[0006] Antibodies that bind Factor XI and/or Factor XIa have been studied. For example, WO 2016/207858 describes one such anti-Factor XI and/or Factor XIa antibody, disclosed in Table 1 of the present application as Antibody 1. The present disclosure adds to these developments and provides further clinical methods, including dosage regimens, to treat patients with specific thromboembolic disorders with desired safety and efficacy. Furthermore, the present disclosure adds to the earlier developments in the field by providing formulations comprising such FXI and/or FXIa antibodies that are sufficiently stable and suitable for administration to patients.

SUMMARY OF THE DISCLOSURE

[0007] The present disclosure relates to methods for detection and measurement of anti-Factor XI and/or activated Factor XI (Factor XIa) antibodies, or antigen-binding fragments thereof.

[0008] Accordingly, in one aspect, provided herein is a method of detecting an anti-drug antibody (ADA) against an anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof, wherein the method comprises: (a) incubating a sample with an acid to dissociate anti-Factor XI and/or anti-Factor XIa antibody – antigen complexes and/or dissociate anti-Factor XI and/or anti-Factor XIa antibody – ADA complexes present in the sample to create an acid digest, (b) incubating the acid digest on a plate coated with the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof, (c) neutralizing the acid digest, and (d) detecting the presence of the ADA using a ruthenylated detector cocktail.

[0009] In some examples, the sample is a sample from a subject. In some examples, the sample is selected from the group consisting of blood, plasma, or serum from a subject. In some examples, the method comprises an initial step of preparing the sample.

[0010] In some examples, the acid is selected from the group consisting of acetic acid, citric acid, phosphoric acid, and mixtures thereof. In some examples, the acid is acetic acid. In some examples, the acetic acid is at a concentration of about 300 mM.

[0011] In some examples, the antigen is Factor XI and/or Factor XIa. In some examples, the plate is coated with streptavidin. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the plate is at a concentration selected from the group consisting of about 0.1 μg/ml, about 0.25 μg/ml, about

0.5 μg/ml, about 0.75 μg/ml, and about 1 μg/ml. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the plate is at a concentration of about 0.25 μg/ml.

[0012] In some examples, the neutralizing allows for the ADA to bind the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the coated plate. In some examples, the neutralizing uses a base at a pH of about 8.0. In some examples, the neutralizing uses a base selected from the group consisting of Tris, Phosphate, HEPES, triethanolamine, and mixtures thereof. In some examples, the base is Tris.

[0013] In some examples, the ruthenylated detector cocktail comprises an antibody. In some examples, the antibody is selected from the group consisting of an anti-human IgG, an anti-human IgM, an anti-human IgE, an anti-rabbit Ig, and any combination thereof. In some examples, the detecting specifically detects the ADA and not Factor XI and/or Factor XIa.

[0014] In some examples, the method further comprises a washing after the incubating.

[0015] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof is at a concentration of about 0.25 μg/mL.

[0016] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) comprising complementary determining regions HCDR1, HCDR2, and HCDR3 in SEQ ID NO: 9 or 29; and a light chain variable region (VL) comprising complementary determining regions LCDR1, LCDR2, LCDR3 in SEQ ID NO: 19 or 39.

[0017] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises: i. a heavy chain variable region CDR1 of SEQ ID NO: 23; a heavy chain variable region CDR2 of SEQ ID NO: 24; a heavy chain variable region CDR3 of SEQ ID NO: 25; a light chain variable region CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 34; and a light chain variable region CDR3 of SEQ ID NO: 35; ii. a heavy chain variable region CDR1 of SEQ ID NO: 26; a heavy chain variable region CDR2 of SEQ ID NO: 27; a heavy chain variable region CDR3 of SEQ ID NO: 28; a light chain variable region CDR1 of SEQ ID NO: 36; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 38; iii. a

heavy chain variable region CDR1 of SEQ ID NO: 43; a heavy chain variable region CDR2 of SEQ ID NO: 44; a heavy chain variable region CDR3 of SEQ ID NO: 45; a light chain variable region CDR1 of SEQ ID NO: 47; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 15; or iv. a heavy chain variable region CDR1 of SEQ ID NO: 46; a heavy chain variable region CDR2 of SEQ ID NO: 4; a heavy chain variable region CDR3 of SEQ ID NO: 5; a light chain variable region CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 14; and a light chain variable region CDR3 of SEQ ID NO: 15.

[0018] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9, 29, and a VH with 90% identity thereto; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19, 39, and a VL with 90% identity thereto. In certain examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9 and 29; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19 and 39.

[0019] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 31, 11, and a heavy chain with 90% identity thereto; and a light chain comprising an amino acid sequence of SEQ ID NO: 41, 21, and a light chain with 90% identity thereto. In certain examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 31 and a light chain comprising an amino acid sequence of SEQ ID NO: 41.

[0020] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody is a human monoclonal antibody. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody is a human IgG1 isotype. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises D265A and P329A substitutions in the Fc domain.

[0021] In another example, provided herein is a method of detecting an anti-drug antibody (ADA) against an anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises: i. a heavy chain variable region CDR1 of SEQ ID NO: 23; a heavy chain variable region CDR2 of SEQ ID NO: 24; a heavy chain variable region CDR3 of SEQ ID NO: 25; a light chain variable region

CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 34; and a light chain variable region CDR3 of SEQ ID NO: 35; ii. a heavy chain variable region CDR1 of SEQ ID NO: 26; a heavy chain variable region CDR2 of SEQ ID NO: 27; a heavy chain variable region CDR3 of SEQ ID NO: 28; a light chain variable region CDR1 of SEQ ID NO: 36; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 38; iii. a heavy chain variable region CDR1 of SEQ ID NO: 43; a heavy chain variable region CDR2 of SEQ ID NO: 44; a heavy chain variable region CDR3 of SEQ ID NO: 45; a light chain variable region CDR1 of SEQ ID NO: 47; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 15; or iv. a heavy chain variable region CDR1 of SEQ ID NO: 46; a heavy chain variable region CDR2 of SEQ ID NO: 4; a heavy chain variable region CDR3 of SEQ ID NO: 5; a light chain variable region CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 14; and a light chain variable region CDR3 of SEQ ID NO: 15 and wherein the method comprises: (a) incubating a sample with an acid to dissociate anti-Factor XI and/or anti-Factor XIa antibody – antigen complexes and/or dissociate anti-Factor XI and/or anti-Factor XIa antibody – ADA complexes present in the sample to create an acid digest, (b) incubating the acid digest on a plate coated with the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof, (c) neutralizing the acid digest, and (d) detecting the presence of the ADA using a ruthenylated detector cocktail.

[0022] In some examples, the sample is a sample from a subject. In some examples, the sample is selected from the group consisting of blood, plasma, or serum from a subject. In some examples, the method comprises an initial step of preparing the sample.

[0023] In some examples, the acid is selected from the group consisting of acetic acid, citric acid, phosphoric acid, and mixtures thereof. In some examples, the acid is acetic acid. In some examples, the acetic acid is at a concentration of about 300 mM.

[0024] In some examples, the antigen is Factor XI and/or Factor XIa. In some examples, the plate is coated with streptavidin. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the plate is at a concentration selected from the group consisting of about 0.1 µg/ml, about 0.25 µg/ml, about 0.5 µg/ml, about 0.75 µg/ml, and about 1 µg/ml. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the plate is at a concentration of about 0.25 µg/ml.

[0025] In some examples, the neutralizing allows for the ADA to bind the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof on the coated plate. In some examples, the neutralizing uses a base at a pH of about 8.0. In some examples, the neutralizing uses a base selected from the group consisting of Tris, Phosphate, HEPES, triethanolamine, and mixtures thereof. In some examples, the base is Tris.

[0026] In some examples, the ruthenylated detector cocktail comprises an antibody. In some examples, the antibody is selected from the group consisting of an anti-human IgG, an anti-human IgM, an anti-human IgE, an anti-rabbit Ig, and any combination thereof. In some examples, the detecting specifically detects the ADA and not Factor XI and/or Factor XIa.

[0027] In some examples, the method further comprises a washing after the incubating.

[0028] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof is at a concentration of about 0.25 μg/mL.

[0029] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9, 29, and a VH with 90% identity thereto; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19, 39, and a VL with 90% identity thereto. In certain examples, the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9 and 29; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19 and 39.

[0030] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 31, 11, and a heavy chain with 90% identity thereto; and a light chain comprising an amino acid sequence of SEQ ID NO: 41, 21, and a light chain with 90% identity thereto. In certain examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 31 and a light chain comprising an amino acid sequence of SEQ ID NO: 41.

[0031] In some examples, the anti-Factor XI and/or anti-Factor XIa antibody is a human monoclonal antibody. In some examples, the anti-Factor XI and/or anti-Factor

XIa antibody is a human IgG1 isotype. In some examples, the anti-Factor XI and/or anti-Factor XIa antibody comprises D265A and P329A substitutions in the Fc domain.

[0032] Other examples and details of the disclosure are presented herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] **FIG. 1** is a schematic depiction of the initial bridging assay for detection of anti-drug antibodies (ADA) in cynomolgus samples.

[0034] **FIG. 2** is a schematic depiction of the modified bridging assay with a Factor XI/Factor XIa depletion step for detection of ADA in cynomolgus samples.

[0035] **FIG. 3** is a schematic depiction of the modified bridging assay with a Ruthenylated anti-monkey Ig for detection of ADA in cynomolgus samples.

[0036] **FIG. 4** is a schematic depiction of the ADA assay for cynomolgus samples.

[0037] **FIG. 5** is a schematic depiction of the ADA assay for human samples.

DETAILED DESCRIPTION

Definitions

[0038] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0039] The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate.

[0040] As used herein, the terms “FXI protein,” “FXI antigen,” and “FXI” are used interchangeably, and refers to the Factor XI protein in different species. Factor XI is the mammalian plasma coagulation factor XI, a glycoprotein present in human plasma at a concentration of 25-30 nM as a zymogen that when converted by limited proteolysis to an active serine protease, participates in the intrinsic pathway of blood coagulation.

[0041] The terms “FXIa protein,” “FXIa antigen,” and “FXIa”, are used interchangeably, and refers to the activated FXI protein in different species. The zymogen Factor XI is converted into its active form, the coagulation factor XIa (FXIa), either via the contact phase of blood coagulation or through thrombin-mediated activation on the platelet surface. During this activation of factor XI, an internal peptide bond is cleaved in each of the two chains, resulting in the activated factor XIa, a serine protease composed of two heavy and two light

CLAIMS

What is claimed is:

1. A method of detecting an anti-drug antibody (ADA) against an anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof, wherein the method comprises:

a. incubating a sample with an acid to dissociate anti-Factor XI and/or anti-Factor XIa antibody – antigen complexes and/or dissociate anti-Factor XI and/or anti-Factor XIa antibody – ADA complexes present in the sample to create an acid digest,

b. incubating the acid digest on a plate coated with the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof,

c. neutralizing the acid digest, and

d. detecting the presence of the ADA using a ruthenylated detector cocktail.

2. The method of claim 1, wherein the sample is selected from the group consisting of human blood, plasma, or serum.

3. The method of claim 1, wherein the method further comprises

a. an initial step of preparing the sample; and/or

b. a washing step after the incubating.

4. The method of any one of claims 1-3, wherein:

a. the acid is selected from the group consisting of acetic acid, citric acid, phosphoric acid, and mixtures thereof;

b. the neutralizing uses a base selected from the group consisting of Tris, Phosphate, HEPES, triethanolamine, and mixtures thereof, optionally at a pH of about 8.0; and/or;

c. the ruthenylated detector cocktail comprises an antibody.

5. The method of claim 4, wherein:

a. the acid is acetic acid, optionally at a concentration of about 300 mM; and/or

b. the base is Tris.

6. The method of any one of claims 1-3, wherein the plate is coated with streptavidin.

7. The method of any one of claims 1-3, wherein the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof is coated on the plate in a buffer at a

concentration selected from the group consisting of about 0.1 µg/mL, about 0.25 µg/mL, about 0.5 µg/mL, about 0.75 µg/mL, and about 1 µg/mL, preferably about 0.25 µg/mL.

8. The method of claim 5, wherein the antibody is selected from the group consisting of an anti-human IgG, an anti-human IgM, an anti-human IgE, an anti-rabbit Ig, and any combination thereof.

9. The method of any one of claims 1-3, wherein the anti-Factor XI and/or anti-Factor XIa antibody or antigen-binding fragment thereof comprises:

i. a heavy chain variable region CDR1 of SEQ ID NO: 23; a heavy chain variable region CDR2 of SEQ ID NO: 24; a heavy chain variable region CDR3 of SEQ ID NO: 25; a light chain variable region CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 34; and a light chain variable region CDR3 of SEQ ID NO: 35;

ii. a heavy chain variable region CDR1 of SEQ ID NO: 26; a heavy chain variable region CDR2 of SEQ ID NO: 27; a heavy chain variable region CDR3 of SEQ ID NO: 28; a light chain variable region CDR1 of SEQ ID NO: 36; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 38;

iii. a heavy chain variable region CDR1 of SEQ ID NO: 43; a heavy chain variable region CDR2 of SEQ ID NO: 44; a heavy chain variable region CDR3 of SEQ ID NO: 45; a light chain variable region CDR1 of SEQ ID NO: 47; a light chain variable region CDR2 of SEQ ID NO: 37; and a light chain variable region CDR3 of SEQ ID NO: 15; or

iv. a heavy chain variable region CDR1 of SEQ ID NO: 46; a heavy chain variable region CDR2 of SEQ ID NO: 4; a heavy chain variable region CDR3 of SEQ ID NO: 5; a light chain variable region CDR1 of SEQ ID NO: 33; a light chain variable region CDR2 of SEQ ID NO: 14; and a light chain variable region CDR3 of SEQ ID NO: 15.

10. The method of any one of claims 1-3, wherein the anti-Factor XI and/or anti-Factor Xia antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9, 29, and a VH with 90% identity thereto; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19, 39, and a VL with 90% identity thereto; or

b. a heavy chain variable region (VH) selected from the group consisting of SEQ ID NO: 9 and 29; and a light chain variable region (VL) selected from the group consisting of SEQ ID NO: 19 and 39.

11. The method of any one of claims 1-3, wherein the anti-Factor XI and/or anti-Factor XIa antibody comprises:

a. a heavy chain comprising an amino acid sequence of SEQ ID NO: 31, 11, and a heavy chain with 90% identity thereto; and a light chain comprising an amino acid sequence of SEQ ID NOs: 41, 21, or a light chain with 90% identity thereto; or

b. a heavy chain comprising an amino acid sequence of SEQ ID NO: 31 and a light chain comprising an amino acid sequence of SEQ ID NO: 41.

12. The method of any one of claims 1-3, wherein the anti-Factor XI and/or anti-Factor XIa antibody is a human monoclonal antibody.

13. The method of claim 12, wherein the anti-Factor XI and/or anti-Factor Xia antibody is a human IgG1 isotype,

optionally comprises an antibody Fc domain comprising D265A and P329A substitutions.