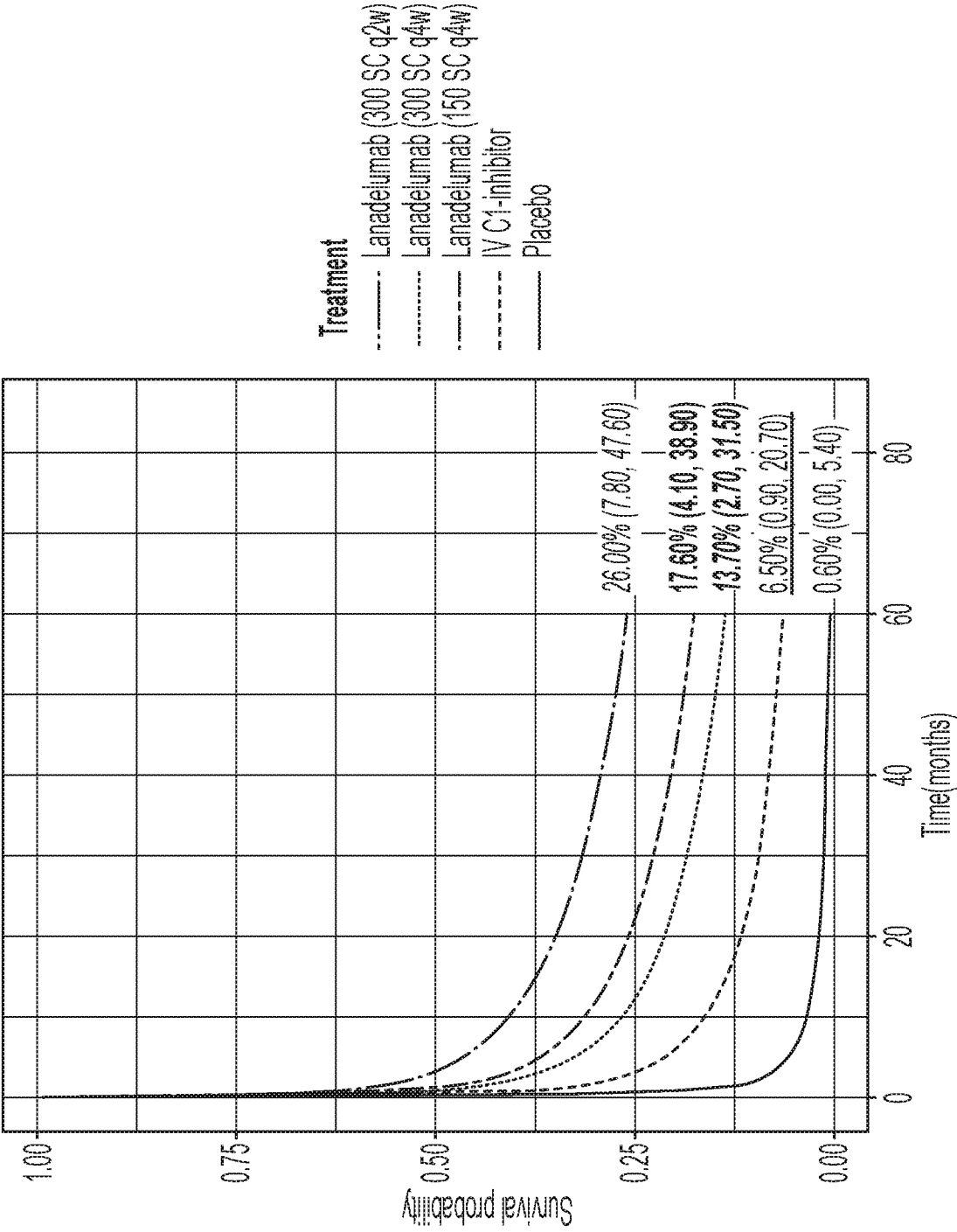


FIG. 5A

7/14

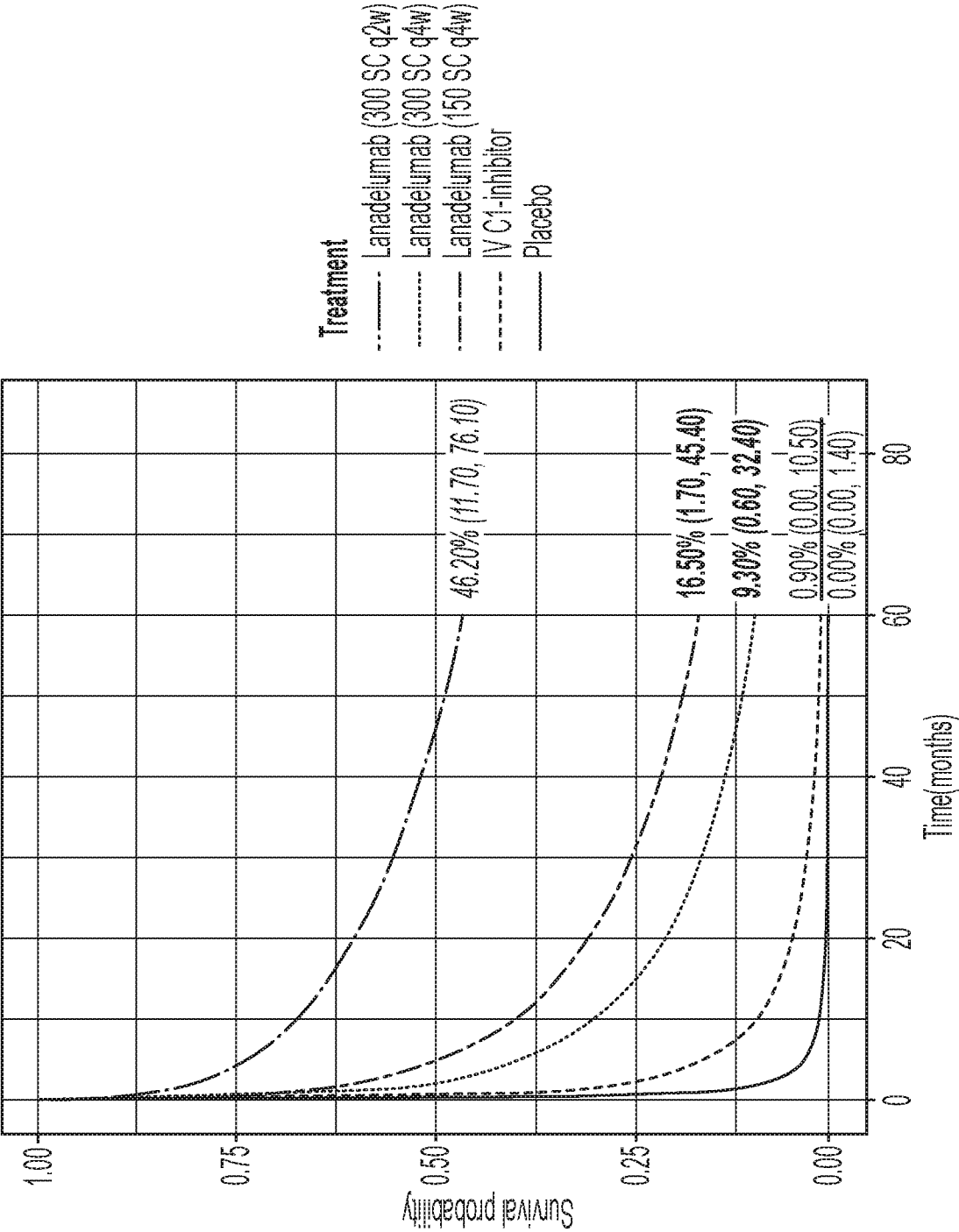


FIG. 5B

8/14

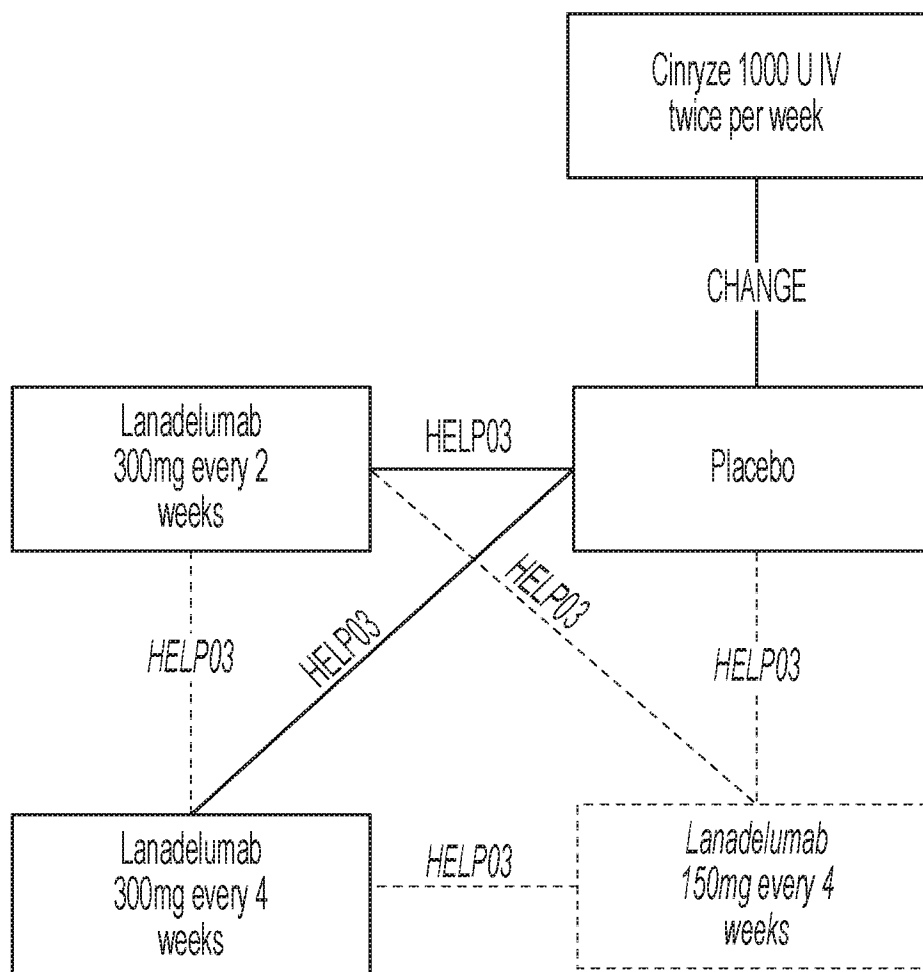


FIG. 6

9/14

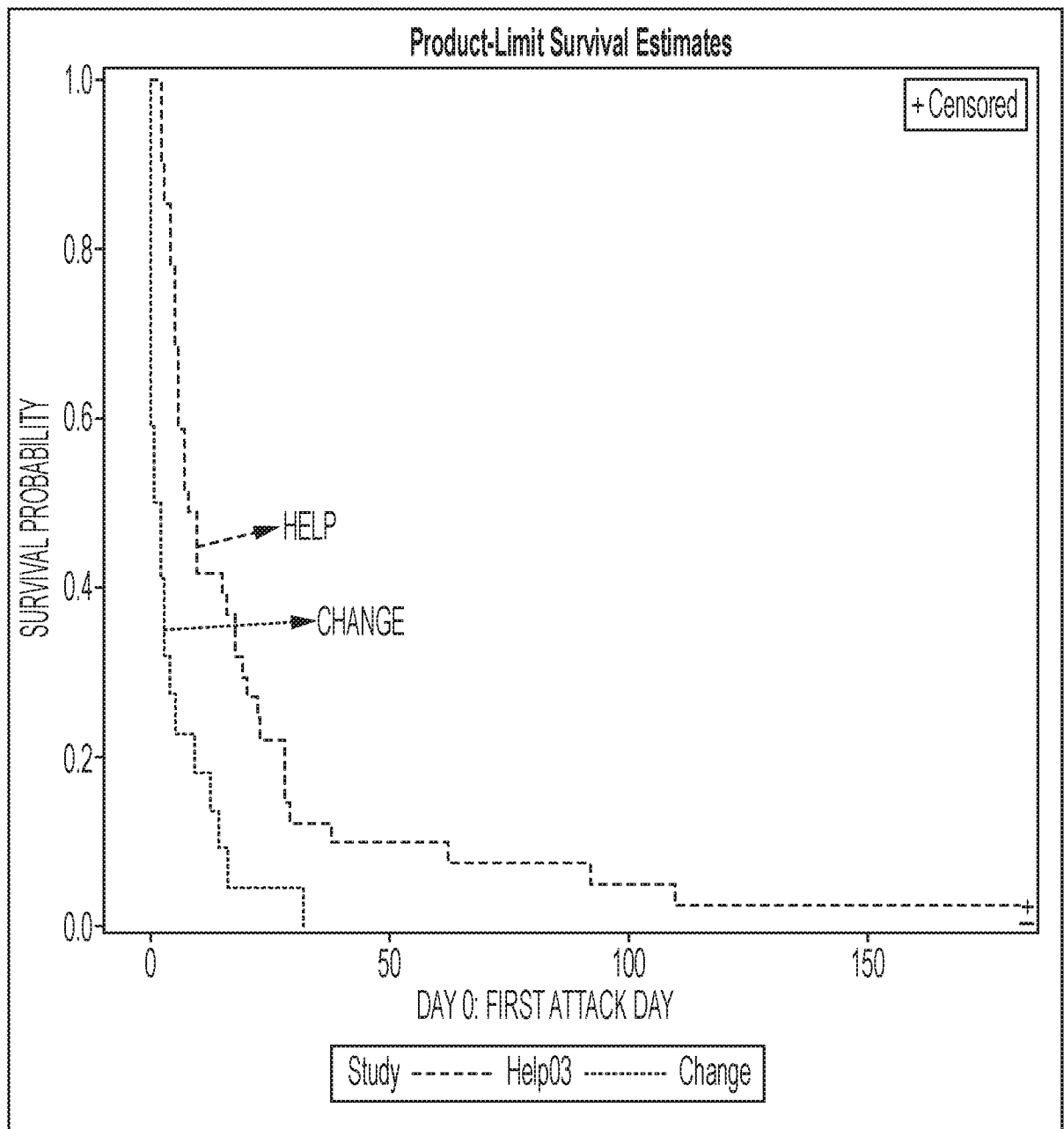


FIG. 7

10/14

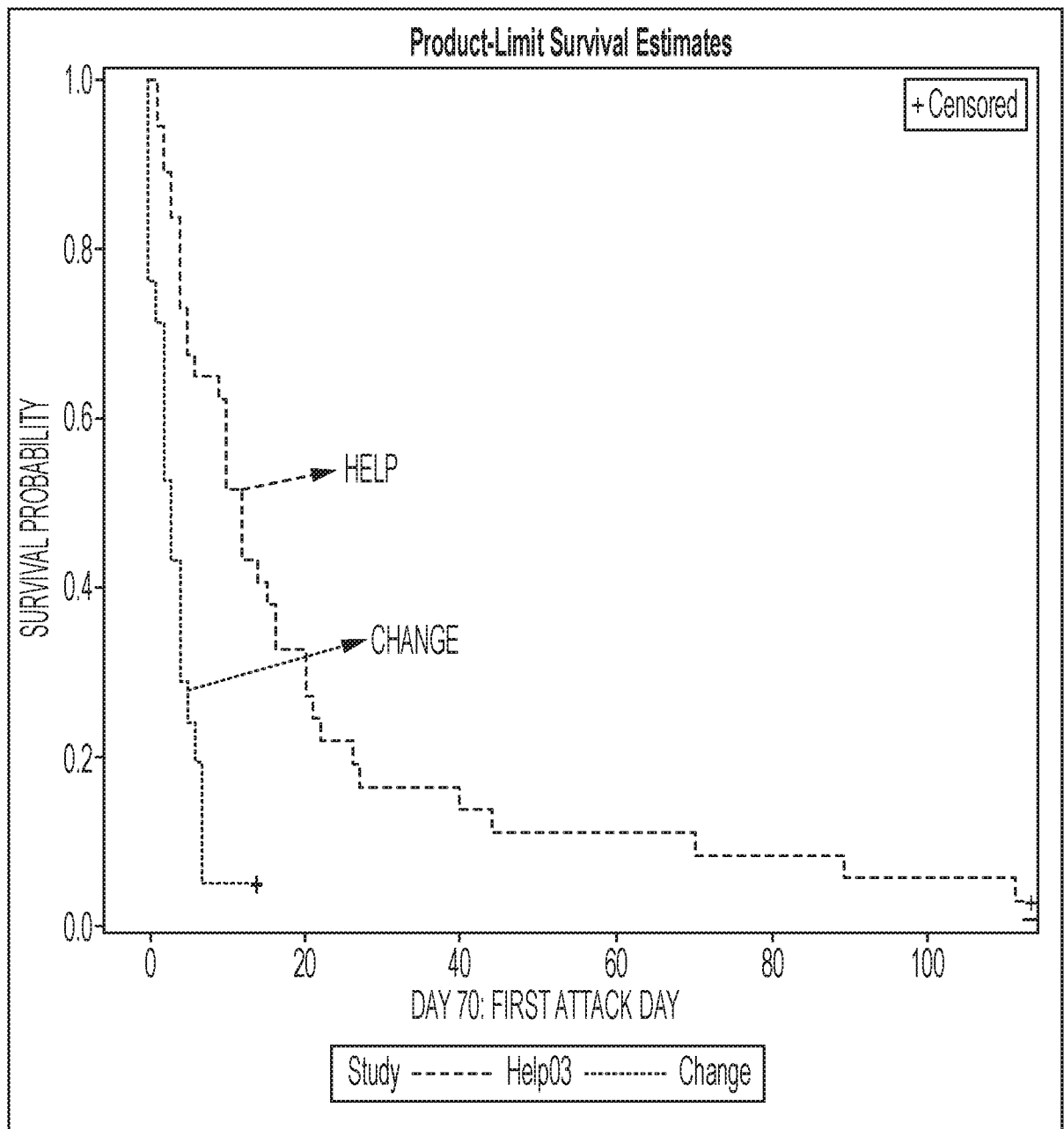


FIG. 8

11/14

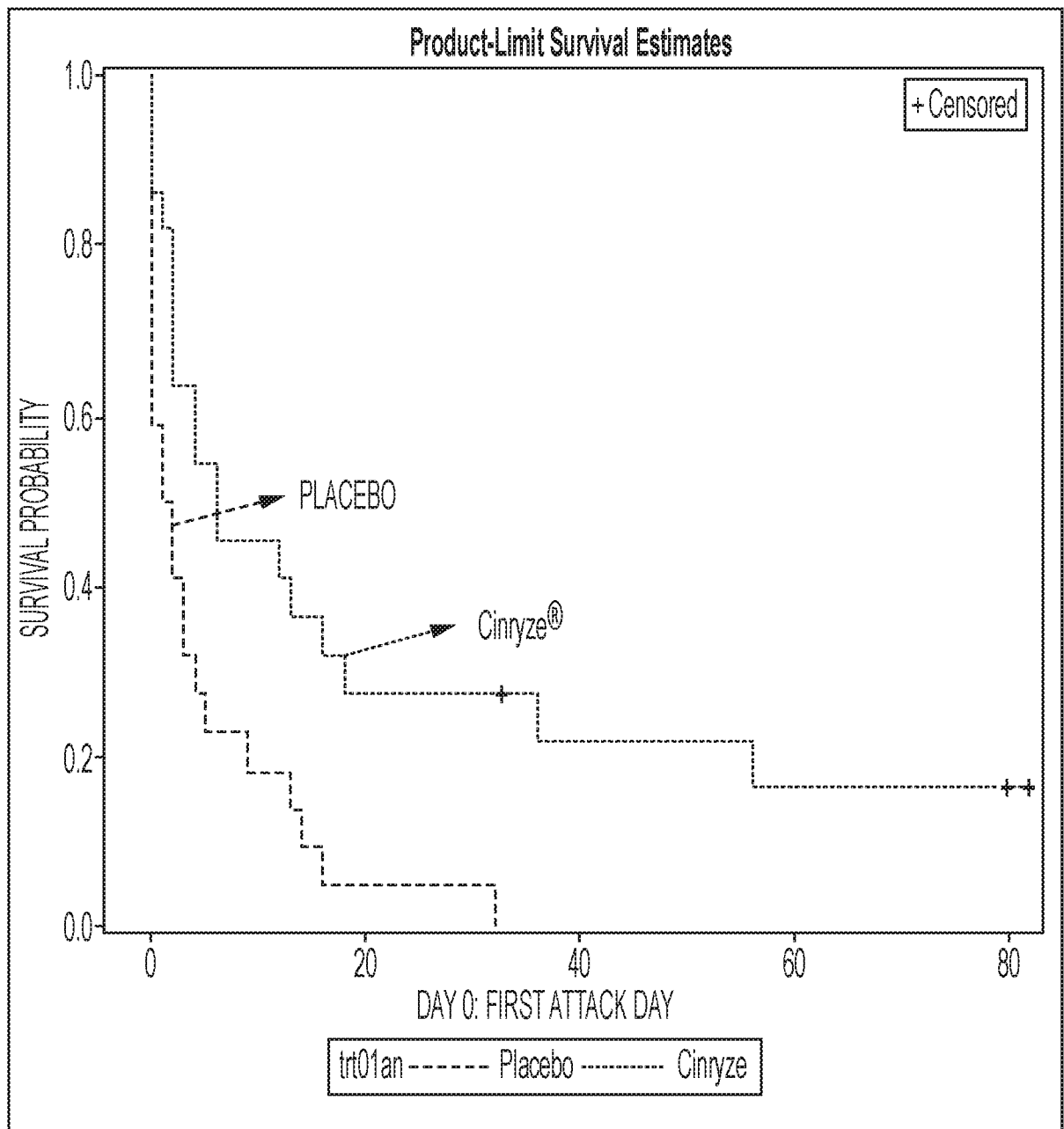


FIG. 9

12/14

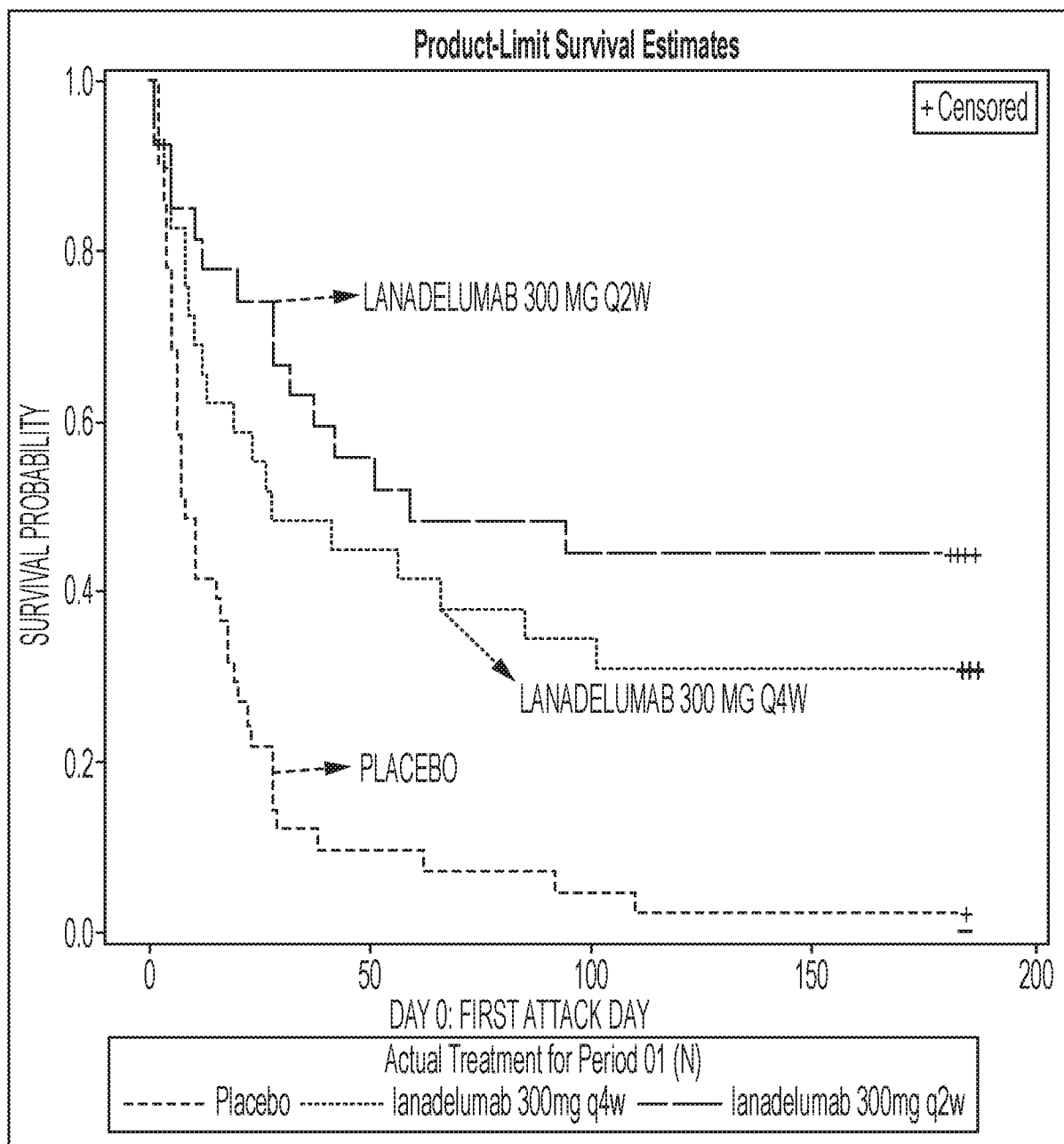


FIG. 10

13/14

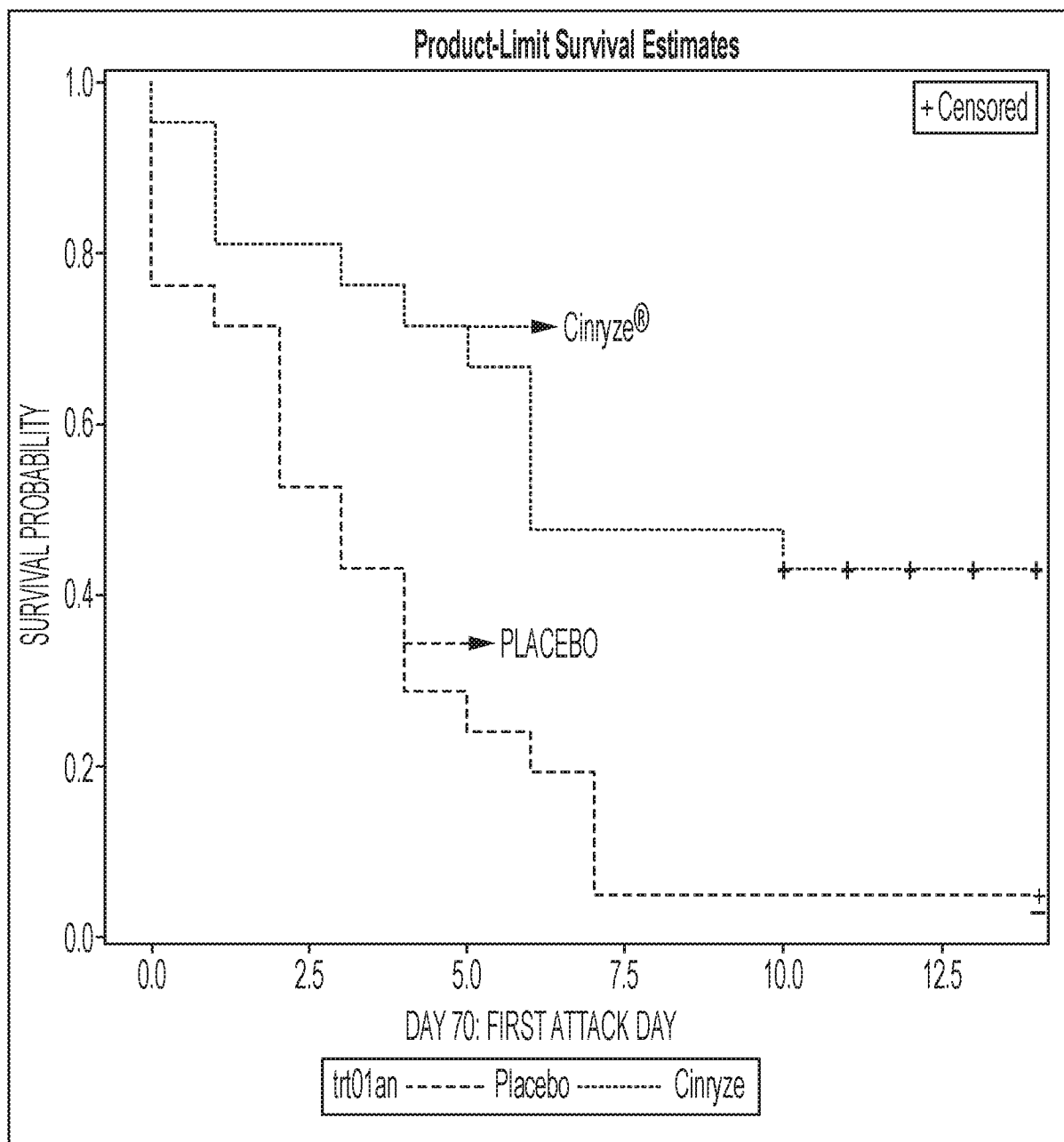


FIG. 11

14/14

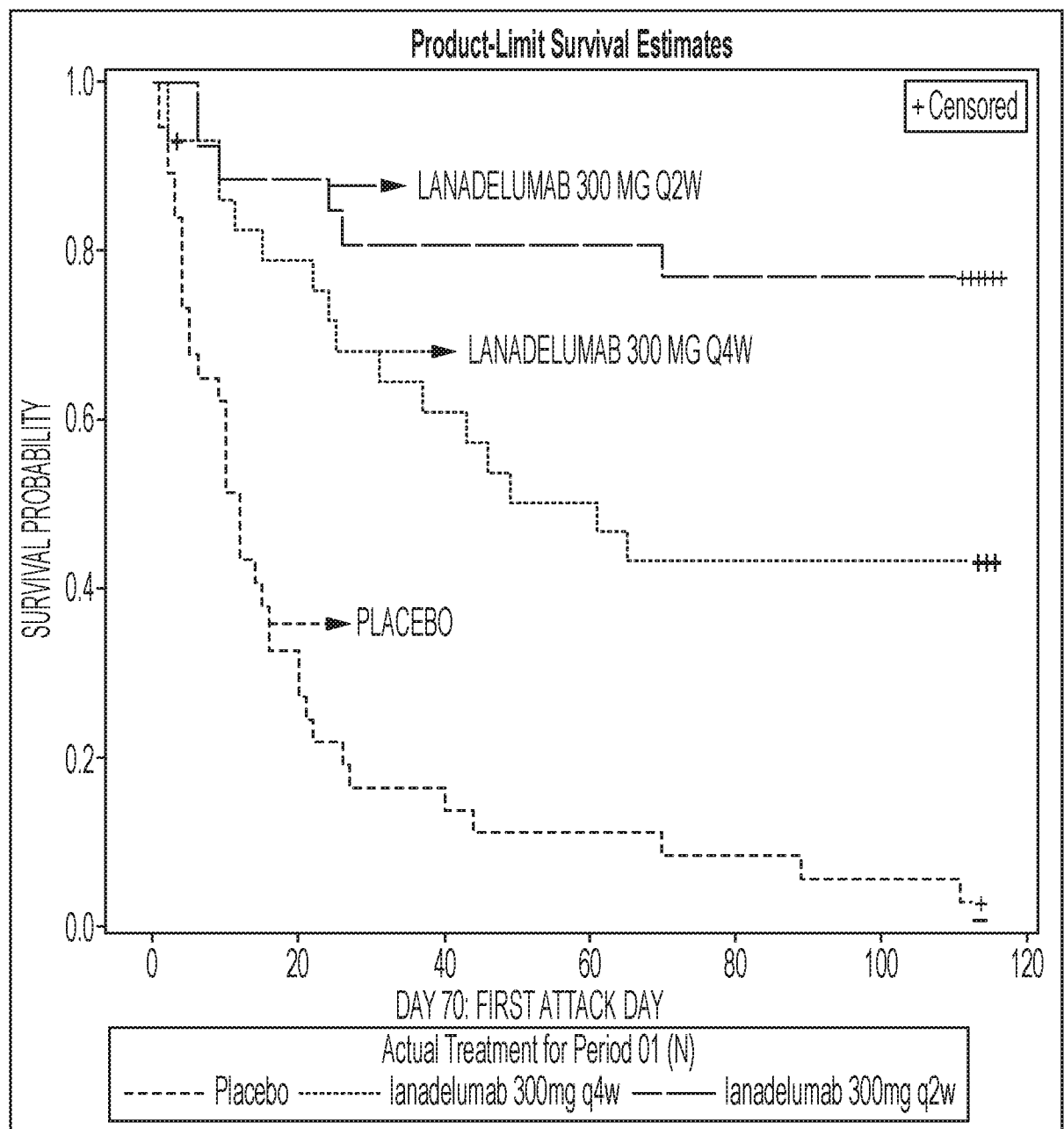


FIG. 12

SEQUENCE LISTING

<110> Dyax Corp.

<120> PLASMA KALLIKREIN INHIBITORS AND USES THEREOF FOR TREATING
HEREDITARY ANGIOEDEMA ATTACK

<130> D0617.70132W000

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> US 62/818,189

<151> 2019-03-14

<160> 10

<170> PatentIn version 3.5

<210> 1

<211> 469

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

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1				5					10					15	

His	Ser	Glu	Val	Gln	Leu	Leu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro
		20					25						30		

Gly	Gly	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser
		35					40					45			

His	Tyr	Ile	Met	Met	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu
	50					55					60				

Trp Val Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp
65 70 75 80

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
85 90 95

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
100 105 110

Tyr Cys Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp
115 120 125

Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu

245

250

255

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 260 265 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 275 280 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 290 295 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
 305 310 315 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 325 330 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 340 345 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 355 360 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
 370 375 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 385 390 395 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 405 410 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
 420 425 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
435 440 445

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
450 455 460

Ser Leu Ser Pro Gly
465

<210> 2

<211> 231

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

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Met Gly Trp Ser Cys Ile Leu Phe Leu Val Ala Thr Ala Thr Gly Ala
1 5 10 15

His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser
20 25 30

Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
35 40 45

Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
50 55 60

Leu Ile Tyr Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe
65 70 75 80

Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu

85

90

95

Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Thr Tyr
 100 105 110

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
 115 120 125

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 130 135 140

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
 145 150 155 160

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
 165 170 175

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
 180 185 190

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
 195 200 205

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
 210 215 220

Ser Phe Asn Arg Gly Glu Cys
 225 230

<210> 3

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 3

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
20 25 30

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

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

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

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

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

Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 4

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 4

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

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

Tyr Lys Ala Ser Thr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Thr Tyr Trp Thr
85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 5

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Polypeptide

<400> 5

His Tyr Ile Met Met
1 5

<210> 6

<211> 17

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 6

Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 7
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
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<400> 7

Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile
1 5 10

<210> 8
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 8

Arg Ala Ser Gln Ser Ile Ser Ser Trp Leu Ala
1 5 10

<210> 9

<211> 7
<212> PRT
<213> Artificial Sequence

<220>
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<400> 9

Lys Ala Ser Thr Leu Glu Ser
1 5

<210> 10
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Polypeptide

<400> 10

Gln Gln Tyr Asn Thr Tyr Trp Thr
1 5