

HUMANIZED ANTI-COMPLEMENT FACTOR BB ANTIBODIES AND USES THEREOF

RELATED APPLICATION

5 This application claims priority to US provisional application serial number 63/012,590, filed April 20, 2020, the entire content of which is incorporated herein by reference.

FIELD OF THE INVENTION

10 The present application relates to humanized anti-factor Bb antibodies and the use of the antibodies.

BACKGROUND

 The complement system is part of the innate immune system. Its primary role is to
15 “complement” the ability of antibodies and phagocytic cells to clear harmful pathogens from an organism. The complement system includes three separate upstream activation pathways (the classical pathway, the alternative pathway and the lectin pathway), all converging on a common terminal pathway. Factor B is a component of the alternative pathway of complement and can be cleaved into factor Ba and factor Bb. Factor B also contains a serine protease (SP)
20 domain, and when activated it provides the catalytic activity of the alternative pathway C3 and C5 convertases. Aberrant activation of the complement system can cause damage to host tissue in a wide variety of pathological settings, ranging from autoimmune disease to organ transplantation. There is still a need for treating diseases or disorders associated with complement system. The present invention addresses this need and others.

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SUMMARY

 The present disclosure provides, in some aspects, humanized anti-factor Bb antibodies, compositions comprising the antibodies, methods of producing the antibodies, and methods of using the antibodies, for example, for treating a complement-mediated disease or disorder. As
30 shown in the data provided herein, the humanized anti-factor Bb antibodies of the present disclosure have a binding affinity within two-fold of that of the parent antibody. Unexpectedly, initial attempts to humanize the parent mouse anti-factor Bb antibody produced a majority of variants lacking an acceptable binding affinity. Thus, to produce humanized versions having suitable binding affinities (*e.g.*, to treat complement-mediated diseases or disorders), multiple

rounds of humanization were required. Further, only certain V_H and V_L domains could be combined to produce an antibody that bound to factor Bb with an acceptable binding affinity *see, e.g.*, antibodies tested in Example 1, Table 9.

Some aspects of the present disclosure provide a humanized antibody that binds
5 specifically to human complement factor Bb protein and comprises a heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO: 19 and a light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO: 27.

Other aspects of the present disclosure provide a humanized antibody that binds specifically to human complement factor Bb protein and comprises a V_H comprising the amino
10 acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the humanized antibody binds specifically to the human complement factor Bb protein with an affinity of 10⁻⁶ to 10⁻⁹ M.

In some embodiments, the humanized antibody inhibits a complement pathway
15 activity. In some embodiments, the complement pathway activity is the alternative pathway (AP) activity.

In some embodiments, the complement AP activity is selected from the group consisting of: AP-mediated terminal membrane attack complex (MAC) deposition, AP-mediated hemolysis, C3 fragment deposition on red blood cells or other cell types, C3b/Bb-mediated cleavage of C3, and C3bBb3b-mediated cleavage of C5.
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In some embodiments, the humanized antibody is a bispecific antibody or a multispecific antibody.

In some embodiments, the humanized antibody is selected from the group consisting of an Ig monomer, a Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, and a Fv.

25 In some embodiments, the humanized antibody comprises a heavy chain constant region of the isotype IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the humanized antibody comprises an IgG4 constant region or a variant thereof.

In some embodiments, the heavy chain constant region comprises an amino acid
30 sequence that is at least 90% identical to any one of SEQ ID NOs: 28-30.

In some embodiments, the humanized antibody comprises a heavy chain comprising the amino acid sequence of any one of SEQ ID NOs: 32-34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, the humanized antibody comprises a heavy chain comprising the amino acid sequence of any one of SEQ ID NOs: 36-38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

Also provided herein are conjugates comprising a humanized antibody of the present
5 disclosure.

In some embodiments, a humanized anti-factor Bb antibody of a conjugate comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a humanized anti-factor Bb antibody of a conjugate comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:
10 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a humanized anti-factor Bb antibody of a conjugate comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a humanized anti-factor Bb antibody of a conjugate comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:
15 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

Further provided herein are pharmaceutical compositions comprising a humanized antibody described herein or a conjugate described herein.

In some embodiments, a pharmaceutical composition comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO:
20 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a pharmaceutical composition comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a pharmaceutical composition comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO:
25 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a pharmaceutical composition comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

30 In some embodiments, a pharmaceutical composition comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a pharmaceutical composition comprises a conjugate that comprises a

humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a pharmaceutical composition comprises a conjugate that
5 comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a pharmaceutical composition comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID
10 NO: 39.

In some embodiments, a pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

Also provided herein are devices comprising a humanized antibody described herein, a conjugate described herein, or a pharmaceutical composition described herein.

15 In some embodiments, a device comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a device comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID
20 NO: 35.

In some embodiments, a device comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a device comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid
25 sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a device comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some
30 embodiments, a device comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a device comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a device comprises a conjugate that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a device comprises a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27.

10 In some embodiments, a device comprises a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a device comprises a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26.

15 In some embodiments, a device comprises a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, the device is an injectable device, for example, a syringe, a pen, or an electronic injection device (e-Device).

Yet other aspects of the present disclosure provide methods of treating a subject having a complement-mediated disease or disorder, the methods comprising administering to the subject an effective amount of a humanized antibody described herein, a conjugate described herein, or a pharmaceutical composition described herein to treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the

subject an effective amount of a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, to treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated
5 disease or disorder comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a
10 subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39, to treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a conjugate
15 that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises
20 administering to the subject an effective amount of a conjugate that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, to treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a conjugate that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L
25 comprising the amino acid sequence of SEQ ID NO: 26, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a conjugate that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39, to
30 treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that

comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a
5 pharmaceutical composition that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, to treat the complement-mediated disease or disorder.

In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a
10 pharmaceutical composition that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26, to treat the complement-mediated disease or disorder. In some embodiments, a method of treating a subject having a complement-mediated disease or disorder comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a heavy chain
15 comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39, to treat the complement-mediated disease or disorder.

In some embodiments, the complement-mediated disease is selected from the group consisting of: IgA nephropathy (Berger's disease), atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH), idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), lupus nephritis, ANCA vasculitis,
20 membranous nephropathy, C3 glomerulonephritis (C3GN), focal segmental glomerulosclerosis (FSGS), multiple sclerosis, macular degeneration, age-related macular degeneration (AMD), rheumatoid arthritis, antiphospholipid antibody syndrome, asthma, ischemia-reperfusion injury, Type II membranoproliferative glomerulonephritis (GN), spontaneous fetal loss, Pauci-
25 immune vasculitis, epidermolysis bullosa, recurrent fetal loss, and traumatic brain injury.

Still other aspects of the present disclosure provide methods of inhibiting a complement pathway activity in a subject. In some embodiments, the complement pathway activity is an alternative pathway (AP) activity. In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an
30 effective amount of a humanized anti-factor Bb antibody, a conjugate comprising a humanized anti-factor Bb antibody, or a pharmaceutical composition comprising a humanized anti-factor Bb antibody, to inhibit the complement pathway activity.

In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27, to inhibit
5 the complement pathway activity. In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, to inhibit the complement pathway activity.

10 In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26, to inhibit the complement pathway activity. In some embodiments, a method of inhibiting a complement
15 pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39, to inhibit the complement pathway activity.

In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a conjugate that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27, to inhibit the complement pathway activity. In some embodiments, a method of
20 inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a conjugate that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, to inhibit the complement pathway activity.

25 In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a conjugate that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26, to inhibit the complement pathway activity. In some embodiments, a method of
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inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a conjugate that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39, to
5 inhibit the complement pathway activity.

In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising
10 the amino acid sequence of SEQ ID NO: 27, to inhibit the complement pathway activity. In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino
15 acid sequence of SEQ ID NO: 35, to inhibit the complement pathway activity.

In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising
20 the amino acid sequence of SEQ ID NO: 26, to inhibit the complement pathway activity. In some embodiments, a method of inhibiting a complement pathway activity (*e.g.*, AP activity) in a subject comprises administering to the subject an effective amount of a pharmaceutical composition that comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino
25 acid sequence of SEQ ID NO: 39, to inhibit the complement pathway activity.

In some embodiments, the complement AP activity is selected from the group consisting of: AP-mediated terminal membrane attack complex (MAC) deposition, AP-mediated hemolysis, C3 fragment deposition on red blood cells or other cell types, C3b/Bb-mediated cleavage of C3, and C3bBb3b-mediated cleavage of C5. In some embodiments, the
30 subject has a complement-mediated disease or disorder.

In some embodiments, a method further comprises administering to the subject a therapeutic agent.

In some embodiments, administering is intravenous, subcutaneous, or intramuscular.

Also provided herein are humanized anti-factor Bb antibodies for use in a method for treating a complement-mediated disease or disorder. Further provided herein are conjugates comprising a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder. Further still provided herein are pharmaceutical compositions comprising a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder. Still further provided herein are devices comprising a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder.

In some embodiments, a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a humanized anti-factor Bb antibody for use in a method for treating a complement-mediated disease or disorder comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a conjugate for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a conjugate for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a conjugate for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a conjugate for use in a method for

treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a pharmaceutical composition for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a pharmaceutical composition for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a pharmaceutical composition for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a pharmaceutical composition for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a device for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a device for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a device for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a device for use in a method for treating a complement-mediated disease or disorder comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, the complement-mediated disease is selected from the group consisting of: IgA nephropathy (Berger's disease), atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH), idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), lupus nephritis, ANCA vasculitis, membranous nephropathy, C3 glomerulonephritis (C3GN), focal segmental glomerulosclerosis (FSGS), multiple sclerosis, macular degeneration, age-related macular degeneration (AMD), rheumatoid arthritis, antiphospholipid antibody syndrome, asthma, ischemia-reperfusion injury, Type II membranoproliferative GN, spontaneous fetal loss, Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, and traumatic brain injury.

Also provided herein are humanized anti-factor Bb antibodies for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity). Further provided herein are conjugates comprising a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity). Further still provided herein are pharmaceutical compositions comprising a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity). Still further provided herein are devices comprising a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity).

In some embodiments, a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a humanized anti-factor Bb antibody for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a conjugate for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising

a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a conjugate for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a conjugate for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a conjugate for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity

(*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a pharmaceutical composition for use in a method for inhibiting a complement pathway activity (*e.g.*, AP activity) comprises a humanized anti-factor Bb antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

Also provided herein are nucleic acids or nucleic acid sets encoding or collectively encoding a humanized antibody described herein; vectors or vector sets comprising a nucleic acid or nucleic acid set described herein; cells expressing a humanized antibody, a nucleic acid or nucleic acid set, or a vector or vector set as described herein.

In some embodiments, a nucleic acid or nucleic acid set encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a nucleic acid or nucleic acid set encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a nucleic acid or nucleic acid set encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a nucleic acid or nucleic acid set encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a vector or vector set comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a vector or vector set comprises a

nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

5 In some embodiments, a vector or vector set comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a vector or vector set comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

10 In some embodiments, a cell comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a cell comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

15 In some embodiments, a cell comprises a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a cell comprises a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

20 In some embodiments, a cell comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a cell comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

30 In some embodiments, a cell comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a cell comprises a nucleic acid or nucleic acid set that

encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a cell comprises a vector or vector set that comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27. In some embodiments, a cell comprises a vector or vector set that comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a cell comprises a vector or vector set that comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a cell comprises a vector or vector set that comprises a nucleic acid or nucleic acid set that encodes or collectively encodes a humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a cell is a mammalian cell, for example, selected from the group consisting of human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, NS0 myeloma cells, SP2 cells, COS cells, and mammary epithelial cells.

Other aspects of the present disclosure provide methods of producing a humanized antibody described herein, the methods comprising culturing a cell described herein to produce the humanized antibody. In some embodiments, a method further comprises isolating the humanized antibody.

In some embodiments, a method of producing a humanized anti-factor Bb antibody comprises culturing a cell that comprises a nucleic acid that encodes or nucleic acid set that collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 19 and a V_L comprising the amino acid sequence of SEQ ID NO: 27 to produce the humanized anti-factor Bb antibody. In some embodiments, a method of producing a humanized anti-factor Bb antibody comprises culturing a cell that comprises a

nucleic acid that encodes or nucleic acid set that collectively encodes humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a method of producing a humanized anti-factor Bb antibody
5 comprises culturing a cell that comprises a nucleic acid that encodes or nucleic acid set that collectively encodes a humanized anti-factor Bb antibody that comprises a V_H comprising the amino acid sequence of SEQ ID NO: 17 and a V_L comprising the amino acid sequence of SEQ ID NO: 16 to produce the humanized anti-factor Bb antibody. In some embodiments, a method
10 of producing a humanized anti-factor Bb antibody comprises culturing a cell that comprises a nucleic acid that encodes or nucleic acid set that collectively encodes humanized anti-factor Bb antibody that comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 28 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other
15 embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings,
20 each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIGs. 1A-1D show binding of humanized antibody variants (Group 1) to complement factor Bb (factor Bb) using single cycle kinetics. Raw sensorgrams and fitted curves (1:1
25 binding model) are shown for humanized variants and control antibodies binding to factor Bb. Kinetic analysis was carried out on a Biacore T200. Each antibody was captured on a Protein A CM5 chip before increasing concentrations of factor Bb were injected and a single off-rate was determined. *Scales are identical for each section, and x is from -200 to 1400 and y from -5 to 40.

30 **FIGs. 2A-2C** show binding of humanized variants (Group 2) to factor Bb using single cycle kinetics. Raw sensorgrams and fitted curves (1:1 binding model) for redesigned variants and control antibodies binding to factor Bb are shown. Kinetic analysis was carried out on a

Biacore T200. Each antibody was captured on a Protein A CM5 chip before increasing concentrations of factor Bb were injected and a single off-rate was determined.

FIG. 3 shows SDS-PAGE gels of Protein A/Post-SEC-purified antibodies. 1 µg of each reduced antibody sample was loaded on a NuPage 4-12% Bis-Tris gel (ThermoFisher, Loughborough, UK) and run at 200 V for 35 minutes. Gels were stained with InstantBlue (Expedeon, Swavesey, UK). Mk: PAGERuler™ Plus pre-stained protein ladder (ThermoFisher, Loughborough, UK).

FIGs. 4A-4C show binding of lead antibodies (all from Group 2) to factor Bb using multi-cycle kinetics. Multiple cycle sensorgrams data and fitted curves (1:1 binding model) are shown for the humanized variants binding to factor Bb. (**FIG. 4A**: Chimeric VH0/Vκ0, VH4/Vκ6; **FIG. 4B**: VH4/Vκ7, VH6/Vκ6; **FIG. 4C**: VH6/Vκ7, VH7/Vκ7).

FIG. 5 shows factor Bb competition enzyme-linked immunosorbent assay (ELISA) of humanized variants against parental antibody. A dilution series of the anti-factor Bb variants was tested against a fixed concentration of murine parental antibody for binding to factor-Bb. Bound murine antibody was detected using anti-mouse peroxidase conjugate and tetramethylbenzidine (TMB) substrate.

FIGs. 6A-6C show activity of humanized anti-factor Bb antibodies in WIESLAB® Complement Alternative Pathway (CAP) (**FIGs. 6A**) and AP-mediated hemolysis (**FIGs. 6B-6C**) using human serum.

FIGs. 7A-7D show specificity of lead humanized variants to active form (factor Bb) of human factor B (**FIGs. 7A and 7B**) or cynomolgus monkey factor B (**FIGs. 7C and 7D**) by surface plasmon resonance.

FIGs. 8A-8B show specificity of binding of chimeric parent antibody VH0/Vκ0-IgG4v1 (**FIG. 8A**) and representative humanized variant antibody VH6/Vκ7-IgG4v2 (**FIG. 8B**) to factor Bb only among various complement proteins.

FIGs. 9A-9B show activity of VH6/Vκ7-IgG4v2 (produced from HEK cells) and VH6/Vκ7-IgG4v2 _CHO (produced from CHO cells) in WIESLAB® Complement Alternative Pathway (CAP) assay using normal human (**FIG. 9A**) and Cyno (**FIG. 9B**) serum.

FIGs. 10A-10B show activity of VH6/Vκ7-IgG4v2 (produced from HEK) and VH6/Vκ7-IgG4v2 _CHO in WIESLAB® Complement Classical Pathway (CCP) assay using normal human (**FIG. 10A**) and Cyno (**FIG. 10B**) serum.

FIG. 11 show hemolysis of Rabbit RBC by VH6/Vκ7-IgG4v2_HEK and VH6/Vκ7-IgG4v2 _CHO in normal human serum.

FIG. 12 show affinity and multiple cycle sensorgrams raw data with fitted curves for V_H6/V_K7-IgG4v2_CHO(left) and V_H6/V_K7-IgG4v2_HEK(right) binding to human Bb protein.

DETAILED DESCRIPTION

5 The present disclosure provides humanized antibodies that bind complement factor Bb protein. These antibodies are referred to herein as “humanized anti-factor Bb antibodies.” The present disclosure also provides nucleic acids encoding humanized anti-factor Bb antibodies, compositions comprising the antibodies, methods of producing the antibodies (*e.g.*, recombinant production methods), and methods of using the antibodies, such as methods of
10 treating a (at least one) complement-mediated disease or disorder.

“Antibody” encompasses antibodies or immunoglobulins of any isotype, including but not limited to humanized antibodies and chimeric antibodies. An antibody may be a single-chain antibody (scAb) or a single domain antibody (dAb) (*e.g.*, a single domain heavy chain antibody or a single domain light chain antibody; *see Holt et al. (2003) Trends Biotechnol.*
15 21:484). The term “antibody” also encompasses fragments of antibodies (antibody fragments) that retain specific binding to an antigen. “Antibody” further includes single-chain variable fragments (scFvs), which are fusion proteins of the variable regions of the heavy (V_H) and light chains (V_L) of antibodies, connected with a short linker peptide, and diabodies, which are noncovalent dimers of scFv fragments that include the V_H and V_L connected by a small peptide
20 linker (Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 (1995)). Other fusion proteins that comprise an antigen-binding portion of an antibody and a non-antibody protein are also encompassed by the term “antibody.”

“Antibody fragments” comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments
25 include an antigen-binding fragment (Fab), Fab', F(ab')₂, a variable domain Fv fragment (Fv), an Fd fragment, and an antigen binding fragment of a chimeric antigen receptor.

Papain digestion of antibodies produces two identical antigen-binding fragments, referred to as “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an
30 F(ab')₂ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

“Fv” is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region includes a dimer of one heavy-chain variable domain and one

light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three
5 CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

“Fab” fragments contain the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including at least one
10 cysteine from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

“scFv” antibody fragments comprise the V_H and V_L of an antibody, wherein these
15 regions are present in a single polypeptide chain. In some cases, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L regions, which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)*.

“Diabody” refers to a small antibody fragment with two antigen-binding sites, which
20 fragments comprise a V_H connected to a V_L in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, Hollinger *et al.*
25 *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

An antibody can be monovalent or bivalent. An antibody can be an Ig monomer, which is a “Y-shaped” molecule that consists of four polypeptide chains: two heavy chains and two light chains connected by disulfide bonds.

Antibodies can be detectably labeled, *e.g.*, with a radioisotope, an enzyme that
30 generates a detectable product, and/or a fluorescent protein. Antibodies can be further conjugated to other moieties, such as members of specific binding pairs, *e.g.*, biotin member of biotin-avidin specific binding pair. Antibodies can also be bound to a solid support, including, but not limited to, polystyrene plates and/or beads.

An “isolated” antibody is one that has been identified and separated and/or recovered from a component of its natural environment (*i.e.*, is not naturally occurring). Contaminant components of its natural environment are materials that would interfere with uses (*e.g.*, diagnostic or therapeutic uses) of the antibody, and can include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some cases, an antibody is purified (1) to greater than 90%, greater than 95%, or greater than 98% by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or non-reducing conditions using Coomassie blue or silver stain. Isolated antibodies encompass antibodies *in situ* within recombinant cells, as at least one component of the antibody's natural environment will not be present. In some embodiments, an isolated antibody is prepared by at least one purification step.

A “monoclonal antibody” is an antibody produced by a group of identical cells, all of which were produced from a single cell by repetitive cellular replication. That is, the clone of cells only produces a single antibody species. While a monoclonal antibody can be produced using hybridoma production technology, other production methods known to those skilled in the art can also be used (*e.g.*, antibodies derived from antibody phage display libraries).

A “complementarity determining region (CDR)” is the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. CDRs have been described by Lefranc *et al.* (2003) *Developmental and Comparative Immunology* 27:55; Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U. S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991); by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); and MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein.

As used herein, the terms “CDR-L1,” “CDR-L2,” and “CDR-L3” refer, respectively, to the first, second, and third CDRs in a light chain variable region. As used herein, the terms “CDR-H1,” “CDR-H2,” and “CDR-H3” refer, respectively, to the first, second, and third CDRs in a heavy chain variable region. As used herein, the terms “CDR-1,” “CDR-2,” and

“CDR-3” refer, respectively, to the first, second and third CDRs of either chain’s variable region.

A “framework” when used in reference to an antibody variable region includes all amino acid residues outside the CDR regions within the variable region of an antibody. A
5 variable region framework is generally a discontinuous amino acid sequence that includes only those amino acids outside of the CDRs. A “framework region” includes each domain of the framework that is separated by the CDRs.

A “humanized antibody” is an antibody comprising portions of antibodies of different origin, wherein at least one portion comprises amino acid sequences of human origin. For
10 example, the humanized antibody can comprise portions derived from an antibody of nonhuman origin with the requisite specificity, such as a mouse, and from antibody sequences of human origin (*e.g.*, chimeric immunoglobulin), joined together chemically by conventional techniques (*e.g.*, synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (*e.g.*, DNA encoding the protein portions of the chimeric antibody can be expressed
15 to produce a contiguous polypeptide chain). Another example of a humanized antibody is an antibody containing at least one chain comprising a CDR derived from an antibody of nonhuman origin and a framework region derived from a light and/or heavy chain of human origin (*e.g.*, CDR-grafted antibodies with or without framework changes). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin.
20 See, *e.g.*, Cabilly *et al.*, U. S. Pat. No. 4,816,567; Cabilly *et al.*, European Patent No. 0,125,023 B1; Boss *et al.*, U. S. Pat. No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M. S. *et al.*, WO 86/01533; Neuberger, M. S. *et al.*, European Patent No. 0,194,276 B1; Winter, U. S. Pat. No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Padlan, E. A. *et al.*, European Patent Application No. 0,519,596 A1. See also, Ladner *et al.*, U. S. Pat. No.
25 4,946,778; Huston, U. S. Pat. No. 5,476,786; and Bird, R. E. *et al.*, Science, 242: 423-426 (1988)), regarding single chain antibodies.

In some embodiments, a humanized antibody is produced using synthetic and/or recombinant nucleic acids to prepare genes (*e.g.*, cDNA) encoding the desired humanized chain. For example, nucleic acid (*e.g.*, DNA) sequences coding for humanized variable regions
30 can be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see *e.g.*, Kamman, M., *et al.*, Nucl. Acids Res., 17: 5404 (1989)); Sato, K., *et al.*, Cancer Research, 53: 851-856 (1993); Daugherty, B. L. *et al.*, Nucleic Acids Res., 19(9): 2471-2476

(1991); and Lewis, A. P. and J. S. Crowe, Gene, 101: 297-302 (1991)). Using these or other suitable methods, variants can also be readily produced. For example, cloned variable regions can be mutagenized, and sequences encoding variants with the desired specificity can be selected (*e.g.*, from a phage library; see *e.g.*, Krebber *et al.*, U. S. Pat. No. 5,514,548;

5 Hoogenboom *et al.*, WO 93/06213, published Apr. 1, 1993).

Humanized Anti-factor Bb Antibodies

The amino acid sequences of the mouse monoclonal anti-factor Bb antibody from which the humanized anti-factor Bb antibodies described herein are derived are provided in
10 Table 1. In some embodiments, a humanized anti-factor Bb antibody comprises a framework region of the heavy chain variable region and/or the light chain variable region that include sequences derived from a human immunoglobulin framework.

Table 1. Mouse Monoclonal Anti-Factor Bb Antibody

Ab	CDRs (Kabat)	CDRs (IMGT)	Variable regions (Kabat CDRs bolded , IMGT CDRs <u>underlined</u>)
Anti-factor Bb mouse monoclonal antibody	CDR-H1: NYAMS (SEQ ID NO: 1)	CDR-H1: GFTFSNYA (SEQ ID NO: 7)	V _H (also referred to as “VH0” herein): EVQLVESGGALVKPG GSLKLSCAAS GFTFSN YAMSWVRQTPEKRLE WVA TISNRGSYTYYP DSVKG RFTISRDNK NTLYLQMSSLRSED ALYYC ARERPMDY W GQGTSTVTSS (SEQ ID NO: 12)
	CDR-H2: TISNRGSYTYYPDSVKG (SEQ ID NO: 2)	CDR-H2: ISNRGSYT (SEQ ID NO: 8)	
	CDR-H3: ERPMDY (SEQ ID NO: 3)	CDR-H3: ARERPMDY (SEQ ID NO: 9)	
	CDR-L1: KASQDVGTAVA (SEQ ID NO: 4)	CDR-L1: QDVGTA (SEQ ID NO: 10)	V _L (also referred to as “Vk0” herein): DIVMTQSHKFMSTSV GDRVSITC KASQDVG TAV AWYQQKPGQSP KLLIY WASTRHT GVP DRFTGSGSGTDFTLTI TNVQSEDLAVYFC HQ HSSNPLT FGAGTKLE LK (SEQ ID NO: 13)
	CDR-L2: WASTRHT (SEQ ID NO: 5)	CDR-L2: WAS (SEQ ID NO: 11)	
	CDR-L3: HQHSSNPLT (SEQ ID NO: 6)	CDR-L3: HQHSSNPLT (SEQ ID NO: 6)	

15

In some embodiments, a humanized anti-factor Bb antibody described herein comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2), and a heavy chain complementarity

determining region 3 (CDR-H3) of a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 12. In some embodiments, a humanized anti-factor Bb antibody described herein comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2), and a light chain
5 complementarity determining region 3 (CDR-L3) of a light chain variable region comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, a humanized anti-factor Bb antibody further comprises a humanized heavy chain framework region and/or a humanized light chain framework region.

In some embodiments, according to the Kabat definition, a humanized anti-factor Bb
10 antibody described herein comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3. In some embodiments, according to the Kabat definition, a humanized anti-factor Bb antibody described herein comprises a CDR-L1
15 comprising the amino acid sequence of SEQ ID NO: 4, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, a humanized anti-factor Bb antibody further comprises a humanized heavy chain framework region and/or a humanized light chain framework region.
In some embodiments, according to the IMGT definition, a humanized anti-factor Bb antibody described herein comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 7,
20 a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, according to the IMGT definition, a humanized anti-factor Bb antibody described herein comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR-L3 comprising the amino acid sequence of SEQ ID
25 NO: 6. In some embodiments, a humanized anti-factor Bb antibody further comprises a humanized heavy chain framework region and/or a humanized light chain framework region.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a V_H containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared
30 with the V_H set forth in SEQ ID NO: 12. In some embodiments, the anti-factor Bb antibody of the present disclosure comprises a V_L containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the V_L set forth in SEQ ID NO: 13.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a V_H comprising an amino acid sequence that is at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the V_H as set forth in SEQ ID NO: 12. In some embodiments, the humanized anti-factor Bb of the present disclosure comprises a V_L comprising an amino acid sequence that is at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the V_L as set forth in SEQ ID NO: 13.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to the Kabat definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_H as set forth in SEQ ID NO: 12. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_L as set forth in SEQ ID NO: 13.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to the Kabat definition system), wherein the framework regions of the V_H are collectively at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_H as set forth in SEQ ID NO: 12. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), wherein the framework

regions of the V_L are collectively at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_L as set forth in any one of SEQ ID NO: 13.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the IMGT definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_H as set forth in SEQ ID NO: 12. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_L as set forth in SEQ ID NO: 13.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the IMGT definition system), wherein the framework regions of the V_H collectively are at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_H as set forth in SEQ ID NO: 12. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), wherein the framework region of the V_L collectively are at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_L as set forth in any one of SEQ ID NO: 13.

Examples of the amino acid sequences and DNA coding sequences of the humanized heavy chain variable regions of the humanized anti-factor Bb antibodies described herein are

provided in Table 2. Examples of the amino acid sequences and DNA coding sequences of the humanized light chain variable regions of the humanized anti-factor Bb antibodies described herein are provided in Table 3.

5 **Table 2. Examples of Humanized Heavy Chain Variable Regions**

Humanized V _H	Amino Acid Sequence	DNA Sequence
V _{H1}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQAPG KGLEWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMS SLRSEDALYYCAR ERPMDYWGQGTSV TVSS (SEQ ID NO: 14)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGG CTCCAGGGAAGGGGCTGGAGTGGGTGCGAACCATTAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTCAACATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAATGAGCAGCCTGAGATCTGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGCCTATGGAC TACTGGGGCCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 41)
V _{H2}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQAPG KGLEWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMN SLRAEDALYYCAR ERPMDYWGQGTTLV TVSS (SEQ ID NO: 15)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGG CTCCAGGGAAGGGGCTGGAGTGGGTGCGAACCATTAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTCAACATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGCCTATGGAC TACTGGGGCCAAGGAACCTGGTCACCGTCTCCTCA (SEQ ID NO: 42)
V _{H3}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQAPG KGLEWVATISNRGS YTYYADSVKGRFTI SRDNAKNSLYLQMN SLRAEDALYYCAR ERPMDYWGQGTTLV TVSS (SEQ ID NO: 16)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGG CTCCAGGGAAGGGGCTGGAGTGGGTGCGAACCATTAGT AATCGTGGTAGTTACACCTACTACGCAGACTCAGTGA AGGGCCGATTCAACATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGCCTATGGAC TACTGGGGCCAAGGAACCTGGTCACCGTCTCCTCA (SEQ ID NO: 43)
V _{H4}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQAPG KRLWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMS SLRSEDALYYCAR ERPMDYWGQGTSV TVSS (SEQ ID NO: 17)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGG CTCCAGGGAAGAGGCTGGAGTGGGTGCGAACCATTAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTCAACATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAATGAGCAGCCTGAGATCTGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGCCTATGGAC TACTGGGGCCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 44)
V _{H5}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQTPE KRLWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMS SLRSEDALYYCAR ERPMDYWGQGTSV	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGA CTCCAGAGAAGAGGCTGGAGTGGGTGCGAACCATTAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTCAACATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAATGAGCAGCCTGAGATCTGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGCCTATGGAC

Humanized V _H	Amino Acid Sequence	DNA Sequence
	TVSS (SEQ ID NO: 18)	TACTGGGGCCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 45)
V _{H6}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQAPG KRLEWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMN SLRAEDTALYYCAR ERPMDYWGQGLV TVSS (SEQ ID NO: 19)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGG CTCCAGGGAAGAGGCTGGAGTGGGTGCGAACCATTAAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGGCCTATGGAC TACTGGGGCCAAGGAACCCCTGGTCACCGTCTCCTCA (SEQ ID NO: 46)
V _{H7}	EVQLVESGGGLVLP GGSLRLSCAASGFTF SNYAMSWVRQTP KRLEWVATISNRGS YTYYPDSVKGRFTI SRDNAKNSLYLQMN SLRAEDTALYYCAR ERPMDYWGQGLV TVSS (SEQ ID NO: 20)	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTCAAG CCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGAT TCACCTTCAGTAACTATGCCATGTCTTGGGTCCGCCAGA CTCCAGAGAAGAGGCTGGAGTGGGTGCGAACCATTAAGT AATCGTGGTAGTTACACCTACTACCCAGACTCAGTGA AGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACT CACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACA CGGCTTTGTATTACTGTGCGAGAGAGAGGGCCTATGGAC TACTGGGGCCAAGGAACCCCTGGTCACCGTCTCCTCA (SEQ ID NO: 47)

CDRs according to the Kabat definition are bolded in the amino acid and DNA sequences

Table 3. Examples of humanized light chain variable regions

Humanized V _L (V _κ)	Amino Acid Sequence	DNA Sequence
V _{κ1}	DIVMTQSPSFLSAS VGDRVITITCKASQ DVGTAVAWYQQK PGQPPKLLIYWAST RHTGVPDRFTGSG SGTDFLTITSLQSE DFAVYFCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 21)	GACATCGTGATGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTAGGAGACAGAGTCACCATCACTTGCAAGGCCAGTCA GGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAAC CAGGGCAACCTCCTAAGCTCCTGATCTATTGGGCATCCAC TCGGCACACTGGGGTCCCAGATAGGTTACAGGCAGTGG ATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGCAG TCTGAAGATTTTGCAGTTTATTTCTGTCAACACATAGCA GCAATCCTCTCACGTTTGCCAGGGGACCAAGCTGGAGA TCAAA (SEQ ID NO: 48)
V _{κ2}	DIVMTQSPSTLSAS VGDRVITITCKASQ DVGTAVAWYQQK PGQPPKLLIYWAST RHTGVPDRFTGSG SGTDFLTITSLQAE DFAVYFCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 22)	GACATCGTGATGACCCAGTCTCCATCCACCCTGTCTGCATC TGTAGGAGACAGAGTCACCATCACTTGCAAGGCCAGTCA GGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAAC CAGGGCAACCTCCTAAGCTCCTGATCTATTGGGCATCCAC TCGGCACACTGGGGTCCCAGATAGGTTACAGGCAGTGG ATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGCAG GCTGAAGATTTTGCAGTTTATTTCTGTCAACACATAGCA GCAATCCTCTCACGTTTGCCAGGGGACCAAGCTGGAGA TCAAA (SEQ ID NO: 49)
V _{κ3}	DIQMTQSPSTLSAS VGDRVITITCKASQ DVGTAVAWYQQK PGQPPKLLIYWAST RHTGVPDRFTGSGS GTDFTLTITSLQAE DFAVYFCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 23)	GACATCCAGATGACCCAGTCTCCATCCACCCTGTCTGCATC CTGTAGGAGACAGAGTCACCATCACTTGCAAGGCCAGTCA AGGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAAC CCAGGGCAACCTCCTAAGCTCCTGATCTATTGGGCATCCA CTCGGCACACTGGGGTCCCAGATAGGTTACAGCGGCAGTG GATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGCA GGCTGAAGATTTTGCAGTTTATTTCTGTCAACACATAGC AGCAATCCTCTCACGTTTGCCAGGGGACCAAGCTGGAG ATCAAA (SEQ ID NO: 50)

Humanized V _L (V _κ)	Amino Acid Sequence	DNA Sequence
V _κ 4	DIQMTQSPSTLSAS VGDRVITITCRASQ DVGTAVAWYQQK PGQPPKLLIYWAST RHTGVPDRFSGSGS GTDFTLTISLQAE DFAVYYCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 24)	GACATCCAGATGACCCAGTCTCCATCCACCCTGTCTGCAT CTGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCTC AGGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAA CCAGGGCAACCTCCTAAGCTCCTGATCTATTGGGCATCCA CTCGGCACACTGGGGTCCCAGATAGGTTACAGCGGCAGTG GATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGCA GGCTGAAGATTTTGCAGTTTATTACTGTACCAACATAGC AGCAATCCTCTCACGTTTGGCCAGGGGACCAAGCTGGAG ATCAAA (SEQ ID NO: 51)
V _κ 5	DIQMTQSPSTLSAS VGDRVITITCRASQ DVGTAVAWYQQK PGQPPKLLIYWAST RHTGVPDRFSGSGS GTDFTLTISLQAE DFAVYYCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 25)	GACATCCAGATGACCCAGTCTCCATCCACCCTGTCTGCAT CTGTAGGAGACAGAGTCACCATCACTTGCCGGGCCAGTCTC AGGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAA CCAGGGCAACCTCCTAAGCTCCTGATCTATTGGGCATCCA CTCGGCACACTGGGGTCCCAGATAGGTTACAGCGGCAGTG GATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGCA GGCTGAAGATTTTGCAGTTTATTACTGTACCAACATAGC AGCAATCCTCTCACGTTTGGCCAGGGGACCAAGCTGGAG ATCAAA (SEQ ID NO: 52)
V _κ 6	DIVMTQSPSFLSAS VGDRVITITCKASQ DVGTAVAWYQQK PGKAPKLLIYWAST RHTGVPDRFTGSG SGTDFTLTISLQSE DFAVYFCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 26)	GACATCGTGATGACCCAGTCTCCATCCTTCCTGTCTGCATC TGTAGGAGACAGAGTCACCATCACTTGCAAGGCCAGTCA GGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAA CAGGGAAAGCCCTAAGCTCCTGATCTATTGGGCATCCA CTCGGCACACTGGGGTCCCAGATAGGTTACAGGCAGTG GATCATGGGACAGATTTCACTCTCACAATCAGCAGCCTGC AGTCTGAAGATTTTGCAGTTTATTCTGTACCAACATAG CAGCAATCCTCTCACGTTTGGCCAGGGGACCAAGCTGGA GATCAAA (SEQ ID NO: 53)
V _κ 7	DIQMTQSPSTLSAS VGDRVITITCKASQ DVGTAVAWYQQK PGKAPKLLIYWAST RHTGVPDRFSGSGS GTDFTLTISLQAE DFAVYFCHQHSSN PLTFGQGTKLEIK (SEQ ID NO: 27)	GACATCCAGATGACCCAGTCTCCATCCACCCTGTCTGCAT CTGTAGGAGACAGAGTCACCATCACTTGCAAGGCCAGTCTC AGGATGTGGGTACTGCTGTAGCCTGGTATCAGCAAAAA CCAGGGAAAGCCCTAAGCTCCTGATCTATTGGGCATCC ACTCGGCACACTGGGGTCCCAGATAGGTTACAGCGGCAGT GGATCTGGGACAGATTTCACTCTCACAATCAGCAGCCTGC AGGCTGAAGATTTTGCAGTTTATTCTGTACCAACATAG CAGCAATCCTCTCACGTTTGGCCAGGGGACCAAGCTGGA GATCAAA (SEQ ID NO: 54)

CDRs according to the Kabat definition are bolded in the amino acid and DNA sequences

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to the Kabat definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_H as set forth in any one of SEQ ID NOs: 14-20. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the Kabat definition system), a CDR-L2

having the amino acid sequence of SEQ ID NO: 5 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the V_L as set forth in any one of SEQ ID NOs: 21-27.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to the Kabat definition system), wherein the framework regions of the V_H are collectively at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_H as set forth in any one of SEQ ID NOs: 14-20. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), wherein the framework regions of the V_L are collectively at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_L as set forth in any one of SEQ ID NOs: 21-27.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the IMGT definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework region as compared with the V_H as set forth in any one of SEQ ID NOs: 14-20. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), and containing no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1

amino acid variation) in the framework region as compared with the V_L as set forth in any one of SEQ ID NOs: 21-27.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the IMGT definition system), wherein the framework region of the V_H is at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_H as set forth in any one of SEQ ID NOs: 14-20. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), wherein the framework region of the V_L are collectively at least 80% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the framework regions of the V_L as set forth in any one of SEQ ID NOs: 21-27.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H as set forth in any one of SEQ ID NOs: 14-20. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_L as set forth in any one of SEQ ID NOs: 21-27. Table 4 provides examples of humanized anti-factor Bb antibodies comprising one of the humanized V_Hs provided in Table 2 and one of the humanized V_Ls provided in Table 3.

Table 4. Examples of Variable Regions of Humanized Anti-factor Bb Antibodies

Antibody	Variable Region Amino Acid Sequence
V _H 6/V _κ 7	V _H : EVQLVESGGGLV ^K PGGSLRLS ^{CAAS} GF ^{TS} NY ^{AMS} WVRQAPGK ^{RLEW} VATIS ^{NRGS} Y^{TYYP}DS^{SVK}GRFTIS RDNAKNSLYLQMNSLRAEDTALYYCAR ER^{PM}DY WGQGT ^{LV} TVSS (SEQ ID NO: 19)
	V _L : DIQMTQSPSTLSASV ^{GDRV} TITCK ASQDV GTAVAWYQKPGKAPKLLIYW ASTRHT GVPDRFSGSGSGTDFTLTIS ^{SL} QAEDFAVYF CHQHSSNPL TFGQGT ^{KLEIK} (SEQ ID NO: 27)
V _H 4/V _κ 6	V _H : EVQLVESGGGLV ^K PGGSLRLS ^{CAAS} GF ^{TS} NY ^{AMS} WVRQAPGK ^{RLEW} VATIS ^{NRGS} Y^{TYYP}DS^{SVK}GRFTIS RDNAKNSLYLQMSSLRSEDALYYCAR ER^{PM}DY WGQGT ^{SVT} VSS (SEQ ID NO: 17)
	V _L :

Antibody	Variable Region Amino Acid Sequence
	DIVMTQSPSFLSASVGDRVTTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHT GVPDRFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 26)
V _H 6/V _κ 6	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYCARERPMDYWGQGTLLV TVSS (SEQ ID NO: 19)
	V _L : DIVMTQSPSFLSASVGDRVTTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHT GVPDRFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 26)
V _H 4/V _κ 7	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMSSLRSEDALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 17)
	V _L : DIQMTQSPSTLSASVGDRVTTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHT GVPDRFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 27)
V _H 7/V _κ 7	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQTPEKRLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYCARERPMDYWGQGTLLV TVSS (SEQ ID NO: 20)
	V _L : DIQMTQSPSTLSASVGDRVTTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHT GVPDRFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 27)
V _H 1/V _κ 1	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMSSLRSEDALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 14)
	V _L : DIVMTQSPSFLSASVGDRVTTITCKASQDVGTAVAWYQQKPGQPPKLLIYWASTRHT GVPDRFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 21)
V _H 1/V _κ 2	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMSSLRSEDALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 14)
	V _L : DIVMTQSPSTLSASVGDRVTTITCKASQDVGTAVAWYQQKPGQPPKLLIYWASTRHT GVPDRFTGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 22)
V _H 1/V _κ 3	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMSSLRSEDALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 14)
	V _L : DIQMTQSPSTLSASVGDRVTTITCKASQDVGTAVAWYQQKPGQPPKLLIYWASTRHT GVPDRFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 23)
V _H 1/V _κ 4	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVATISNRGS YTYYPDSVKGRFTISRDNKNSLYLQMSSLRSEDALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 14)
	V _L :

Antibody	Variable Region Amino Acid Sequence
	DIQMTQSPSTLSASVGDRTTITCRASQDVGTAVAWYQQKPGQPPKLLIYWASTRHT GVPDRFSGSGSGTDFTLTISLQAEDFAVYYCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 24)
V _H 1/Vκ5	V _H : EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVATISNRGS YTYYPDSVKGR FTISRDNKNSLYLQMSSLRSEDTALYYCARERPMDYWGQGTSTV VSS (SEQ ID NO: 14)
	V _L : DIQMTQSPSTLSASVGDRTTITCRASQDVGTAVAWYQQKPGQPPKLLIYWASTRHT GVPDRFSGSGSGTDFTLTISLQAEDFATYYCHQHSSNPLTFGQGTKLEIK (SEQ ID NO: 25)

- CDRs according to the Kabat definition are bolded in the amino acid sequences

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 19 and a humanized V_L comprising the amino acid sequence of SEQ ID NO: 27.

- 5 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 17 and a humanized V_L comprising the amino acid sequence of SEQ ID NO: 26.

- In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 19 and a
10 humanized V_L comprising the amino acid sequence of SEQ ID NO: 26.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 17 and a humanized V_L comprising the amino acid sequence of SEQ ID NO: 27.

- In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
15 comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 20 and a humanized V_L comprising the amino acid sequence of SEQ ID NO: 27.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 14 and a humanized V_L comprising the amino acid sequence of any one of SEQ ID NOs: 21.

- 20 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 14 and a humanized V_L comprising the amino acid sequence of any one of SEQ ID NOs: 22.

- In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 14 and a
25 humanized V_L comprising the amino acid sequence of any one of SEQ ID NOs: 23.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 14 and a humanized V_L comprising the amino acid sequence of any one of SEQ ID NOs: 24.

5 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure comprises a humanized V_H comprising the amino acid sequence of SEQ ID NO: 14 and a humanized V_L comprising the amino acid sequence of any one of SEQ ID NOs: 25.

In some embodiments, a humanized anti-factor Bb antibody described herein is a full-length IgG, an Ig monomer, a Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, or a Fv. In some embodiments, a humanized anti-factor Bb antibody described herein is a full-length IgG.

10 In some embodiments, the heavy chain of any of the humanized anti-factor Bb antibodies as described herein comprises a heavy chain constant region (CH) or a portion thereof (*e.g.*, CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can of any suitable origin, *e.g.*, human, mouse, rat, or rabbit. In some embodiments, the heavy chain constant region is from a human IgG (a gamma heavy chain), *e.g.*, IgG1, IgG2, or IgG4.

15 In some embodiments, mutations can be introduced into the heavy chain constant region of any one of the humanized anti-factor Bb antibodies described herein. In some embodiments, one, two or more mutations (*e.g.*, amino acid substitutions) are introduced into the heavy chain constant region (*e.g.*, in a CH2 domain (residues 231-340 of human IgG1) and/or CH3 domain (residues 341-447 of human IgG1) and/or the hinge region, with numbering according to the

20 Kabat numbering system (*e.g.*, the EU index in Kabat)) to increase or decrease the affinity of the antibody for an Fc receptor (*e.g.*, an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc

25 receptor of an antibody that can be made to alter the affinity of the antibody for an Fc receptor are described in, *e.g.*, Smith P *et al.*, (2012) PNAS 109: 6181-6186, U. S. Pat. No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, which are incorporated herein by reference.

30 In some embodiments, one, two or more mutations (*e.g.*, amino acid substitutions) are introduced into the hinge region of the heavy chain constant region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.*, increased or decreased) as described in, *e.g.*, U. S. Pat. No. 5,677,425. The number of cysteine residues in the hinge region of the CH1 domain can be altered to, *e.g.*, facilitate assembly of the light and heavy

chains, or to alter (*e.g.*, increase or decrease) the stability of the antibody or to facilitate linker conjugation.

In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof to alter (*e.g.*, decrease or increase) half-life of the antibody *in vivo*. In some
5 embodiments, the one or more mutations are introduced into an Fc or hinge-Fc domain fragment. See, *e.g.*, International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U. S. Pat. Nos. 5,869,046; 6,121,022; 6,277,375; and 6,165,745 for examples of mutations that will alter (*e.g.*, decrease or increase) the half-life of an antibody *in vivo*.

10 In some embodiments, the constant region antibody described herein is an IgG1 constant region and comprises a methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU index as in Kabat. See U. S. Pat. No. 7,658,921, which is incorporated herein by reference. This type of mutant IgG, referred to
15 as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (see Dall'Acqua W F *et al.*, (2006) J Biol Chem 281: 23514-24). In some embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU index as in Kabat.
20 Additional mutations that may be introduced to the heavy chain constant region that would increase the half-life of the antibody are known in the art, *e.g.*, the M428L/N434S (EU numbering; M459L/N466S Kabat numbering) mutations as described in Zalevsky *et al.*, Nat Biotechnol. 2010 Feb; 28(2): 157-159.

In some embodiments, one, two or more amino acid substitutions are introduced into an
25 IgG constant domain Fc region to alter the effector function(s) of the antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U. S. Pat. Nos. 5,624,821 and 5,648,260. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating antibody
30 thereby increasing tumor localization. See, *e.g.*, U. S. Pat. Nos. 5,585,097 and 8,591,886 for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In some embodiments, at least one amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation

sites on Fc region, which may reduce Fc receptor binding (see, *e.g.*, Shields R L *et al.*, (2001) J Biol Chem 276: 6591-604).

In some embodiments, at least one amino acid in the constant region can be replaced with a different amino acid residue such that the antibody has altered Clq binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U. S. Pat. No. 6,194,551 (Idusogie *et al.*). In some embodiments, at least one amino acid residue in the N-terminal region of the CH2 domain of an antibody described herein is altered to thereby alter the ability of the antibody to fix complement. This approach is described further in International Publication No. WO 94/29351. In some embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor. This approach is described further in International Publication No. WO 00/42072.

In some embodiments, to avoid potential complications due to Fab-arm exchange, which is known to occur with native IgG4 mAbs, the antibodies provided herein may comprise a stabilizing ‘Adair’ mutation (Angal S., *et al.*, “A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody,” Mol Immunol 30, 105-108; 1993), where serine 228 (EU numbering; residue 241 Kabat numbering) is converted to proline resulting in an IgG1-like hinge sequence. In some embodiments, to reduce residual antibody-dependent cellular cytotoxicity, a L235E (EU numbering, corresponding to L248E in Kabat numbering) mutation is introduced to the heavy chain constant region, *e.g.*, as described in Benhnia *et al.*, JOURNAL OF VIROLOGY, Dec. 2009, p. 12355–12367.

In some embodiments, the heavy chain constant region in any one of the humanized anti-factor Bb antibodies described herein is an IgG4 constant region, or a variant thereof. Examples of IgG4 constant regions and variants are provided in Table 5.

Table 5. Examples of Heavy Chain Constant Regions

Heavy Chain Constant Region	Amino Acid Sequence
IgG4 constant region WT (also referred to herein as “IgG4wt”)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRVESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLGLK (SEQ ID NO: 28)

Heavy Chain Constant Region	Amino Acid Sequence
IgG4 constant region variant 1 (also referred to herein as "IgG4v1")	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK (SEQ ID NO: 29)
IgG4 constant region variant 2 (also referred to herein as "IgG4v2")	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHSHYTQKSLSLGLK (SEQ ID NO: 30)

In some embodiments, the light chain of any of the humanized anti-factor Bb antibodies described herein may further comprise a light chain constant region (C_L). In some examples, the C_L is a kappa light chain. In other examples, the C_L is a lambda light chain. In some embodiments, the C_L is a kappa light chain, the sequence of which is provided below:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 31)

Other antibody heavy and light chain constant regions are well known in the art, *e.g.*, those provided in the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php., both of which are incorporated by reference herein.

In some embodiments, a humanized anti-factor Bb antibody described herein comprises a heavy chain comprising any one of the V_H as listed in Table 2 or any variants thereof and a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any one of SEQ ID NOs: 28-30. In some embodiments, a humanized anti-factor Bb antibody described herein comprises a heavy chain comprising any one of the V_H as listed in Table 2 or any variants thereof and a heavy chain constant region that contains no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with any one of SEQ ID NOs: 28-30. In some embodiments, a humanized anti-factor Bb antibody described herein comprises a heavy chain comprising any one of the V_H as listed in Table 2 or any variants thereof and a heavy chain constant region comprising the amino acid sequence of any one of SEQ ID NOs: 28-30.

In some embodiments, a humanized anti-factor Bb antibody described herein comprises a light chain comprising any one of the V_L as listed in Table 3 or any variants thereof and a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 31. In some embodiments, a humanized anti-factor Bb antibody described herein comprises a light chain comprising any one of the V_L as listed in Table 3 or any variants thereof and a light chain constant region that contains no more than 20 amino acid variations (*e.g.*, no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with SEQ ID NO: 31. In some embodiments, a humanized anti-factor Bb antibody described herein comprises a light chain comprising any one of the V_L as listed in Table 3 or any variants thereof and a light chain constant region comprising the amino acid sequence of SEQ ID NO: 31.

Examples of the amino acid sequences of the heavy chain and light chain of the humanized anti-factor Bb antibodies described herein are provided in Table 6.

Table 6. Examples of the heavy chain and light chain of the humanized anti-factor Bb antibodies

Antibody	Amino Acid Sequence
V _H 6/V _K 7-IgG4wt	Heavy chain <i>EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYCARERPMQDYWGQGTLVTVSSASTKG</i> PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVHLQDNLNGKEYKKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMEALHNHYTQKSLSLGLK (SEQ ID NO: 32)
	Light Chain <i>DIQMTQSPSTLSASVGDRVTITCKASQDVGTAFAWYQQKPGKAPKLLIYWASTRITGVPD</i> <i>RFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLT LSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 35)
V _H 6/V _K 7-IgG4v1	Heavy chain <i>EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYCARERPMQDYWGQGTLVTVSSASTKG</i> PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPSCPAPEFEGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVHLQDNLNGKEYKKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMEALHNHYTQKSLSLGLK (SEQ ID NO: 33)
	Light Chain <i>DIQMTQSPSTLSASVGDRVTITCKASQDVGTAFAWYQQKPGKAPKLLIYWASTRITGVPD</i> <i>RFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLT LSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 35)
V _H 6/V _K 7-IgG4v2	Heavy chain <i>EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYCARERPMQDYWGQGTLVTVSSASTKG</i>

Antibody	Amino Acid Sequence
	PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGKTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLF PPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVLHEALHSHYTQKSLSLSLGK (SEQ ID NO: 34)
	Light Chain <i>DIQMTQSPSTLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHTGVPD</i> <i>RFSGSGSGTDFTLTISLQAEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 35)
V _H 4/V _κ 6- IgG4wt	Heavy Chain <i>EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMS</i> <i>WVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDN</i> <i>AKNSLYLQMSLRSEDTALYYCARE</i> <i>RPMDYWGQGTSTVTVSSASTKGP</i> SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS SSVVTVPSSSLGKTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLF PKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMEALHNHYTQKSLSLSLGK (SEQ ID NO: 36)
	Light Chain <i>DIVMTQSPSFLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHTGVPD</i> <i>RFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 39)
V _H 4/V _κ 6 - IgG4v1	Heavy Chain <i>EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMS</i> <i>WVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDN</i> <i>AKNSLYLQMSLRSEDTALYYCARE</i> <i>RPMDYWGQGTSTVTVSSASTKGP</i> SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS SSVVTVPSSSLGKTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLF PKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMEALHNHYTQKSLSLSLGK (SEQ ID NO: 37)
	Light Chain <i>DIVMTQSPSFLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHTGVPD</i> <i>RFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 39)
V _H 4/V _κ 6 - IgG4v2	Heavy Chain <i>EVQLVESGGGLVKPGGSLRLSCAASGFTFSNYAMS</i> <i>WVRQAPGKRLEWVATISNRGSYTTY</i> <i>PDSVKGRFTISRDN</i> <i>AKNSLYLQMSLRSEDTALYYCARE</i> <i>RPMDYWGQGTSTVTVSSASTKGP</i> SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS SSVVTVPSSSLGKTKYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLF PKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVLHEALHSHYTQKSLSLSLGK (SEQ ID NO: 38)
	Light Chain <i>DIVMTQSPSFLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWASTRHTGVPD</i> <i>RFTGSGSGTDFTLTISLQSEDFAVYFCHQHSSNPLTFGQGTKLEIKRTVAAPSVFIFPPSD</i> EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 39)

In Table 6:

- bolded: CDRs accordingly to Kabat definition

- Italic: V_H/V_L

In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 32 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

5 In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 33 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

10 In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 36 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 37 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

15 In some embodiments, a humanized anti-factor Bb antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NO: 38 and a light chain comprising the amino acid sequence of SEQ ID NO: 39.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure binds a factor Bb protein (*e.g.*, a factor Bb protein from a mammal, fish, or invertebrate that has a complement system). In some embodiments, a humanized anti-factor Bb antibody of the present disclosure binds a mammalian factor Bb protein. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure binds a human factor Bb protein. In some
25 protein having the amino acid sequence of SEQ ID NO: 40.

Homo sapiens factor Bb protein (SEQ ID NO: 40)

30 KIVLDPSGSMNIYLVLDGSDSIGASNFTGAKKCLVNLIEKVASYGVKPRYGLVITYATY
PKIWVKVSEADSSNADWVTKQLNEINYEDHKLKSGTNTKKALQAVYSMMSPDDVP
PEGWNRTRHVILMTDGLHNMGGDPITVIDEIRDLLYIGKDRKNPREYLDVYVFGVG
PLVNQVNINALASKKDNEQHVFVKDMENLEDVFYQMIDESQSLSLCGMVWEHRKG
TDYHKQPWQAKISVIRPSKGHESCMGAVVSEYFVLTAHCFTVDDKEHSIKVSVGGE
KRDLEIEVVLFHPNYNINGKKEAGIPEFYDYDVALIKLKNKLKYGQTIRPICLPCTEGTT
RALRLPPTTTCQQQKEELLPAQDIKALFVSEEEKLTRKEVYIKNGDKKGCERDAQY

APGYDKVKDISEVVTPRFLCTGGVSPYADPNTCRGDSGGPLIVHKRSRFIQVGVISWG
VVDVCKNQKRQKQVPAHARDFHINLFQVLPWLKEKLQDEDLGFL

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
5 binds a complement Bb protein with an affinity of about 10^{-6} to 10^{-11} nM, *e.g.*, from about 10^{-6}
M to about 10^{-7} M, about 10^{-7} M to about 10^{-8} M, about 10^{-8} M to about 10^{-9} M, from about
 10^{-9} M to about 10^{-10} M, or from about 10^{-10} M to about 10^{-11} M. The terms “about” preceding a
numerical value mean $\pm 10\%$ of the recited numerical value.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
10 exhibits preferential binding for factor Bb, compared with binding for factor B. In some
embodiments, a humanized anti-factor Bb antibody of the present disclosure binds to factor
Bb, but does not substantially bind to soluble Factor B. In some embodiments, a humanized
anti-factor Bb antibody of the present disclosure binds to factor Bb with an affinity that is at
least 2-fold, at least 2.5-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7.5-fold, at
15 least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 50-fold, at least 75-fold,
or at least 100-fold, higher than the affinity of the antibody for factor B. In some embodiments,
a humanized anti-factor Bb antibody of the present disclosure binds to factor Bb with an
affinity that is 2-fold to 2.5-fold, 2.5-fold to 5-fold, 5-fold to 10-fold, 10-fold to 15-fold, 15-
fold to 20-fold, 20-fold to 25-fold, 25-fold to 50-fold, 50-fold to 75-fold, or 75-fold to 100-
20 fold, higher than the affinity of the antibody for factor B.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
inhibits the complement pathway activity by at least 10%, at least 20%, at least 30%, at least
40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least
85%, at least 90%, at least 95%, or 100%, compared to the level of complement activity in the
25 absence of a humanized anti-factor Bb antibody.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
inhibits alternative pathway (AP) activity by at least 10%, at least 20%, at least 30%, at least
40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least
85%, at least 90%, at least 95%, or 100%, compared to the level of AP activity in the absence
30 of a humanized anti-factor Bb antibody. In some embodiments, a humanized anti-factor Bb
antibody of the present disclosure inhibits AP activity with an IC_{50} of 10^{-7} M to 10^{-9} M, *e.g.*, an
 IC_{50} of 10^{-7} M to 5×10^{-7} M, 5×10^{-7} M to 10^{-8} M, 10^{-8} M to 5×10^{-8} M, or 5×10^{-8} M to 10^{-9} M. In some embodiments, the complement AP activity is selected the group consisting of AP-
mediated terminal membrane attack complex (MAC) deposition, AP-mediated hemolysis, C3

fragment deposition on red blood cells or other cell types, C3b/Bb-mediated cleavage of C3, and C3bBb3b-mediated cleavage of C5. In some embodiments, inhibition of complement AP activity by the humanized anti-factor Bb antibodies can be measured using the Complement System Alternative Pathway WIESLAB® kit.

5 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits formation of membrane attack complex (MAC) by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the amount of MAC formed in the absence of the anti-factor Bb antibody.

10 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3b/Bb-mediated cleavage of C3. C3b/Bb is also known as “C3 convertase.” In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3b/Bb-mediated cleavage of C3 by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,
15 at least 95%, or 100%, compared to the cleavage of C3 in the absence of the anti-factor Bb antibody. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3b/Bb-mediated cleavage of C3 with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

20 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3b/Bb-mediated cleavage of C3, thereby reducing production of a C3 cleavage product. For example, a humanized anti-factor Bb antibody of the present disclosure may inhibit C3b/Bb-mediated cleavage of C3, thereby reducing production of a C3 cleavage product (*e.g.*, C3a and/or C3b) by at least 10%, at least 20%, at least 30%, at least 40%, at least
25 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the production of the C3 cleavage product in the absence of the anti-factor Bb antibody.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits complement AP-mediated cell lysis by at least 10%, at least 20%, at least 30%, at least
30 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the degree of cell lysis in the absence of the anti-factor Bb antibody. A cell lysis assay can be used to determine the degree of inhibition of AP-mediated cell lysis. In some embodiments, a humanized anti-factor Bb

antibody of the present disclosure inhibits AP-mediated cell lysis with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure
5 inhibits complement AP-mediated hemolysis by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the degree of hemolysis in the absence of the anti-factor Bb antibody. A rabbit red blood cell (RBC) hemolysis assay can be used to determine the degree of inhibition of AP-mediated hemolysis. In some embodiments, a
10 humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated hemolysis with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated deposition of C3b, C3d, or other C3 split product on a cell or tissue. For
15 example, a humanized anti-factor Bb antibody of the present disclosure may inhibit AP-mediated deposition of C3b, C3d, or other C3 split product on a cell or tissue by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the amount of deposition of C3b, C3d, or other C3 split product on the cell or tissue in the absence
20 of administration of the anti-factor Bb antibody, or before administration of the anti-factor Bb antibody. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated deposition of C3b, C3d, or other C3 split product on a cell or tissue with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

25 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated C3b deposition on a cell or tissue by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the amount of C3b deposition on the cell or tissue in the absence of the humanized anti-factor Bb antibody. In
30 some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated C3b deposition on a cell or tissue with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated C3b deposition on red blood cells (RBCs) or other cell types by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, compared to the amount of C3b deposition on RBCs in the absence of the humanized anti-factor Bb antibody. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits AP-mediated C3b deposition on RBCs with an IC_{50} of from 10^{-7} M to 10^{-9} M, *e.g.*, an IC_{50} of from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to 10^{-8} M, from 10^{-8} M to 5×10^{-8} M, or from 5×10^{-8} M to 10^{-9} M.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure, when administered to a subject in need thereof, reduces the amount of factor Bb in circulation in the subject. For example, a humanized anti-factor Bb antibody of the present disclosure, when administered to a subject in need thereof, may reduce the amount of factor Bb in circulation in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, compared to the amount of factor Bb in circulation in the subject in the absence of administering the humanized anti-factor Bb antibody, or compared to the amount of factor Bb in circulation in the subject before administration of the humanized anti-factor Bb antibody.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3bBb3b-mediated cleavage of C5. In some embodiments, a humanized anti-factor Bb antibody of the present disclosure inhibits C3bBb3b-mediated cleavage of C5 by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, compared to C3bBb3b-mediated cleavage of C5 in the absence of a humanized anti-factor Bb antibody.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure is a bispecific or multispecific antibody. For example, a humanized anti-factor Bb antibody can be a bispecific antibody comprising a first antigen-binding portion that specifically binds an epitope in a complement Bb protein, and a second antigen-binding portion that binds a second antigen.

Immunoconjugates

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure is conjugated with another agent to form an immunoconjugate. For example, a humanized anti-factor Bb antibody may comprise a free thiol (-SH) group at the carboxyl terminus, where the

5 free thiol group can be used to attach the antibody to a second polypeptide (*e.g.*, another antibody, including a humanized anti-factor Bb antibody), a scaffold, a carrier, *etc.* In some embodiments, a humanized anti-factor Bb antibody comprises at least one non-naturally occurring amino acids. In some embodiments, the non-naturally encoded amino acid comprises a carbonyl group, an acetyl group, an aminooxy group, a hydrazine group, a hydrazide group, a

10 semicarbazide group, an azide group, or an alkyne group. See, *e.g.*, U. S. Patent No. 7,632,924 for suitable non-naturally occurring amino acids. Inclusion of a non-naturally occurring amino acid can provide for linkage to a polymer, a second polypeptide, a scaffold, *etc.* For example, a humanized anti-factor Bb antibody linked to a water-soluble polymer can be made by reacting a water-soluble polymer (*e.g.*, PEG) that comprises a carbonyl group to the antibody, where the

15 antibody comprises a non-naturally encoded amino acid that comprises an aminooxy, hydrazine, hydrazide or semicarbazide group. In some embodiments, a humanized anti-factor Bb antibody linked to a water-soluble polymer can be made by reacting the antibody that comprises an alkyne-containing amino acid with a water-soluble polymer (*e.g.*, PEG) that comprises an azide moiety. In some embodiments, the azide or alkyne group is linked to the

20 PEG molecule through an amide linkage.

In some embodiments, a humanized anti-factor Bb antibody is linked (*e.g.*, covalently linked) to a polymer (*e.g.*, a polymer other than a polypeptide). Suitable polymers include, *e.g.*, biocompatible polymers, and water-soluble biocompatible polymers. Suitable polymers include synthetic polymers and naturally-occurring polymers. Suitable polymers can have an

25 average molecular weight in a range of from 500 Da to 50,000 Da, *e.g.*, from 5,000 Da to 40,000 Da, or from 25,000 to 40,000 Da.

In some embodiments, a humanized anti-factor Bb antibody comprises a "radiopaque" label, *e.g.*, a label that can be easily visualized using for example x-rays. Radiopaque materials are well known to those of skill in the art. The most common radiopaque materials include

30 iodide, bromide or barium salts. Other radiopaque materials are also known and include but are not limited to organic bismuth derivatives (see, *e.g.*, U. S. Pat. No. 5,939,045), radiopaque multiurethanes (see U. S. Pat. No. 5,346,981), organobismuth composites (see, *e.g.*, U. S. Pat.

No. 5,256,334), and/or radiopaque barium multimer complexes (see, *e.g.*, U. S. Pat. No. 4,866,132).

In some embodiments, a humanized anti-factor Bb antibody is covalently linked to a second moiety (*e.g.*, a lipid, a polypeptide other than a humanized anti-factor Bb antibody, a synthetic polymer, and/or a carbohydrate) using for example, glutaraldehyde, a homobifunctional cross-linker, or a heterobifunctional cross-linker.

In some embodiments, a humanized anti-factor Bb antibody is immobilized on a solid support. Suitable supports are well known in the art and comprise, *inter alia*, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, nylon membranes, sheets, duracytes, wells of reaction trays (*e.g.*, multi-well plates), plastic tubes, *etc.* A solid support can comprise any of a variety of substances, including, *e.g.*, glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amylose, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. Suitable methods for immobilizing a humanized anti-factor Bb antibody onto a solid support are well known and include, but are not limited to ionic, hydrophobic, and/or covalent interactions. Solid supports can be soluble or insoluble, *e.g.*, in aqueous solution. In some embodiments, a suitable solid support is generally insoluble in an aqueous solution.

In some embodiments, a humanized anti-factor Bb antibody comprises a detectable label. Suitable detectable labels include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Suitable include, but are not limited to, magnetic beads (*e.g.* DYNABEADS™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas Red, rhodamine, a green fluorescent protein, a red fluorescent protein, and/or a yellow fluorescent protein), radiolabels (*e.g.*, ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase, luciferase, and others commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.* polystyrene, polypropylene, latex, *etc.*) beads.

In some embodiments, a humanized anti-factor Bb antibody comprises a contrast agent or a radioisotope, where the contrast agent or radioisotope is one that is suitable for use in imaging, *e.g.*, imaging procedures carried out on humans. Non-limiting examples of labels include radioisotope such as ¹²³I (iodine), ¹⁸F (fluorine), ⁹⁹Tc (technetium), ¹¹¹In (indium), and ⁶⁷Ga (gallium), and contrast agent such as gadolinium (Gd), dysprosium, and iron. Radioactive

Gd isotopes (^{153}Gd) also are available and suitable for imaging procedures in non-human mammals. A humanized anti-factor Bb antibody can be labeled using standard techniques. For example, a humanized anti-factor Bb antibody can be iodinated using chloramine T or 1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycouril. For fluorination, fluorine is added to a humanized anti-

5 factor Bb antibody during the synthesis by a fluoride ion displacement reaction. See, Muller-Gartner, H., TIB Tech., 16:122-130 (1998) and Saji, H., Crit. Rev. Ther. Drug Carrier Syst., 16(2):209-244 (1999) for a review of synthesis of proteins with such radioisotopes. A humanized anti-factor Bb antibody can also be labeled with a contrast agent through standard techniques. For example, a humanized anti-factor Bb antibody can be labeled with Gd by

10 conjugating low molecular Gd chelates such as Gd diethylene triamine pentaacetic acid (GdDTPA) or Gd tetraazacyclododecanetetraacetic (GdDOTA) to the antibody. See, Caravan *et al.*, Chem. Rev. 99:2293-2352 (1999) and Lauffer *et al.*, J. Magn. Reson. Imaging, 3:11-16 (1985). A humanized anti-factor Bb antibody can be labeled with Gd by, for example, conjugating polylysine-Gd chelates to the antibody. See, for example, Curtet *et al.*, Invest.

15 Radiol., 33(10):752-761 (1998). Alternatively, in some embodiments, a humanized anti-factor Bb antibody can be labeled with Gd by incubating paramagnetic polymerized liposomes that include Gd chelator lipid with avidin and biotinylated antibody. See, for example, Sipkins *et al.*, Nature Med., 4:623-626 (1998).

Suitable fluorescent proteins include, but are not limited to, green fluorescent protein

20 (GFP) or variants thereof, blue fluorescent variant of GFP (BFP), cyan fluorescent variant of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPS65T, Emerald, Topaz (TYFP), Venus, Citrine, mCitrine, GFPuv, destabilised EGFP (dEGFP), destabilised ECFP (dECFP), destabilised EYFP (dEYFP), mCFPm, Cerulean, T-Sapphire, CyPet, YPet, mKO, HcRed, t-HcRed, DsRed,

25 DsRed2, DsRed-monomer, J-Red, dimer2, t-dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein and kindling protein, Phycobiliproteins and Phycobiliprotein conjugates including B-Phycoerythrin, R-Phycoerythrin and Allophycocyanin. Other examples of fluorescent proteins include mHoneydew, mBanana, mOrange, dTomato, tdTomato, mTangerine, mStrawberry, mCherry, mGrape1, mRaspberry,

30 mGrape2, and/or mPlum (Shaner *et al.* (2005) Nat. Methods 2:905-909). Any of a variety of fluorescent and colored proteins from Anthozoan species, as described in, *e.g.*, Matz *et al.* (1999) Nature Biotechnol. 17:969-973, is suitable for use.

In some embodiments, a humanized anti-factor Bb antibody is conjugated to a therapeutic agent. Any of the humanized anti-factor Bb antibodies disclosed herein can be used to form an antibody-agent conjugate. The agent can be attached to the N terminus of the light chain, the C terminus of the light chain, the N terminus of the heavy chain, or the C terminus of the heavy chain. In some embodiments, the agent is attached to the hinge of the antibody or to at least one other sites on the antibody. For a single chain antibody, the agent can be attached to the N or C terminus of the single chain antibody. The agent can be conjugated to the antibody directly or via a linker using techniques known to those skilled in the art. The linker can be cleavable or non-cleavable. Examples of such therapeutic agents (*e.g.*, for use in therapy) are known to those skilled in the art.

In some embodiments, a humanized anti-factor Bb antibody is linked to (*e.g.*, covalently or non-covalently linked) a fusion partner, *e.g.*, a ligand; an epitope tag; a peptide; and/or a protein other than an antibody. Suitable fusion partners include peptides and polypeptides that confer enhanced stability *in vivo* (*e.g.*, enhanced serum half-life); provide ease of purification, *e.g.*, (His)_n, *e.g.*, 6His; provide for secretion of the fusion protein from a cell; provide an epitope tag, *e.g.*, GST, hemagglutinin (HA; *e.g.*, YPYDVPDYA; SEQ ID NO: 55), FLAG (*e.g.*, DYKDDDDK; SEQ ID NO: 56), and/or c-myc (*e.g.*, EQKLISEEDL; SEQ ID NO: 57); provide a detectable signal, *e.g.*, an enzyme that generates a detectable product (*e.g.*, β -galactosidase, luciferase), or a protein that is itself detectable, *e.g.*, a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, *etc.*; and/or provides for multimerization, *e.g.*, a multimerization domain such as an Fc portion of an immunoglobulin. The fusion can also include an affinity domain, including peptide sequences that can interact with a binding partner, *e.g.*, such as one immobilized on a solid support, useful for identification or purification. Consecutive single amino acids, such as histidine, when fused to a protein, can be used for one-step purification of the fusion protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO: 58), HisX6 (HHHHHH) (SEQ ID NO:59), C-myc (EQKLISEEDL) (SEQ ID NO:60), Flag (DYKDDDDK) (SEQ ID NO:61), StrepTag (WSHPQFEK) (SEQ ID NO:62), hemagglutinin, *e.g.*, HA Tag (YPYDVPDYA; SEQ ID NO:63), glutathione-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:66), Phe-His-His-Thr (SEQ ID NO:64), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:65), metal binding domains, *e.g.*, zinc binding domains or calcium binding domains such as those from calcium-binding proteins, *e.g.*,

calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, leucine zipper sequences, and maltose binding protein.

5

Methods of Producing Humanized Anti-Factor Bb Antibodies

In some embodiments, fully human antibodies can be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human
10 antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XenomouseRTM from Amgen, Inc. (Fremont, CA) and HuMAb-MouseRTM and TC MouseTM from Medarex, Inc. (Princeton, NJ) or H2L2 mice from Harbour Antibodies BV (Holland). In another alternative, antibodies may be made recombinantly by phage display or yeast technology. See, for example, U. S. Pat. Nos.
15 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter *et al.*, (1994) Annu. Rev. Immunol. 12:433-455. Alternatively, the phage display technology (McCafferty *et al.*, (1990) Nature 348:552-553) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors.

Antigen-binding fragments of an intact antibody (full-length antibody) can be prepared
20 via routine methods. For example, F(ab')₂ fragments can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by reducing the disulfide bridges of F(ab')₂ fragments. Genetically engineered antibodies, such as humanized antibodies, chimeric antibodies, single-chain antibodies, and bispecific antibodies, can be produced via, *e.g.*, conventional recombinant technology. In one example, DNA encoding a monoclonal
25 antibodies specific to a target antigen can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into at least one expression vector, which is then transfected into host cells such as *Escherichia coli* (*E.*
30 *coli*) cells, simian COS cells, Chinese hamster ovary (CHO) cells, human HEK293 cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in recombinant host cells. See, *e.g.*, PCT Publication No. WO 87/04462. The DNA can then be modified, for example, by substituting the coding sequence

for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison *et al.*, (1984) Proc. Nat. Acad. Sci. 81:6851, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, genetically engineered antibodies, such as
5 “chimeric” or “hybrid” antibodies; can be prepared that have the binding specificity of a target antigen.

A single-chain antibody can be prepared via recombinant technology, for example, by linking a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. In some embodiments, a flexible linker is
10 incorporated between the two variable regions.

Alternatively, techniques described for the production of single chain antibodies (U. S. Patent Nos. 4,946,778 and 4,704,692) can be adapted, for example, to produce a phage or yeast scFv library and scFv clones specific to factor Bb can be identified from the library following routine procedures. Positive clones can be subjected to further screening to identify those that
15 has high factor Bb binding affinity.

In some embodiments, a humanized anti-factor Bb antibody is prepared by recombinant technology as exemplified below. Nucleic acids encoding the heavy and light chain of an anti-factor Bb antibody as described herein can be cloned into one expression vector, each nucleotide sequence being in operable linkage to a suitable promoter. In one example, each of
20 the nucleotide sequences encoding the heavy chain and light chain is in operable linkage to a distinct promoter. Alternatively, the nucleotide sequences encoding the heavy chain and the light chain can be in operable linkage with a single promoter, such that both heavy and light chains are expressed from the same promoter. When necessary, an internal ribosomal entry site (IRES) can be inserted between the heavy chain and light chain encoding sequences.

25 In some examples, the nucleotide sequences encoding the two chains of the antibody are cloned into two vectors, which can be introduced into the same or different cells. When the two chains are expressed in different cells, each of them can be isolated from the host cells expressing such and the isolated heavy chains and light chains can be mixed and incubated under suitable conditions allowing for the formation of the antibody.

30 Generally, a nucleic acid sequence encoding one or all chains of an antibody can be cloned into a suitable expression vector in operable linkage with a suitable promoter using methods known in the art. For example, the nucleotide sequence and vector can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each

molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of a gene. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/promoters would depend on the type of host cells for use in producing the antibodies.

A variety of promoters can be used for expression of the antibodies described herein, including, but not limited to, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the Rous sarcoma virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, *E. coli* lac UV promoter, and the herpes simplex tk virus promoter.

Regulatable promoters can also be used. Such regulatable promoters include those using the lac repressor from *E. coli* as a transcription modulator to regulate transcription from lac operator bearing mammalian cell promoters [Brown, M. *et al.*, Cell, 49:603-612 (1987)], those using the tetracycline repressor (tetR) [Gossen, M., and Bujard, H., Proc. Natl. Acad. Sci. USA 89:5547-5551 (1992); Yao, F. *et al.*, Human Gene Therapy, 9:1939-1950 (1998); Shockelt, P., *et al.*, Proc. Natl. Acad. Sci. USA, 92:6522-6526 (1995)]. Other systems include FK506 dimer, VP16 or p65 using estradiol, RU486, diethylstilbestrol, or rapamycin. Inducible systems are available from Invitrogen, Clontech and Ariad, among others.

Regulatable promoters that include a repressor with the operon can be used. In one embodiment, the lac repressor from *E. coli* can function as a transcriptional modulator to regulate transcription from lac operator-bearing mammalian cell promoters [M. Brown *et al.*, Cell, 49:603-612 (1987)]; Gossen and Bujard (1992); [M. Gossen *et al.*, Natl. Acad. Sci. USA, 89:5547-5551 (1992)] combined the tetracycline repressor (tetR) with the transcription activator (VP 16) to create a tetR-mammalian cell transcription activator fusion protein, tTa (tetR-VP16), with the tetO bearing minimal promoter derived from the human cytomegalovirus (hCMV) promoter to create a tetR-tet operator system to control gene expression in mammalian cells. In one embodiment, a tetracycline inducible switch is used. The tetracycline repressor (tetR) alone, rather than the tetR-mammalian cell transcription factor fusion derivatives can function as potent trans-modulator to regulate gene expression in mammalian cells when the tetracycline operator is properly positioned downstream for the TATA element of the CMVIE promoter (Yao *et al.*, Human Gene Therapy). One particular advantage of this tetracycline inducible switch is that it does not require the use of a tetracycline repressor-mammalian cells transactivator or repressor fusion protein, which in some instances can be toxic to cells (Gossen *et al.*, Natl. Acad. Sci. USA, 89:5547-5551

(1992); Shockett *et al.*, Proc. Natl. Acad. Sci. USA, 92:6522-6526 (1995)), to achieve its regulatable effects.

Additionally, the vector can contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient
5 transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense
10 RNA. Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art. Examples of polyadenylation signals useful to practice the methods described herein include, but are not limited to, human collagen I polyadenylation signal, human collagen II polyadenylation signal, and SV40 polyadenylation signal.

At least one vector (*e.g.*, expression vectors) comprising nucleic acids encoding any of
15 the antibodies may be introduced into suitable host cells for producing the antibodies. The host cells can be cultured under suitable conditions for expression of the antibody or any polypeptide chain thereof. Such antibodies or polypeptide chains thereof can be recovered by the cultured cells (*e.g.*, from the cells or the culture supernatant) via a conventional method, *e.g.*, affinity purification. If necessary, polypeptide chains of the antibody can be incubated
20 under suitable conditions for a suitable period of time allowing for production of the antibody.

In some embodiments, methods for preparing an antibody described herein involve a recombinant expression vector that encodes both the heavy chain and the light chain of an anti-factor Bb antibody, as also described herein. The recombinant expression vector can be introduced into a suitable host cell (*e.g.*, a dihydrofolate reductase (DHFR)-CHO cell) by a
25 conventional method, *e.g.*, calcium phosphate mediated transfection. Positive transformant host cells can be selected and cultured under suitable conditions allowing for the expression of the two polypeptide chains that form the antibody, which can be recovered from the cells or from the culture medium. When necessary, the two chains recovered from the host cells can be incubated under suitable conditions allowing for the formation of the antibody.

30 In some embodiments, two recombinant expression vectors are provided, one encoding the heavy chain of the anti-factor Bb antibody and the other encoding the light chain of the anti-factor Bb antibody. Both of the two recombinant expression vectors can be introduced into

a suitable host cell (*e.g.*, DHFR-CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection.

Alternatively, each of the expression vectors can be introduced into a suitable host cells. Positive transformants can be selected and cultured under suitable conditions allowing
5 for the expression of the polypeptide chains of the antibody. When the two expression vectors are introduced into the same host cells, the antibody produced therein can be recovered from the host cells or from the culture medium. If necessary, the polypeptide chains can be recovered from the host cells or from the culture medium and then incubated under suitable conditions allowing for formation of the antibody. When the two expression vectors are
10 introduced into different host cells, each of them can be recovered from the corresponding host cells or from the corresponding culture media. The two polypeptide chains can then be incubated under suitable conditions for formation of the antibody.

Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recovery of
15 the antibodies from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

Any of the nucleic acids encoding the heavy chain, the light chain, or both of an anti-factor Bb antibody as described herein (*e.g.*, as provided in Tables 2 and 3), vectors (*e.g.*, expression vectors) containing such; and host cells comprising the vectors are within the scope
20 of the present disclosure.

Pharmaceutical Compositions and Therapeutic Methods

Other aspects of the present disclosure provide compositions, including pharmaceutical compositions comprising any one of the humanized anti-factor Bb antibodies described herein.
25 In general, a pharmaceutical composition, also referred to herein as a formulation, comprises an effective amount of any one of the humanized anti-factor Bb antibodies described herein. An “effective amount” means a dosage sufficient to produce a desired result, *e.g.*, reduction in an adverse symptom associated with a complement-mediated disease or disorder, amelioration of a symptom of a complement-mediated disease or disorder, slowing progression of a
30 complement-mediated disease or disorder, *etc.* Generally, the desired result is at least a reduction in a symptom of a complement-mediated disease or disorder, as compared to a control.

In a method of the present disclosure, a humanized anti-factor Bb antibody can be administered to a subject using a convenient means capable of resulting in the desired therapeutic effect or diagnostic effect. Thus, a humanized anti-factor Bb antibody can be incorporated into a variety of formulations for therapeutic administration. More particularly, a humanized anti-factor Bb antibody can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers, pharmaceutically acceptable diluents, or other pharmaceutically acceptable excipients. In some embodiments, a pharmaceutical composition comprises a humanized anti-factor Bb antibody and a pharmaceutically acceptable excipient.

In pharmaceutical dosage forms, a humanized anti-factor Bb antibody can be administered in the form of their pharmaceutically acceptable salts, or they can also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, a humanized anti-factor Bb antibody can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules.

A humanized anti-factor Bb antibody can be formulated into preparations for injection by dissolving, suspending or emulsifying the antibody in an aqueous or nonaqueous solvent; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Pharmaceutical compositions comprising a humanized anti-factor Bb antibody are prepared by mixing a humanized anti-factor Bb antibody having the desired degree of purity with optional physiologically acceptable carriers, other excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, other excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed. In some embodiments, the compositions comprise a buffer, an antioxidant, an amino acid or a combination thereof.

The pharmaceutical composition can be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however, solutions comprising antibacterial agents can be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

A tonicity agent can be included in the antibody formulation to modulate the tonicity of the formulation. In some embodiments, the aqueous formulation is isotonic, although hypertonic or hypotonic solutions can be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as a physiological salt solution or serum.

A surfactant can also be added to the antibody formulation to reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the formulation and/or reduce adsorption.

A lyoprotectant can also be added in order to protect the labile active ingredient (*e.g.*, a protein) against destabilizing conditions during the lyophilization process.

In some embodiments, a subject formulation includes a humanized anti-factor Bb antibody, and at least one of the above-identified agents (*e.g.*, a surfactant, a buffer, a stabilizer, a tonicity agent) and is essentially free of at least one preservative.

A humanized anti-factor Bb antibody can be utilized in aerosol formulation to be administered via inhalation. A humanized anti-factor Bb antibody can be formulated into pressurized acceptable propellants.

Furthermore, a humanized anti-factor Bb antibody can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. A humanized anti-factor Bb antibody can be administered rectally via a suppository.

Other modes of administration will also find use with a method of the present disclosure. For instance, a humanized anti-factor Bb antibody can be formulated in suppositories and, in some embodiments, aerosol and intranasal compositions. For suppositories, the vehicle composition may include traditional binders and carriers.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed. The nasal formulations can also contain a preservative. A surfactant can be present to enhance absorption of a humanized anti-factor Bb antibody by the nasal mucosa.

A humanized anti-factor Bb antibody can be administered as an injectable formulation. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation can also be emulsified, or the antibody encapsulated in liposome vehicles.

In some embodiments, a humanized anti-factor Bb antibody is formulated in a controlled release formulation. Controlled release within the scope of the present disclosure can be taken to mean any one of a number of extended-release dosage forms.

5 A humanized anti-factor Bb antibody is administered to a subject using a method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

10 Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, intranasal, intramuscular, intratracheal, intrathecal, intracranial, subcutaneous, intradermal, topical, intravenous, intraperitoneal, intraarterial (*e.g.*, via the carotid artery), spinal or brain delivery, rectal, nasal, oral, and other enteral and parenteral routes of administration.

15 An antibody of the present disclosure can be administered to a subject using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the present disclosure include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

20 In some embodiments, a humanized anti-factor Bb antibody is administered by injection and/or delivery, *e.g.*, to a site in a brain artery or directly into brain tissue. A humanized anti-factor Bb antibody can also be administered directly to a target site *e.g.*, by biolistic delivery to the target site.

A variety of subjects may be treated in accordance with the methods provided herein. Generally, such subjects are “mammals” or “mammalian,” where these terms are used broadly to describe organisms which are within the class Mammalia, including the orders carnivores (*e.g.*, cats), herbivores (*e.g.*, cattle, horses, and sheep), omnivores (*e.g.*, dogs, goats, and pigs), 25 rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In some embodiments, a subject has a complement system, such as a mammal, fish, or invertebrate. In some embodiments, a subject is a complement system-containing mammal, fish, or invertebrate companion animal, agricultural animal, work animal, zoo animal, or lab animal. In some embodiments, a subject is a human.

30 “Treatment” refers to at least an amelioration of the symptoms associated with pathological condition afflicting a subject, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.*, symptom, associated with the pathological condition being treated, such as a complement-mediated disease or disorder. As

such, treatment also includes situations where the pathological condition, or at least symptoms and/or secondary effects associated therewith, are completely inhibited, *e.g.*, prevented from happening, or stopped, *e.g.*, terminated, such that subject no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

5 In some embodiments, a “subject” is a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (*e.g.*, equines, bovinos, ovines, porcines, caprines), *etc.* Also encompassed by these terms are any animal that has a complement system, such as mammals, fish, and some invertebrates. As such these terms include complement system-containing mammal, fish, and invertebrate companion animals,
10 agricultural animals, work animals, zoo animals, and lab animals.

 A “biological sample” encompasses a variety of sample types obtained from a subject and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes
15 samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term “biological sample” encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples. The term “biological sample” includes urine, saliva, cerebrospinal fluid, interstitial fluid, ocular fluid,
20 synovial fluid, and/or blood fractions such as plasma and serum. The term “biological sample” also includes solid tissue samples, tissue culture samples, and cellular samples.

 The present disclosure provides a device or container suitable for containing a composition comprising a humanized anti-factor Bb antibody for administration to a subject. For example, a humanized anti-factor Bb antibody can be disposed within a container suitable
25 for containing a pharmaceutical composition. The container can be, for example, a bottle (*e.g.*, with a closure device, such as a cap), a blister pack (*e.g.*, which can provide for enclosure of at least one doses per blister), a vial, flexible packaging (*e.g.*, sealed Mylar or plastic bags), an ampule (for single doses in solution), a dropper, a syringe, thin film, and/or a tube. In some embodiments, a container, such as a sterile container, comprises a subject pharmaceutical
30 composition. In some embodiments, the container is a bottle or a syringe. In some embodiments, the container is a bottle. In some embodiments, the container is a syringe. In some embodiments, the device is an injectable device, such as a syringe (*e.g.*, a pre-filled syringe), a pen (*e.g.*, a pre-filled pen), or an electronic injection device (e-Devices).

The present disclosure provides methods of treating a complement-mediated disease or disorder. The methods generally involve administering an effective amount of a humanized anti-factor Bb antibody of the present disclosure to a subject in need thereof. In some embodiments, administration of a humanized anti-factor Bb antibody modulates the activity of the complement pathway activity in a cell, a tissue, or a fluid of a subject, and treats the complement-mediated disease or disorder.

An “effective amount” refers to the amount of an anti-complement factor Bb antibody that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the anti-complement Bb antibody, the disease and its severity and the age, weight, *etc.*, of the subject to be treated.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit complement pathway activity in a cell, tissue, or fluid of the subject.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit formation of MAC in a cell, tissue, or fluid of the subject.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit C3b/Bb-mediated cleavage of C3 in a cell, tissue, or fluid of the subject.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit C3b/Bb-mediated cleavage of C3, and thereby to reduce production of a C3 cleavage product.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit complement AP-mediated lysis of a cell in the subject.

In some embodiments, an effective amount of the humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit complement AP-mediated hemolysis in a cell, tissue, or fluid (*e.g.*, RBC-containing fluid) of the subject.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit production of an anaphylatoxin.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit AP-mediated deposition of C3b, C3d, or other C3 split product on a cell or tissue in the subject.

5 In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit AP-mediated C3b deposition on a cell or tissue in the subject.

In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit AP-mediated deposition of C3b, C3d, or other C3 split product on RBCs in the subject.

10 In some embodiments, an effective amount of a humanized anti-factor Bb antibody of the present disclosure is an amount that is effective to reduce or inhibit AP-mediated C3b deposition on RBCs in the subject.

In some embodiments, a humanized anti-factor Bb antibody of the present disclosure, when administered in at least one doses to a subject in need thereof, reduces the amount of factor Bb in circulation in the subject. For example, a humanized anti-factor Bb antibody of the present disclosure, when administered in at least one doses to a subject in need thereof, may reduce the amount of factor Bb in circulation in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, compared to the amount of factor Bb in circulation in the subject in the absence of administering the humanized anti-factor Bb antibody, or compared to the amount of factor Bb in circulation in the subject before administration of the humanized anti-factor Bb antibody.

25 In some embodiments, a humanized anti-factor Bb antibody of the present disclosure, when administered in at least one doses to a subject in need thereof, reduces the amount of factor Bb in plasma in the subject. For example, a humanized anti-factor Bb antibody of the present disclosure, when administered in at least one doses to a subject in need thereof, may reduce the amount of factor Bb in plasma in the subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%, compared to the amount of factor Bb in plasma in the subject in the absence of administering the humanized anti-factor Bb antibody, or compared to the amount of factor Bb in plasma in the subject before administration of the humanized anti-factor Bb antibody.

In some embodiments, a method of the present disclosure to treat a subject having a complement-mediated disease or disorder comprises administering to the subject a humanized anti-factor Bb antibody of the present disclosure or a pharmaceutical composition comprising: a) a humanized anti-factor Bb antibody of the present disclosure; and b) a pharmaceutically acceptable excipient suitable for administration to such subject. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. Administering can be by any route known to those skilled in the art, including those disclosed herein. In some embodiments, administering is intravenous. In some embodiments, administering is intrathecal. In some embodiments, administering is intramuscular. In some embodiments, administering is subcutaneous.

Complement-mediated diseases and disorders that are suitable for treatment with a humanized anti-factor Bb antibody of the present disclosure include diseases and disorders associated with the alternative complement pathway. Studies in preclinical animal models and clinical trials indicate alternative pathway plays an important role in the development of tissue injury and pathogenesis of several conditions (Holers et al., Immunological Reviews 223: 300-316; Cao et al., (2016) Haematologica 101(11): 1319-1326; Schubart et al., (2019) PNAS 116(16): 7926-7931; Thurman, (2015) Am J Kidney Dis 65(1): 156-168; Vriese et al., (2015) Am J Kidney Dis 65(1): 156-168; and Gold et al., (2006) Nat. Genet. 38(4): 458-462). In some embodiments, complement-mediated diseases that are suitable for treatment with a humanized anti-factor Bb antibody of the present disclosure include, but are not limited to, IgA nephropathy (Berger's disease), atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH), idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), lupus nephritis, ANCA vasculitis, membranous nephropathy, C3 glomerulonephritis (C3GN), focal segmental glomerulosclerosis (FSGS), multiple sclerosis, macular degeneration, age-related macular degeneration (AMD), rheumatoid arthritis, antiphospholipid antibody syndrome, asthma, ischemia-reperfusion injury, Type II membranoproliferative GN, spontaneous fetal loss, Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, and traumatic brain injury.

A humanized anti-factor Bb antibody of the present disclosure can be administered to a subject in need thereof alone (*e.g.*, as monotherapy); or in combination therapy with at least one additional therapeutic agents.

"In combination with" as used herein refers to uses where, for example, the first compound is administered during the entire course of administration of the second compound;

where the first compound is administered for a period of time that is overlapping with the administration of the second compound, *e.g.* where administration of the first compound begins before the administration of the second compound and the administration of the first compound ends before the administration of the second compound ends; where the
5 administration of the second compound begins before the administration of the first compound and the administration of the second compound ends before the administration of the first compound ends; where the administration of the first compound begins before administration of the second compound begins and the administration of the second compound ends before the administration of the first compound ends; where the administration of the second
10 compound begins before administration of the first compound begins and the administration of the first compound ends before the administration of the second compound ends. As such, “in combination” can also refer to regimen involving administration of two or more compounds.” In combination with” as used herein also refers to administration of two or more compounds that can be administered in the same or different formulations, by the same or different routes,
15 and in the same or different dosage form type.

Subjects suitable for treatment with a humanized anti-factor Bb antibody include subjects who have been diagnosed as having a complement-mediated disease or disorder; subjects at greater risk than the general population for developing a complement-mediated disease or disorder (*e.g.*, subjects having a genetic predisposition to developing a complement-
20 mediated disease or disorder). Also included are subjects having any one of the complement-mediated diseases or disorders listed hereinabove. In some embodiments, the subject is an adult human. In some embodiments, the subject is a human child.

EXAMPLES

25 **Example 1: Humanization of a mouse monoclonal anti-factor Bb antibody**

Variable region genes from the parent anti-factor Bb antibody (see Table 1) hybridoma were amplified, cloned and sequenced, resulting in the identification of a single unique VH domain and a single unique Vκ domain.

Initially, three humanized VH regions and five humanized Vκ regions designed using
30 COMPOSITE HUMAN ANTIBODY™ technology were cloned into IgG4v1 heavy chain and kappa light chain vectors. The parent antibody, two control antibodies and all 15 humanized antibody combinations were expressed transiently in HEK EBNA cells.

In order to assess the binding of all humanized variants, single cycle kinetic analysis was performed on supernatants from transfected cell cultures. Kinetic experiments were performed on a Biacore T200 (serial no. 1909913) running Biacore T200 Control software V2.0.1 and Evaluation software V3.0 (GE Healthcare, Uppsala, Sweden). All single cycle kinetic experiments were run at 25°C with HBS-P+ running buffer (pH 7.4) (GE Healthcare, Little Chalfont, UK).

Antibodies were diluted in running buffer to a final concentration of 1 µg/ml, based on concentrations assessed by ELISA titer. At the start of each cycle, antibodies were loaded onto Fc2, Fc3 and Fc4 of the Protein A chip (GE Healthcare, Little Chalfont, UK). IgGs were captured at a flow rate of 10 µl/min to give an immobilization level (RL) of ~ 63 RU, the theoretical value to obtain an R_{max} of ~ 50 RU. The surface was then allowed to stabilize. Single cycle kinetic data was obtained with factor Bb (CompTech, Tyler, USA) as the analyte at a flow rate of 30 µl/min to minimize any potential mass transport limitations. Multiple repeats with the reference chimeric antibody were performed to check the stability of the surface and analyte over the kinetic cycles. The signal from the reference channel Fc1 (no antibody) was subtracted from that of Fc2, Fc3 and Fc4 to correct for differences in non-specific binding to a reference surface. A four point, two-fold dilution range from 0.78 nM to 6.25 nM factor Bb without regeneration between each concentration was used. The association phase for the four injections of increasing concentrations of factor Bb was monitored for 200 seconds each time and a single dissociation phase was measured for 200 seconds following the last injection of factor Bb. Regeneration of the Protein A surface was conducted using two injections of 10 mM glycine-HCL pH 1.5.

The sensorgrams and fitted data for the single cycle kinetics are shown in FIGs. 1A-1D and the kinetic parameters measured for the interaction of factor Bb with each antibody are shown in Table 7. The relative K_D was calculated by dividing the K_D of the VH0/Vκ0 reference antibody by that of the humanized variant assayed in the same experiment.

Table 7. Single Cycle Kinetic Parameters of the Humanized Variants and Reference Antibodies Binding to Factor Bb.

Antibody	k _a (1/Ms)	k _d (1/s)	K _D (M)	Chi ² (RU ²)	Relative K _D
VH0/Vκ0 (Chimeric)	5.85 x10 ⁵	1.03 x10 ⁻³	1.76 x10 ⁻⁹	0.0134	1.00
VH0/Vκ1 (control)	5.86 x10 ⁵	1.57 x10 ⁻³	2.68 x10 ⁻⁹	0.00688	1.52

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	χ^2 (RU ²)	Relative K_D
VH1/V κ 0 (control)	3.76×10^5	1.16×10^{-3}	3.09×10^{-9}	0.00605	1.76
VH1/V κ 1	5.74×10^5	2.87×10^{-3}	5.01×10^{-9}	0.00228	2.85
VH1/V κ 2	4.81×10^5	2.51×10^{-3}	5.22×10^{-9}	0.00381	2.97
VH1/V κ 3	6.75×10^5	2.63×10^{-3}	3.90×10^{-9}	0.00488	2.22
VH1/V κ 4	4.51×10^5	2.94×10^{-3}	6.52×10^{-9}	0.0029	3.70
VH1/V κ 5	2.25×10^5	2.01×10^{-3}	8.96×10^{-9}	0.00803	5.09
VH2/V κ 1	1.80×10^5	3.73×10^{-3}	2.07×10^{-8}	0.00991	11.78
VH2/V κ 2	2.34×10^5	3.26×10^{-3}	1.39×10^{-8}	0.00327	7.90
VH2/V κ 3	2.52×10^5	3.07×10^{-3}	1.22×10^{-8}	0.00259	6.93
VH2/V κ 4	3.39×10^5	3.68×10^{-3}	1.09×10^{-8}	0.00377	6.19
VH2/V κ 5	2.25×10^5	3.75×10^{-3}	1.66×10^{-8}	0.00241	9.43
VH3/V κ 1	2.91×10^5	4.04×10^{-3}	1.39×10^{-8}	0.00393	7.90
VH3/V κ 2	2.36×10^5	3.77×10^{-3}	1.60×10^{-8}	0.0019	9.09
VH3/V κ 3	2.20×10^5	3.29×10^{-3}	1.49×10^{-8}	0.00359	8.47
VH3/V κ 4	2.67×10^5	4.60×10^{-3}	1.73×10^{-8}	0.00332	9.83
VH3/V κ 5	2.85×10^5	4.51×10^{-3}	1.58×10^{-8}	0.00257	8.98

Biacore analysis showed that all humanized variants bound to factor Bb, however, in all cases the relative K_D was greater than two-fold different than the chimeric suggesting that some binding affinity had been lost. In order to address this, an additional four heavy chain (VH4 to VH7) and two light chain (V κ 6 to V κ 7) sequences were designed and cloned into the appropriate expression vector. Variant sequences are shown in Tables 2 and 3.

Expression and Single Cycle Kinetic Analysis of Redesigned Variants

Five control antibodies (VH0/V κ 6, VH0/V κ 7, VH5/V κ 0, VH6/V κ 0, VH7/V κ 0) and combinations of humanized heavy and light chains (a total of eight humanized pairings, Table 8) were transiently transfected into HEK EBNA adherent cells (ATCC® Cat. No. CRL-10852™) using a PEI transfection method. IgG supernatant titres were monitored by IgG ELISA (Table 9) and transfections were cultured for up to 10 days prior to harvesting supernatants.

Table 8. Antibody Titers of Variants Produced by Transient Transfection ($\mu\text{g}/\text{mL}$).

	V κ 0	V κ 6	V κ 7
VH0	-	60.6	29.9
VH4	-	4.71	4.75
VH5	26.9	38.1	35.5
VH6	5.8	8.5	9.7
VH7	17.9	36.1	39.8

Single cycle kinetics using cell culture supernatants were performed as described above. The sensorgrams and fitted data for the single cycle kinetics are shown in FIGs. 2A-2C.

- 5 All variants were shown to bind to factor Bb. Single cycle kinetics data (Table 9) demonstrated that five antibodies (VH4/V κ 6, VH4/V κ 7, VH6/V κ 6, VH6/V κ 7 and VH7/V κ 7) bound to factor Bb within ~two-fold of the reference chimeric antibody. The relative K_D was calculated by dividing the K_D of the VH0/V κ 0 reference antibody by that of the humanized variant assayed in the same experiment.

10

Table 9. Single Cycle Kinetic Parameters of the Humanized Variants and Reference Antibodies Binding to Factor Bb.

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	χ^2 (RU ²)	Relative K_D
VH0/V κ 0	5.35×10^5	9.49×10^{-4}	1.77×10^{-9}	0.00854	1.00
VH0/V κ 3	5.63×10^5	1.28×10^{-3}	2.27×10^{-9}	0.00587	1.28
VH0/V κ 6	5.34×10^5	1.26×10^{-3}	2.37×10^{-9}	0.00766	1.34
VH0/V κ 7	4.96×10^5	1.05×10^{-3}	2.11×10^{-9}	0.00335	1.19
VH4/V κ 6	3.87×10^5	8.59×10^{-4}	2.22×10^{-9}	0.00473	1.25
VH4/V κ 7	3.86×10^5	9.03×10^{-4}	2.34×10^{-9}	0.00497	1.32
VH5/V κ 0	3.49×10^3	1.45×10^{-3}	4.16×10^{-7}	0.11	235.03
VH5/V κ 6	1.33×10^5	1.73×10^{-2}	1.30×10^{-7}	0.173	73.45
VH5/V κ 7	3.20×10^3	1.66×10^{-3}	5.21×10^{-7}	0.153	294.35
VH6/V κ 0	5.98×10^5	1.97×10^{-3}	3.29×10^{-9}	0.00485	1.86
VH6/V κ 6	4.12×10^5	1.58×10^{-3}	3.84×10^{-9}	0.00588	2.17
VH6/V κ 7	6.30×10^5	2.21×10^{-3}	3.51×10^{-9}	0.00377	1.98
VH7/V κ 0	2.74×10^5	2.07×10^{-3}	7.53×10^{-9}	0.00634	4.25
VH7/V κ 6	1.68×10^5	3.03×10^{-3}	1.80×10^{-8}	0.00587	10.17
VH7/V κ 7	4.83×10^6	1.17×10^{-2}	2.42×10^{-9}	0.0384	1.37

Purification of Antibodies

VH4/V κ 6, VH4/V κ 7, VH6/V κ 6 VH6/V κ 7 and VH7/V κ 7 as well as the chimeric antibody were purified from cell culture supernatants on Protein A sepharose columns (GE Healthcare, Little Chalfont, UK), followed by size exclusion chromatography using a 16/60 Superdex 200 column (GE Healthcare, Little Chalfont, UK) using PBS pH 7.4 as the mobile phase. Antibodies were quantified by OD_{280nm} using an extinction coefficient based on the predicted amino acid sequence. Reduced antibodies were analyzed using SDS-PAGE by loading 1 μ g of each antibody on the gel (FIG. 3) and bands corresponding to the profile of a typical antibody were observed.

Multicycle Kinetics Analysis of Antibodies

In order to establish an accurate affinity for factor Bb, multicycle kinetics analysis was performed on the purified chimeric antibody and the five lead antibodies using a Biacore T200 (serial no. 1909913) instrument running Biacore T200 Evaluation Software V3.0.1 (Uppsala, Sweden). Antibodies were diluted in running buffer to a final concentration of 0.5 μ g/ml. At the start of each cycle, antibodies were loaded onto Fc2, Fc3 and Fc4 of the Protein A chip (GE Healthcare, Little Chalfont, UK). IgGs were captured at a flow rate of 10 μ l/min to give an immobilisation level (RL) of ~ 63 RU, the theoretical value to obtain an R_{max} of ~ 50 RU. The surface was then allowed to stabilize. Kinetic data was obtained with factor Bb as analyte and using a flow rate of 30 μ l/min to minimize any potential mass transfer effects. Multiple repeats of a blank and a repeat of a single concentration of the analyte were programmed into the kinetic run in order to check the stability of both the surface and analyte over the kinetic cycles. For kinetic analysis, a two-fold dilution range was selected from 12.5 nM to 0.391 nM factor Bb. The association phase of factor Bb was monitored for 360 seconds and the dissociation phase was monitored for 600 seconds. Regeneration of the Protein A surface was conducted using two injections of 10 mM glycine-HCL pH 1.5. The signal from the reference channel Fc1 was subtracted from that of Fc2, Fc3 and Fc4 to correct for differences in non-specific binding to a reference surface, and a global R_{max} parameter was used in the 1-to-1 binding model.

The sensorgrams and fitted data for the binding of chimeric antibodies and humanized variants to factor Bb are shown in FIGS. 4A-4C. The relative K_D was calculated by dividing the K_D of the humanized variants by that of the chimeric antibody on the same chip. The kinetic

parameters measured for the interaction of factor Bb with chimeric antibodies and humanized variants are shown in Table 10. Two humanized variants, VH4/Vκ6 and VH4/Vκ7 (bold), showed relative K_D s within two-fold of the reference chimeric antibody.

5 **Table 10. Multiple Cycle Kinetic Parameters of the Humanized Variants and Reference Antibodies Binding to Factor Bb**

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	χ^2 (RU ²)	Relative K_D
VH0/Vκ0	4.87×10^5	9.69×10^{-4}	1.99×10^{-9}	0.0515	1.00
VH4/Vκ6	3.76×10^5	8.05×10^{-4}	2.14×10^{-9}	0.0389	1.08
VH4/Vκ7	3.22×10^5	8.41×10^{-4}	2.61×10^{-9}	0.645	1.31
VH0/Vκ0	5.00×10^5	9.54×10^{-4}	1.91×10^{-9}	0.033	1.00
VH6/Vκ6	2.51×10^5	1.30×10^{-3}	5.17×10^{-9}	0.0307	2.71
VH6/Vκ7	2.18×10^5	1.24×10^{-3}	5.70×10^{-9}	0.0692	2.98
VH0/Vκ0	4.95×10^5	9.40×10^{-4}	1.90×10^{-9}	0.0442	1.00
VH7/Vκ7	2.22×10^5	2.11×10^{-3}	9.51×10^{-9}	0.0219	5.01

Factor Bb competition ELISA

Lead purified variants and chimeric antibodies were tested for their binding to factor Bb using competition against murine parental antibody.

Factor Bb was diluted in 1x PBS to 1.0 μ g/ml and 100 μ l/well was coated overnight at 4°C on a 96-well ELISA plate. The following day the plates were blocked for two hours at room temperature with 1% casein/PBS before washing 2x with PBS pH 7.4. In a 96-well dilution plate a fixed concentration of murine parental antibody (0.5 μ g/ml, final concentration) was added in equal volume to a four-fold titration series of test antibody (starting from 45 μ g/ml to 0.01 μ g/ml, final concentration) diluted in blocking buffer. After washing the plate 3x with PBS-T, 100 μ l of chimeric/test antibody mix was added to the ELISA plate. After incubating at room temperature for one hour, the plate was washed 3x with PBS-T and 100 μ l of anti-mouse IgG Fc-Specific HRP (Sigma, Dorset, UK) diluted 1:1000 in PBS-T was applied for one hour at room temperature to detect bound mouse antibody. For color development, the plate was washed 3x with PBS-T following which 100 μ l of TMB substrate was added and incubated for approximately five minutes at room temperature. The reaction was stopped with 50 μ l of 3.0 M hydrochloric acid and absorbance was read immediately using a DYNEX® plate reader at 450 nm.

The results were plotted and are shown in FIG. 5. IC₅₀ values were calculated for each variant and relative IC₅₀ values were calculated by dividing the IC₅₀ of the humanized variant by that of the chimeric antibody assayed on the same plate (Table 11). All lead variants demonstrated IC₅₀ values within two-fold of the parent antibody.

5

Table 11. Half Maximal Inhibitory Concentration (IC₅₀) Values Obtained from Competition ELISA of Humanized Leads to Factor Bb.

Variant	IC ₅₀ (nM)	Relative IC ₅₀ (nM)
VH0/Vκ0	1.23	1.00
VH4/Vκ6	1.02	0.83
VH4/Vκ7	0.75	0.61
VH6/Vκ6	0.93	0.75
VH6/Vκ7	0.82	0.66
VH7/Vκ7	1.43	1.16

Example 2: Activity of humanized variants

10 Inhibition of WIESLAB® AP by anti-factor Bb humanized variants in human serum

The ability of the humanized variants to inhibit complement AP activity was measured using the Complement System Alternative Pathway WIESLAB® kit. In this plate-based assay, lipopolysaccharide (LPS) coated wells lead to specifically activation of the alternative pathway with detection of membrane attack complex (MAC) deposition serving as the readout. 5. 56% normal human serum (NHS) was incubated with a dilution series of the parental antibody and humanized variants along with a human IgG4 control antibody starting at 100 µg/mL. OD 405 nm was measured and compared to the kit positive and negative controls. Data were plotted relative to the plate positive control (FIG. 6A). All humanized variants showed inhibition of AP-mediated MAC deposition similar to the parental antibody in human serum (Table 13).

20

Table 12. Activity of Humanized anti-factor Bb Antibodies in WIESLAB® AP Assay.

Variant	AP Assay IC ₅₀ (M)
VH0/Vκ0	2.4 x 10 ⁻⁸
VH4/Vκ6	2.2 x 10 ⁻⁸
VH4/Vκ7	2.3 x 10 ⁻⁸

VH6/Vk6	1.9×10^{-8}
VH6/Vk7	2.1×10^{-8}
VH7/Vk7	2.5×10^{-8}

Inhibition of AP-mediated hemolysis by humanized variants in human serum

Inhibition of AP pathway-mediated hemolysis was determined using human or cynomolgus monkey serum and rabbit erythrocytes in EGTA-containing buffer to inhibit the classical pathway. A dilution series of the parental antibody and humanized variants starting at 200 $\mu\text{g/mL}$ were incubated with 20% human serum and 10×10^6 rabbit red blood cells (RBCs) for one hour at 37°C. The amount of lysis was determined by measuring the absorbance of the supernatant at 540 nm and subtracting the background absorbance in control wells containing ethylene diamine tetraacetic acid (EDTA). Results are shown in FIG. 6B. In FIG. 6C, the A540, representing the amount of hemolysis, is shown for each variant at 100 $\mu\text{g/mL}$. VH4/VK6 and VH6/VK7 show the greatest inhibition of hemolysis as reflected by the largest reduction in A540.

Binding of The Parent Antibody and Humanized Variants to Cynomolgus Monkey

Factor Bb

To ensure that the humanization process did not affect species cross-reactivity, the parental antibody and humanized derivatives were tested for binding to human and cynomolgus monkey factor Bb by Biolayer Interferometry (BLI) using an Octet Red. Briefly, biotinylated antibodies were loaded onto SA Biosensors equilibrated in PBS, 0.1% BSA, 0.02% Tween-20 (assay buffer). After a 60 second baseline in assay buffer, antibodies were loaded onto the probes for 180 seconds, followed by another 60 second baseline. Association to human (Comptech) or cynomolgus monkey factor Bb (purified in-house) was measured for 300 seconds, followed by a 300 second dissociation. Kinetic parameters were calculated by the Octet Analysis software using a 1:1 binding model. Data are summarized in Table 13 and show that the humanization did not affect cross-reactivity to factor Bb from cynomolgus monkey.

Table 13. Binding of Humanized Variants to Human and Cynomolgus Bb by BLI.

Antibody	KD (M) for Human Bb	KD (M) for Cyno Bb
Anti-factor Bb antibody in Table 1	1.9×10^{-9}	1.1×10^{-8}
VH0/Vk0	2.0×10^{-9}	1.0×10^{-8}

VH4/Vk6	1.6×10^{-9}	8.6×10^{-9}
VH4/Vk7	1.9×10^{-9}	1.1×10^{-8}
VH6/Vk6	1.9×10^{-9}	1.1×10^{-8}
VH6/Vk7	1.9×10^{-9}	1.1×10^{-8}
VH7/Vk7	2.9×10^{-9}	1.6×10^{-8}

Binding of VH4/VK6-IgG4v2 and VH6/VK7-IgG4v2 to human and cynomolgus monkey factor Bb

- To determine whether the modification of the Fc portion of the antibodies affected affinity and species cross-reactivity, VH4/VK6-IgG4v2 and VH6/VK7-IgG4v2 were tested alongside their parental antibodies, VH4/VK6 and VH6/VK7, respectively, for their ability to bind human and cynomolgus monkey factor Bb by BLI. VH4/VK6-IgG4v2 and VH6/VK7-IgG4v2 contain mutations in the Fc region that increase the affinity for an Fc receptor. The experiment was carried out as described in the example above and results are summarized in Table 14. As expected, modification of the Fc did not affect binding.

Table 14. Binding Humanized Anti-Factor Bb Antibodies to Human and Cynomolgus Factor Bb by BLI.

Antibody	KD (M) for Human Bb	KD (M) for Cyno Bb
V ₄ 6/V _K 6-IgG4v1	1.6×10^{-9}	1.1×10^{-8}
V _H 4/V _K 6-IgG4v2	1.6×10^{-9}	1.1×10^{-8}
V _H 6/V _K 7-IgG4v1	1.7×10^{-9}	1.0×10^{-8}
V _H 6/V _K 7-IgG4v2	1.7×10^{-9}	1.0×10^{-8}

Specificity of VH4/VK6 and VH6/VK7 for factor Bb (the activated form of factor B)

- The ability of VH4/VK6 and VH6/VK7 to bind to zymogen factor B and factor Bb from both human and cynomolgus monkey was determined by surface plasmon resonance using a Biacore T200. Human factor B and factor Bb were purchased from Comptech and cynomolgus monkey factor B and factor Bb were purified in-house. Briefly, the monoclonal antibodies were captured on a Biacore Series S Protein A chip in HBSP+ (10 mM HEPES, 150 mM NaCl, 0.05% P20 pH 7.4) at a flow rate of 30 μ L/min. A five-point concentration series of each analyte was tested for binding using single cycle kinetics with a contact time of 180 seconds per concentration followed by a 600 sec dissociation at 25°C and a flow rate of 60 μ L/sec. Both human and cynomolgus monkey factor B started at a concentration of 500 nM followed

by 2-fold dilutions, while human and cynomolgus monkey factor Bb started at concentrations of 30 nM and 150 nM, respectively, followed by 2-fold dilutions. Data were analyzed using the Biacore evaluation software using a 1:1 binding model. Sensorgrams are shown in FIGs. 7A-7D. Results are summarized in Table 15. Briefly, both humanized variants show roughly 10-fold higher affinities for human factor Bb compared to cynomolgus monkey factor Bb. Binding to human or cynomolgus monkey factor B was barely detectable and the small signal that was observed was dominated by non-specific binding.

Table 15. Specificity of Lead Humanized Variants to Human or Cynomolgus Monkey Factor Bb

Antibody	Antigen	k_a (1/Ms)	k_d (1/s)	KD (M)	R _{max} (RU)	Chi ² (RU ²)
V _H 4/V _K 6-IgG4v2	Human Factor Bb	4.10×10^5	9.25×10^{-4}	2.26×10^{-9}	108.2	0.636
	Human Factor B	n/a	n/a	n/a	n/a	n/a
	Cyno Factor Bb	3.49×10^5	6.83×10^{-3}	1.96×10^{-8}	118.7	2.42
	Cyno Factor B	n/a	n/a	n/a	n/a	n/a
V _H 6/V _K 7-IgG4v2	Human Factor Bb	2.32×10^5	1.38×10^{-3}	5.92×10^{-9}	91.8	0.114
	Human Factor B	n/a	n/a	n/a	n/a	n/a
	Cyno Factor Bb	1.55×10^5	8.77×10^{-3}	5.65×10^{-8}	104.6	0.457
	Cyno Factor B	n/a	n/a	n/a	n/a	n/a

Binding of chimeric parent and representative humanized variants to various complement proteins

To ensure that humanization did not introduce non-specific binding or cross-reactivity to other complement or plasma proteins, the chimeric parent antibody and a representative humanized variant, V_H6/V_K7-IgG4v2, were tested for binding to aC1s (Comptech A104), C1r (Comptech A102), C2 (Comptech A112), C2a (prepared from C2), thrombin (EMD Millipore 605195), elastase (EMD Millipore 324682), factor D (Comptech A136), and factor Bb (Comptech A155). Briefly, complement and plasma proteins were coated onto ELISA plates at 2.5 µg/mL in PBS overnight at 4°C. Plates were then blocked with Casein for 1 hour at room temperature and washed four times with 1X DPBS/0.05% Tween-20 followed by a single wash with 1X DPBS. Serial dilutions starting at 50 µg/ml of biotinylated chimeric parent or V_H6/V_K7-IgG4v2 were added to the plate in PBS/0.1%casein/0.1%Tween-20 and incubated for 2 hours at room temperature. Plates were washed as described and a 1:10,000 dilution of

streptavidin-HRP (Southern Biotech 7100-05) in PBS/0.1% casein/0.1% Tween-20 was added to the plate and incubated for 30 minutes at room temperature. Plates were again washed and Ultra TMP ELISA (Thermo 34028) was added to the plate for 1 minute, followed by stop solution). OD 450 nm was read in a plate reader and background at 620 nm was subtracted.

- 5 Both the chimeric parent (FIG. 8A) and the humanized variant (FIG. 8B) show complete specificity for factor Bb.

Example 3: V_H6/V_K7-IgG4v2 produced from CHO versus HEK behave the same

- To determine whether V_H6/V_K7-IgG4v2 produced in HEK cells behaved the same
10 when produced in CHO cells, V_H6/V_K7-IgG4v2 was generated by transient expression in HEK cells or by stable expression in CHO cells using standard methods.

- The complement alternative pathway (CAP) and classical pathway (CCP) activities of the antibodies were determined using the Complement System Alternative Pathway and Classical Pathway WIESLAB® Kits, respectively, in accordance with the manufacturer's
15 instructions. To determine CAP activity, antibodies were tested in 5.56% normal human serum (FIG. 9A) and 5.56% normal cynomolgus monkey serum (FIG. 9B). To determine CCP activity, antibodies were tested in 1% normal human serum (FIG. 10A) and 1% normal cynomolgus monkey serum (FIG. 10B). OD_{405nm} was measured and results were normalized to serum activity prior to antibody injection. V_H6/V_K7-IgG4v2 produced in CHO and HEK
20 showed similar activity. IC₅₀ values for both sets of antibodies were calculated and are shown in FIGs. 10A-10B. V_H6/V_K7-IgG4v2 produced in CHO showed similar potency to V_H6/V_K7-IgG4v2 produced in HEK. AP pathway-mediated hemolysis of V_H6/V_K7-IgG4v2 produced in CHO and HEK was also compared. Hemolysis was determined using 20% normal human serum and rabbit erythrocytes in buffer containing EGTA to inhibit the classical pathway. The
25 amount of lysis was determined by measuring the of the supernatant and subtracting the background absorbance in control wells containing ethylene diamine tetraacetic acid (EDTA). V_H6/V_K7-IgG4v2 produced in CHO and HEK showed similar % hemolysis (FIG. 11).

- Multicycle kinetic analysis was performed on CHO and HEK-produced V_H6/V_K7-IgG4v2 antibodies using biolayer interferometry. Antibodies were diluted to 10 µg/mL in
30 PBS+ 0.02% Tween20, 0.1% BSA, 0.05% sodium azide and loaded onto anti-hIgG Fc sensors (pre-equilibrated in the same buffer) for 90 seconds followed by a 60-minute baseline in buffer. Binding of a concentration series of human factor Bb (Complement Technologies, #A155), ranging from 100 nM to 0.14 nM in 2-fold dilutions, was measured in a 300 second

association in the same buffer, followed by a 300 second dissociation step. The binding curves and fitted data, as well as calculated K_{ds} for both antibodies are shown in FIG. 12.

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- 30 All publications, patents, patent applications, publication, and database entries (*e.g.*,
sequence database entries) mentioned herein, *e.g.*, in the Background, Summary, Detailed
Description, Examples, and/or References sections, are hereby incorporated by reference in
their entirety as if each individual publication, patent, patent application, publication, and

database entry was specifically and individually incorporated herein by reference. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS AND SCOPE

5 Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents of the embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

 Articles such as “a,” “an,” and “the” may mean at least one than one unless indicated to
10 the contrary or otherwise evident from the context. Claims or descriptions that include “or” between two or more members of a group are considered satisfied if one, more than one, or all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes “or” between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in
15 which more than one members of the group are present, and embodiments in which all of the group members are present. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

 It is to be understood that the disclosure encompasses all variations, combinations, and
20 permutations in which at least one limitation, element, clause, or descriptive term, from at least one of the claims or from at least one relevant portion of the description, is introduced into another claim. For example, a claim that is dependent on another claim can be modified to include at least one of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that
25 methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

 Where elements are presented as lists, *e.g.*, in Markush group format, it is to be
30 understood that every possible subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term “comprising” is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where an embodiment, product, or method is referred

to as comprising particular elements, features, or steps, embodiments, products, or methods that consist, or consist essentially of, such elements, features, or steps, are provided as well. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically
5 claimed or disclaimed.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” 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
10 or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111. 03.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific
15 value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the
20 understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

Where websites are provided, URL addresses are provided as non-browser-executable codes, with periods of the respective web address in parentheses. The actual web addresses do
25 not contain the parentheses.

In addition, it is to be understood that any particular embodiment of the present disclosure may be explicitly excluded from any at least one of the claims. Where ranges are given, any value within the range may explicitly be excluded from any at least one of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or
30 methods of the disclosure, can be excluded from any at least one claims. For purposes of brevity, all of the embodiments in which at least one elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

The terms “about” and “substantially” preceding a numerical value mean $\pm 10\%$ of the recited numerical value.