

[0008] CD3 is a homodimeric or heterodimeric antigen expressed on T cells in association with the T cell receptor complex (TCR) and is required for T cell activation. Functional CD3 is formed from the dimeric association of two of four different chains: epsilon, zeta, delta and gamma. The CD3 dimeric arrangements include gamma/epsilon, delta/epsilon, and zeta/zeta. Antibodies against CD3 have been shown to cluster CD3 on T cells, thereby causing T cell activation in a manner similar to the engagement of the TCR by peptide-loaded MHC molecules. Thus, anti-CD3 antibodies have been proposed for therapeutic purposes involving the activation of T cells.

[0009] Antigen-binding molecules that target FcεR1α, as well as bispecific antigen-binding molecules that bind both FcεR1α and CD3 would be useful in therapeutic settings in which specific targeting and T cell-mediated killing of cells that express FcεR1α is desired.

BRIEF SUMMARY OF THE INVENTION

[0009a] In one aspect, the present disclosure provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human and/or cynomolgus FcεR1α, wherein:

the first antigen-binding domain comprises a heavy chain complementarity determining region (HCDR)1 having the amino acid sequence of SEQ ID NO:44, an HCDR2 having the amino acid sequence of SEQ ID NO:46, an HCDR3 having the amino acid sequence of SEQ ID NO:48, a light chain complementarity determining region (LCDR)1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; and the second antigen-binding domain comprises: (i) an HCDR1 having the amino acid sequence of SEQ ID NO:4, an HCDR2 having the amino acid sequence of SEQ ID NO:6, an HCDR3 having the amino acid sequence of SEQ ID NO:8, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or (ii) an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or (iii) an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

[0009b] In another aspect, the present disclosure provides a bispecific antibody comprising a first binding arm that binds human CD3 and a second binding arm that binds human FcεR1α, wherein the first binding arm comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 and a light chain comprising the amino acid sequence of SEQ ID NO: 40, and wherein the second binding arm comprises a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, and 54, and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

[\[0009c\]](#) In another aspect, the present disclosure provides a nucleic acid molecule encoding the bispecific antigen-binding molecule as disclosed herein.

[\[0009d\]](#) In another aspect, the present disclosure provides a vector comprising the nucleic acid molecule as disclosed herein.

[\[0009e\]](#) In another aspect, the present disclosure provides a host cell comprising the vector as disclosed herein.

[\[0009f\]](#) In another aspect, the present disclosure provides a pharmaceutical composition comprising the bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier or diluent.

[0010] In a first [aspectexample](#), the present [inventiondisclosure](#) provides antibodies and antigen-binding fragments thereof that bind to human FcεR1α. The antibodies according to this [aspectexample](#) are useful, *inter alia*, for targeting cells expressing FcεR1α. The present [inventiondisclosure](#) also provides bispecific antibodies and antigen-binding fragments thereof that bind human FcεR1α and human CD3. The bispecific antibodies according to this [aspectexample](#) are useful, *inter alia*, for targeting T cells expressing CD3, and for stimulating T cell activation, e.g., under circumstances where T cell-mediated killing of cells expressing FcεR1α is beneficial or desirable. For example, the bispecific antibodies can direct CD3-mediated T cell activation to specific FcεR1α-expressing cells, such as mast cells or basophils.

[0011] Exemplary anti-FcεR1α antibodies of the present [inventiondisclosure](#) are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs) and light chain variable regions (LCVRs), as well as heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), heavy chain (HC), and light chain (LC) of the exemplary anti-FcεR1α antibodies. Table 2 sets forth the sequence identifiers of the nucleic acid molecules encoding the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2, LCDR3, HC and LC of the exemplary anti-FcεR1α antibodies.

[0012] The present [inventiondisclosure](#) provides antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0013] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0014] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain [embodimentexamples](#), the present [inventiondisclosure](#) provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain [embodimentexamples](#), the HCVR/LCVR amino acid sequence pair is of SEQ ID NOs: 2/26, 10/26, or 18/26 (e.g., mAb17110, mAb17111, or mAb17112)

[0015] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0016] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0017] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0018] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0019] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0020] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0021] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain [embodimentexamples](#), the present [inventiondisclosure](#) provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino

acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain [embodimentexamples](#), the HCDR3/LCDR3 amino acid sequence pair is of SEQ ID NOs: 8/32, 16/32, or 24/32 (e.g., mAb17110, mAb17111, or mAb17112).

[0022] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (i.e., HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain [embodimentexamples](#), the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set is selected from the group consisting of SEQ ID NOs: 4-6-8-28-30-32, 12-14-16-28-30-32, or 20-22-24-28-30-32 (e.g., mAb17110, mAb17111, or mAb17112).

[0023] In a related [embodimentexample](#), the present [inventiondisclosure](#) provides antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (i.e., HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1. For example, the present [inventiondisclosure](#) includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair of SEQ ID NOs: 2/26, 10/26, or 18/26 (e.g., mAb17110, mAb17111, or mAb17112). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, e.g., the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, e.g., Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0024] The present [inventiondisclosure](#) also provides nucleic acid molecules encoding anti-FcεR1α antibodies or portions thereof. For example, the present [inventiondisclosure](#) provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0025] The present [inventiondisclosure](#) also provides nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain [embodimentexamples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences

listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0026] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0027] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0028] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0029] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0030] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0031] The present [invention disclosure](#) also provides nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain [embodiment examples](#) the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0032] The present [invention disclosure](#) also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1.

[0033] The present [inventiondisclosure](#) also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-FcεR1α antibodies listed in Table 1.

[0034] The present [inventiondisclosure](#) also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain [embodimentexamples](#), the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain [embodimentexamples](#) according to this [aspectexample](#) of the [inventiondisclosure](#), the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same anti-FcεR1α antibody listed in Table 1.

[0035] The present [inventiondisclosure](#) also provides recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-FcεR1α antibody. For example, the present [inventiondisclosure](#) includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present [inventiondisclosure](#) are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0036] The present [inventiondisclosure](#) includes anti-FcεR1α antibodies having a modified glycosylation pattern. In some [embodimentexamples](#), modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fructose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0037] In another [aspectexample](#), the [inventiondisclosure](#) provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds FcεR1α and a pharmaceutically acceptable carrier. In a related [aspectexample](#), the [inventiondisclosure](#) features a composition which is a combination of an anti-FcεR1α antibody and a second therapeutic agent. In one [embodimentexample](#), the second therapeutic agent is any agent that is advantageously combined with

an anti-FcεR1α antibody. Additional combination therapies and co-formulations involving the anti-FcεR1α antibodies of the present [inventiondisclosure](#) are disclosed elsewhere herein.

[0038] In another [aspectexample](#), the [inventiondisclosure](#) provides therapeutic methods for targeting/killing FcεR1α-expressing cells (e.g., mast cells, or basophils) using an anti-FcεR1α antibody of the [inventiondisclosure](#), wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-FcεR1α antibody of the [inventiondisclosure](#) to a subject in need thereof. In some cases, the anti-FcεR1α antibodies (or antigen-binding fragments thereof) can be used for treating allergy, or may be modified to be more cytotoxic by methods, including but not limited to, modified Fc domains to increase ADCC (see e.g. Shield et al. (2002) JBC 277:26733), radioimmunotherapy, antibody-drug conjugates, or other methods for increasing the efficiency of FcεR1α expressing cells killing.

[0039] The present [inventiondisclosure](#) also includes the use of an anti-FcεR1α antibody of the [inventiondisclosure](#) in the manufacture of a medicament for the treatment of a disease or disorder (e.g., allergy) related to or caused by FcεR1α-expressing cells.

[0040] In yet another [aspectexample](#), the [inventiondisclosure](#) provides monospecific anti-FcεR1α antibodies for diagnostic applications, such as, e.g., imaging reagents.

[0041] In yet another [aspectexample](#), the [inventiondisclosure](#) provides therapeutic methods for stimulating T cell activation using an anti-CD3 antibody or antigen-binding portion of an antibody of the [inventiondisclosure](#), wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody of the present [inventiondisclosure](#).

[0042] In another [aspectexample](#), the present [inventiondisclosure](#) provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α or binds cynomolgus (*Macaca fascicularis*) FcεR1α with a binding dissociation equilibrium constant (K_D) of less than about 250 nM as measured in a surface plasmon resonance assay at 25°C. In yet another [aspectexample](#), the present [inventiondisclosure](#) provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α with a dissociative half-life ($t_{1/2}$) of greater than about 0.54 minute or binds cynomolgus FcεR1α with a dissociative half-life ($t_{1/2}$) of greater than about 0.6 minute as measured in a surface plasmon resonance assay at 25°C.

[0043] The [inventiondisclosure](#) further provides an antibody or antigen-binding fragment that competes for binding to human FcεR1α with a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in Table 1. In another [aspectexample](#), the [inventiondisclosure](#) provides an antibody or antigen-binding fragment that competes for binding to human FcεR1α with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26; and 18/26.

[0044] The [inventiondisclosure](#) furthermore provides an antibody or antigen-binding fragment, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in

Table 1. In another [aspectexample](#), the antibody or antigen-binding fragment binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26, and 18/26.

[0045] The [inventiondisclosure](#) further provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1; and the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in Table 1. In another [aspectexample](#), the isolated antibody or antigen-binding fragment comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair selected from the group consisting of: 2/26; 10/26; and 18/26. In yet another [aspectexample](#), the isolated antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-28-30-32; 12-14-16-28-30-32; and 20-22-24-28-30-32

[0046] In another [aspectexample](#), the [inventiondisclosure](#) provides an isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10, and 18; and (b) a light chain variable region (LCVR) having an amino acid sequence SEQ ID NO: 26. In a further [aspectexample](#), the isolated antibody or antigen-binding fragment an HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/26; 10/26; and 18/26.

[0047] According to another [aspectexample](#), the present [inventiondisclosure](#) provides bispecific antigen-binding molecules (e.g., antibodies) that bind FcεR1α and CD3. Such bispecific antigen-binding molecules are also referred to herein as "anti-FcεR1α/anti-CD3 bispecific molecules," "anti-CD3/anti-FcεR1α bispecific molecules," "anti-FcεR1α x CD3," "anti-CD3 x FcεR1α," or "FcεR1αxCD3 bsAbs." The anti-FcεR1α portion of the anti-FcεR1α/anti-CD3 bispecific molecule is useful for targeting cells that express FcεR1α (e.g., mast cells or basophils), and the anti-CD3 portion of the bispecific molecule is useful for activating T-cells. The simultaneous binding of FcεR1α on a mast cells or basophils and CD3 on a T-cell facilitates directed killing (cell lysis) of the targeted mast cells or basophils by the activated T-cell. The anti-FcεR1α/anti-CD3 bispecific molecules of the [inventiondisclosure](#) are therefore useful, *inter alia*, for treating diseases and disorders related to or caused by FcεR1α-expressing cells (e.g., allergy).

[0048] The bispecific antigen-binding molecules according to this [aspectexample](#) of the present [inventiondisclosure](#) comprise a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds FcεR1α. The present [inventiondisclosure](#) includes anti-FcεR1α/anti-CD3 bispecific molecules (e.g., bispecific antibodies) wherein each antigen-binding domain comprises a heavy chain variable region (HCVR) paired with a light chain variable region (LCVR). In certain exemplary [embodimentexamples](#) of the [inventiondisclosure](#), the anti-CD3

antigen-binding domain and the anti-FcεR1α antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. For example, as illustrated in Example 1 herein, bispecific antibodies were constructed comprising a first antigen-binding domain that specifically binds CD3, wherein the first antigen-binding domain comprises an HCVR and an LCVR, each derived from an anti-CD3 antibody; and a second antigen-binding domain that specifically binds FcεR1α, wherein the second antigen-binding domain comprises an HCVR derived from an anti-FcεR1α antibody paired with the same LCVR. In such [embodimentexamples](#), the first and second antigen-binding domains comprise distinct anti-CD3 and anti-FcεR1α HCVRs but share a common LCVR. The amino acid sequence of this LCVR is shown, *e.g.*, in SEQ ID NO: 26, and the amino acid sequences of the corresponding CDRs (*i.e.*, LCDR1-LCDR2-LCDR3) are shown in SEQ ID NOs: 28, 30, and 32, respectively. Genetically modified mice can be used to produce fully human bispecific antigen-binding molecules comprising two different heavy chains that associate with an identical light chain that comprises a variable domain derived from one of two different human light chain variable region gene segments. Alternatively, variable heavy chains may be paired with one common light chain and expressed recombinantly in host cells. As such, the antibodies of the [inventiondisclosure](#) can comprise immunoglobulin heavy chains associated with a single rearranged light chain. In some [embodimentexamples](#), the light chain comprises a variable domain derived from a human Vk1-39 gene segment or a Vk3-20 gene segment. In other [embodimentexamples](#), the light chain comprises a variable domain derived from a human Vk1-39 gene segment rearranged with a human Jk5 or a human Jk1 gene segment (WO 2017/053856, herein incorporated by reference).

[0049] The present [inventiondisclosure](#) provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences, any of the LCVR amino acid sequences, any of the HCVR/LCVR amino acid sequence pairs, any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences, or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in US publication 2014/0088295 published March 27, 2014 and WO 2018/067331 published April 12, 2018.

[0050] In addition, the present [inventiondisclosure](#) provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the HCVR amino acid sequences as set forth in Table 3 herein. The first antigen-binding domain that specifically binds CD3 may also comprise any of the LCVR amino acid sequences as set forth in Tables 1, and 3 herein. The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises any of the heavy chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Table 3, and/or any of the light chain CDR1-CDR2-CDR3 amino acid sequences as set forth in Tables 1, and 3 herein.

[0051] According to certain [embodimentexamples](#), the present [inventiondisclosure](#) provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds

CD3 comprises a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1 herein or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0052] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a light chain variable region (LCVR) having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0053] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises an HCVR and LCVR (HCVR/LCVR) amino acid sequence pair as set forth in Table 3 herein.

[0054] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence as set forth in Table 3 herein, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0055] In certain [embodimentexamples](#), the first antigen-binding domain that specifically binds CD3 comprises an HCDR3/LCDR3 amino acid sequence pair as set forth in Table 3 herein.

[0056] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, wherein the first antigen-binding domain that specifically binds CD3 comprises a heavy chain CDR1 (HCDR1) domain having an amino acid as set forth in Table 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid as set forth in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR3 (HCDR3) domain having an amino acid as set forth in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR2 (LCDR2) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity, and a light chain CDR3 (LCDR3) domain having an amino acid sequence as set forth in Tables 1, and 3 herein, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0057] Certain non-limiting, exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the [inventiondisclosure](#) include a first antigen-binding domain that specifically binds CD3 comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, having the amino acid sequences as set forth in Table 3 herein.

[0058] The present [inventiondisclosure](#) further provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence as set forth in Table 3 and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence as set forth in Tables 1, and 3.

[0059] In another [aspectexample](#), the [inventiondisclosure](#) provides a bispecific antigen-binding molecule wherein the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence of SEQ ID NO: 42., and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.

[0060] The [inventiondisclosure](#) further provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 comprising the amino acid sequences of SEQ ID Nos: 44-46-48-28-30-32.

[0061] In a further [aspectexample](#), the [inventiondisclosure](#) provides a bispecific antigen-binding molecule, wherein the first antigen-binding domain that specifically binds human CD3 comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair of SEQ ID NO: 42/26.

[0062] In more [embodimentexamples](#), exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the [inventiondisclosure](#) include a bispecific antigen-binding molecule wherein the first antigen-binding domain that specifically binds human CD3 comprises an HCVR comprising HCDR1-HCDR2-HCDR3 having the amino acid sequences of SEQ ID NOs: 44-46-48.

[0063] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain variable region (HCVR) having the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10, and 18, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0064] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO: 26, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0065] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises an HCVR and LCVR (HCVR/LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26, 10/26, and 18/26.

[0066] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 16, and 24, or a substantially similar sequence thereto having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain having an amino acid sequence of SEQ ID NO: 32, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0067] In certain [embodimentexamples](#), the second antigen-binding domain that specifically binds FcεR1α comprises an HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOs: 8/32, 16/32, and 24/32.

[0068] The present [inventiondisclosure](#) also provides anti-CD3/anti-FcεR1α bispecific antigen-binding molecules, wherein the second antigen-binding domain that specifically binds FcεR1α comprises a heavy chain CDR1 (HCDR1) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 12, and 20, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 14, and 22, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 16, and 24, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain having an amino acid sequence of SEQ ID NOs: 28, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain having an amino acid sequence of SEQ ID NO: 30, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; ; and a light chain CDR3 (LCDR3) domain having an amino acid sequence of SEQ ID NOs: 32, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0069] Certain non-limiting, exemplary anti-CD3/anti-FcεR1α bispecific antigen-binding molecules of the [inventiondisclosure](#) include a second antigen-binding domain that specifically binds FcεR1α comprising HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively,

having the amino acid sequences selected from the group consisting of: SEQ ID NOs: 4-6-8-28-30-32, 12-14-16-28-30-32, and 20-22-24-28-30-32.

[0070] In a related [embodimentexample](#), the [inventiondisclosure](#) includes anti-CD3/anti-FcεR1α bispecific antigen-binding molecules wherein the second antigen-binding domain that specifically binds FcεR1α comprises the heavy and light chain CDR domains contained within heavy and light chain variable region (HCVR/LCVR) sequences selected from the group consisting of SEQ ID NOs: 2/26, 10/26, and 18/26.

[0071] In another [aspectexample](#), the [inventiondisclosure](#) provides a bispecific antigen-binding molecule comprising: (a) a first antigen-binding domain that comprises three heavy chain complementarity determining regions HCDR1, HCDR2 and HCDR3, respectively, comprising the amino acid sequences of SEQ ID NOs: 44, 46 and 48, and three light chain complementarity determining regions LCDR1, LCDR2 and LCDR3, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the first antigen-binding domain specifically binds human CD3; and (b) a second antigen-binding domain that comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3); wherein HCDR1 comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 4, 12, and 20; HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 14, and 22; HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 16, and 24; LCDR1 comprises an amino acid sequence of SEQ ID NO: 28; LCDR2 comprises an amino acid sequence of SEQ ID NO: 30; and LCDR3 comprises an amino acid sequence of SEQ ID NO: 32, wherein the second antigen-binding arm specifically binds human FcεR1α.

[0072] In another [aspectexample](#), the [inventiondisclosure](#) provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that binds human CD3 and a second antigen-binding domain that binds human FcεR1α, wherein the second antigen-binding domain is derived from the antibody or antigen-binding fragment of any one of the anti-FcεR1α antibodies of the [inventiondisclosure](#). In a further [aspectexample](#), the [inventiondisclosure](#) provides a bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that specifically binds human FcεR1α.

[0073] The [inventiondisclosure](#) further provides a bispecific antigen-binding molecule which binds human cells expressing human CD3. In another [aspectexample](#), the bispecific antigen-binding molecule binds human cells expressing human FcεR1α and/or cells expressing cynomolgus FcεR1α.

[0074] In another [aspectexample](#) the [inventiondisclosure](#) provides a bispecific antigen-binding molecule which inhibits allergic reaction in a subject (e.g., mice) expressing human FcεR1α. The [inventiondisclosure](#) further provides bispecific antigen-binding molecules which deplete basophils or other FcεR1α-expressing cells in a subject (e.g., mice) expressing human FcεR1α.

[0075] In another [aspectexample](#) the [inventiondisclosure](#) provides a bispecific antigen-binding molecule comprising a second antigen-binding domain that specifically binds a target cell expressing human FcεR1α with a binding ratio greater than 200 in the presence or absence of IgE or binds a target cell expressing cynomolgus FcεR1α with a binding ratio greater than 140 in the presence or absence of IgE, wherein such binding ratio is measured in an *in vitro* FACS binding assay.

[0076] In some [embodimentexamples](#), the antigen-binding molecule induces T cell-mediated killing of FcεR1α-expressing with an EC₅₀ value of less than about 20 nM, as measured in an *in vitro* T cell-mediated cell killing assay, for example, where the FcεR1α expressing cells are basophils.

[0077] In some applications, the second antigen-binding domain binds human or cynomolgus FcεR1α with a K_D value of less than about 467 nM, as measured in an *in vitro* surface plasmon resonance binding assay at 25 °C. In some instances, the second antigen-binding domain binds each of human FcεR1α and cynomolgus FcεR1α with an K_D value of less than about 450 nM, less than about 400 nM, less than about 350 nM, less than about 300 nM, less than about 250 nM, less than about 200 nM, less about 150 nM, less than about 100 nM, or less than about 50 nM.

[0078] In certain [embodimentexamples](#), anti-FcεR1α antibodies of the [inventiondisclosure](#), antigen-binding fragments and bispecific antibodies thereof were made by replacing amino acid residues of a parental in a stepwise manner based on differences between the germline sequence and the parental antibody sequence.

[0079] In another [aspectexample](#), the present [inventiondisclosure](#) provides an isolated bispecific antigen-binding molecule that competes for binding to FcεR1α, or binds to the same epitope on FcεR1α as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 2/26, 10/26 or 18/26.

[0080] In another [aspectexample](#), the present [inventiondisclosure](#) provides an isolated bispecific antigen-binding molecule that competes for binding to human CD3, or binds to the same epitope on human CD3 as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR pair comprising the amino acid sequences of SEQ ID NOs: 2/26, 10/26 or 18/26.

[0081] Any of the bispecific antigen-binding molecules discussed above or herein may be a bispecific antibody. In some [embodimentexamples](#), the bispecific antibody comprises a human IgG heavy chain constant region. In one [embodimentexample](#), the human IgG heavy chain constant region is isotype IgG1. In one [embodimentexample](#), the human IgG heavy chain constant region is isotype IgG4.

In various [embodimentexamples](#), the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

[0082] The present [inventiondisclosure](#) also provides antibodies, or antigen-binding fragments thereof, comprising a heavy chain (HC) and a light chain (LC) amino acid sequence pair (HC/LC) comprising any of the HC amino acid sequences listed in Table 1 paired with any of the LC amino acid sequences listed in Table 1. According to certain [embodimentexamples](#), the present [inventiondisclosure](#) provides antibodies, or antigen-binding fragments thereof, comprising an HC/LC amino acid sequence pair contained within any of the exemplary anti-FcεR1α antibodies listed in Table 1. In certain [embodimentexamples](#), the HC/LC amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 34/40, 36/40, and 38/40.

[0083] The present [inventiondisclosure](#) also provides bispecific antibodies, or antigen-binding fragments thereof comprising a first heavy chain, a second heavy chain and a common light chain comprising any of the HC or LC amino acid sequences listed in Table 7. In certain [embodimentexamples](#), the bispecific antibodies comprise a first HC comprising an amino acid sequence of SEQ ID NO: 56; a second HC comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52 and 54; and a common light chain comprising the amino acid sequence of SEQ ID NO: 40.

[0084] In one [aspectexample](#), the [inventiondisclosure](#) provides a pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule and a pharmaceutically acceptable carrier or diluent. The [inventiondisclosure](#) further provides a method for treating an FcεR1α-related disease, allergy or an IgE-related disease in a subject, the method comprising administering to the subject the pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule and a pharmaceutically acceptable carrier or diluent. In some [embodimentexamples](#), the allergy or other IgE-related diseases are selected from the group consisting of allergic asthma, allergic rhinitis, hay fever, anaphylaxis, atopic dermatitis, chronic urticarial, food allergy, perennial allergy, drug allergy, and pollen allergy. In one [embodimentexample](#), the allergy is severe allergy. In some cases, the allergy leads to anaphylaxis. In certain [embodimentexamples](#), the FcεR1α-related disease comprises severe allergy, mast cell activation disorder or mastocytosis. In certain [embodimentexamples](#), the method for treating allergy comprises administering to the subject the pharmaceutical composition comprising an anti-FcεR1α antigen-binding molecule or anti-FcεR1α/anti-CD3 bispecific antigen-binding molecule at a certain dose, as described elsewhere herein.

[0085] In another [aspectexample](#), the present [inventiondisclosure](#) provides nucleic acid molecules encoding any of the HCVR, LCVR or CDR sequences of the anti-FcεR1α, and anti-CD3/anti-FcεR1α bispecific antigen-binding molecules disclosed herein, including nucleic acid molecules comprising the polynucleotide sequences as set forth in Tables 2, and 4 herein, as well as nucleic acid

molecules comprising two or more of the polynucleotide sequences as set forth in Tables 2, and 4 in any functional combination or arrangement thereof. Recombinant expression vectors carrying the nucleic acids of the [inventiondisclosure](#), and host cells into which such vectors have been introduced, are also encompassed by the [inventiondisclosure](#), as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0086] The present [inventiondisclosure](#) provides nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 7. The present [inventiondisclosure](#) also provides nucleic acid molecules encoding any of the light chain amino acid sequences listed in Table 7.

[0087] The present [inventiondisclosure](#) includes anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecules wherein any of the aforementioned antigen-binding domains that specifically bind CD3 are combined, connected or otherwise associated with any of the aforementioned antigen-binding domains that specifically bind Fc ϵ R1 α to form a bispecific antigen-binding molecule that binds CD3 and Fc ϵ R1 α .

[0088] The present [inventiondisclosure](#) includes anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecules having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fructose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0089] In another [aspectexample](#), the [inventiondisclosure](#) provides a pharmaceutical composition comprising an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule as disclosed herein and a pharmaceutically acceptable carrier. In a related [aspectexample](#), the [inventiondisclosure](#) features a composition which is a combination of an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule and a second therapeutic agent. In one [embodimentexample](#), the second therapeutic agent is any agent that is advantageously combined with an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule. Exemplary agents that may be advantageously combined with an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule are discussed in detail elsewhere herein.

[0090] In yet another [aspectexample](#), the [inventiondisclosure](#) provides therapeutic methods for targeting/ablating cells expressing Fc ϵ R1 α using an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule of the [inventiondisclosure](#), wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule of the [inventiondisclosure](#) to a subject in need thereof. The antibody or fragment thereof may be administered sub-cutaneously, intravenously, intradermally, intraperitoneally, orally or intramuscularly. In certain [embodimentexamples](#), an antibody of the [inventiondisclosure](#) is administered at a dose of about 0.001 mg/kg body weight to about 200 mg/kg body weight of

the subject. In certain [embodiment examples](#), an antibody of the [invention disclosure](#) is administered at a dose comprising between 1 mg to 2500 mg of the antibody to a subject in need thereof.

[0091] The present [invention disclosure](#) also includes the use of an anti-CD3/anti-Fc ϵ R1 α bispecific antigen-binding molecule of the [invention disclosure](#) in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by Fc ϵ R1 α -expressing cells.

[0092] Other [embodiment examples](#) will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0093] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0094] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

[0095] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[0096] The expression "CD3," as used herein, refers to an antigen which is expressed on T cells as part of the multimolecular T cell receptor (TCR) and which consists of a homodimer or heterodimer formed from the association of two of four receptor chains: CD3-epsilon, CD3-delta, CD3-zeta, and CD3-gamma. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "CD3" means human CD3 unless specified as being from a non-human species, e.g., "mouse CD3," "monkey CD3," etc. Human CD3-epsilon comprises the amino acid sequence set forth as SEQ ID NO: 59; human CD3-delta comprises the amino acid sequence set forth as SEQ ID NO: 60; CD3-zeta comprises the amino acid sequence set forth as SEQ ID NO: 61; and CD3-gamma comprises the amino acid sequence set forth as SEQ ID NO: 62.

Claims

What is claimed is:

- ~~1. — An isolated antibody or antigen-binding fragment thereof that binds human FcεR1α or binds cynomolgus FcεR1α with a binding dissociation equilibrium constant (KD) value of less than about 470 nM as measured in a surface plasmon resonance assay at 25°C.~~
- ~~2. — An isolated antibody or antigen-binding fragment thereof that binds human FcεR1α with a dissociative half-life (t_{1/2}) of greater than about 0.54 minutes or binds cynomolgus FcεR1α with a dissociative half-life (t_{1/2}) of greater than about 0.6 minutes as measured in a surface plasmon resonance assay at 25°C.~~
- ~~3. — The antibody or antigen-binding fragment of any one of claims 1 to 2, wherein the antibody or antigen-binding fragment thereof competes for binding to human FcεR1α with a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in Table 1.~~
- ~~4. — The antibody or antigen-binding fragment of claim 3, wherein the reference antibody comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26; and 18/26.~~
- ~~5. — The antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair as set forth in Table 1.~~
- ~~6. — The antibody or antigen-binding fragment of claim 5, wherein the antibody or antigen-binding fragment thereof binds to the same epitope on human FcεR1α as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/26; 10/26; and 18/26.~~
- ~~7. — An isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: (a) the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having an amino acid sequence as set forth in Table 1; and (b) the CDRs of a light chain variable region (LCVR) having an amino acid sequence as set forth in Table 1.~~

~~8. The isolated antibody or antigen-binding fragment of claim 7, wherein the antibody or antigen-binding fragment comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/26, 10/26; and 18/26.~~

~~9. The isolated antibody or antigen-binding fragment of claim 8, wherein the antibody or antigen-binding fragment comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains, respectively, selected from the group consisting of: SEQ ID NOs: 4-6-8-28-30-32; 12-14-16-28-30-32; and 20-22-24-28-30-32.~~

~~10. An isolated antibody or antigen-binding fragment thereof that binds human FcεR1α, wherein the antibody or antigen-binding fragment comprises: (a) a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10 and 18; and (b) a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NOs: 26.~~

~~11. The isolated antibody or antigen-binding fragment of claim 10, wherein the antibody or antigen-binding fragment comprises an HCVR/LCVR amino acid sequence pair selected from the group consisting of: SEQ ID NOs: 2/26; 10/26; and 18/26.~~

~~121.~~ A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3_ε and a second antigen-binding domain that specifically binds human and/or cynomolgus FcεR1α, wherein:

the first antigen-binding domain comprises a heavy chain complementarity determining region (HCDR)1 having the amino acid sequence of SEQ ID NO:44, an HCDR2 having the amino acid sequence of SEQ ID NO:46, an HCDR3 having the amino acid sequence of SEQ ID NO:48, a light chain complementarity determining region (LCDR)1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; and

the second antigen-binding domain comprises:

(i) an HCDR1 having the amino acid sequence of SEQ ID NO:4, an HCDR2 having the amino acid sequence of SEQ ID NO:6, an HCDR3 having the amino acid sequence of SEQ ID NO:8, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or

(ii) an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32; or

(iii) an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32is derived from the antibody or antigen-binding fragment of any one of claims 1-11.

~~13. — A bispecific antigen-binding molecule comprising a first antigen-binding domain that specifically binds human CD3, and a second antigen-binding domain that binds human and/or cynomolgus FcεR1α.~~

2. — A bispecific antibody comprising a first binding arm that binds human CD3 and a second binding arm that binds human FcεR1α, wherein the first binding arm comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 and a light chain comprising the amino acid sequence of SEQ ID NO: 40, and wherein the second binding arm comprises a heavy chain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 52, and 54, and a light chain comprising the amino acid sequence of SEQ ID NO: 40.

~~14. — The bispecific antigen-binding molecule of claim 12 or claim 13, wherein the antigen-binding molecule binds both human CD3 and human FcεR1α and induces T cell-mediated cell killing of FcεR1α-expressing cells with an EC50 value of less than about 20 nM.~~

~~15. — The bispecific antigen-binding molecule of claim 12 or claim 13, wherein the antigen-binding molecule inhibits allergic reaction in a subject expressing human FcεR1α.~~

~~16. — The bispecific antigen-binding molecule of any one of claims 12-15, wherein the antigen-binding molecule activates CD3 signaling with an EC50 value of less than about 4.9 nM.~~

~~17. — The bispecific antigen-binding molecule of any one of claims 12-16, wherein the second antigen-binding domain specifically binds human FcεR1α or binds cynomolgus FcεR1α with a KD value of less than about 470 nM, as measured in an in vitro surface plasmon resonance binding assay at 25 °C.~~

~~18. The bispecific antigen-binding molecule of any one of claims 12-17, wherein the second antigen-binding domain binds human FcεR1α with an KD value of less than about 300 nM, less than about 200 nM, less than about 150 nM, or less than about 100 nM, as measured in an in vitro surface plasmon resonance binding assay at 25 °C.~~

~~19. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the second antigen-binding domain that specifically binds human FcεR1α comprises the heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 10, and 18; and the light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.~~

~~203. The bispecific antigen-binding molecule of claim 1 ~~any one of claims 12-19~~, wherein the second antigen-binding domain comprises an ~~that binds human FcεR1α~~ ~~comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises~~ anhaving the amino acid sequence ~~selected from the group consisting of~~ SEQ ID NOs: 4, ~~12, and 20~~; an HCDR2 having the ~~comprises an~~ amino acid sequence ~~selected from the group consisting of~~ SEQ ID NOs: 6, ~~14, and 22~~; an HCDR3 ~~comprises anhaving the~~ amino acid sequence ~~selected from the group consisting of~~ SEQ ID NOs: 8, ~~16, and 24~~; an LCDR1 ~~comprises~~ having the an amino acid sequence of SEQ ID NO: 28, an; LCDR2 ~~comprises anhaving the~~ amino acid sequence of SEQ ID NO: 30; and an LCDR3 ~~comprises anhaving the~~ amino acid sequence of SEQ ID NOs: 32.~~

4. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises an HCDR1 having the amino acid sequence of SEQ ID NO:12, an HCDR2 having the amino acid sequence of SEQ ID NO:14, an HCDR3 having the amino acid sequence of SEQ ID NO:16, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

5. The bispecific antigen-binding molecule of claim 1, wherein the second antigen-binding domain comprises an HCDR1 having the amino acid sequence of SEQ ID NO:20, an HCDR2 having the amino acid sequence of SEQ ID NO:22, an HCDR3 having the amino acid sequence of SEQ ID NO:24, an LCDR1 having the amino acid sequence of SEQ ID NO:28, an

LCDR2 having the amino acid sequence of SEQ ID NO:30, and an LCDR3 having the amino acid sequence of SEQ ID NO:32.

~~21. The bispecific antigen-binding molecule of claim 20, wherein the second antigen-binding domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 domains selected from the group consisting of SEQ ID NOs: 4-6-8-28-30-32, 12-14-16-28-30-32, and 20-22-24-28-30-32.~~

~~226. The bispecific antigen-binding molecule of any one of claims 1-21, wherein the second antigen-binding domain that specifically binds human FcγR1α comprises: (a) a heavy chain variable region (HCVR) having the amino acid sequence of and light chain CDRs of an HCVR/LCVR pair comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs: 2/26; SEQ ID NO: 10 or SEQ ID NO: 26; and 18; and (b) a light chain variable region (LCVR) having an amino acid sequence of SEQ ID NO: 26/26.~~

~~7. The bispecific antigen-binding molecule of claim 6, wherein the second antigen-binding domain comprises the HCVR/LCVR amino acid sequence pair of: SEQ ID NOs: 18/26.~~

~~23. The bispecific antigen-binding molecule of any one of claims 12-22, wherein the first antigen-binding domain that specifically binds human CD3 comprises heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) from a heavy chain variable region (HCVR) comprising an amino acid sequence of SEQ ID NO: 42, and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) from a light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 26.~~

~~24. The bispecific antigen-binding molecule of any one of claims 12-23, wherein the first antigen-binding domain that specifically binds human CD3 comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein HCDR1 comprises an amino acid sequence of SEQ ID NO: 44; HCDR2 comprises an amino acid sequence of SEQ ID NO: 46; HCDR3 comprises an amino acid sequence of SEQ ID NO: 48; LCDR1 comprises an amino acid sequence of SEQ ID NO:28; LCDR2 comprises an amino acid sequence of SEQ ID NO:30; and LCDR3 comprises an amino acid sequence of SEQ ID NO:32.~~

~~25. The bispecific antigen-binding molecule of any one of claims 12-24, wherein the first antigen-binding domain that specifically binds human CD3 comprises the heavy and light chain CDRs of an HCVR/LCVR amino acid sequence pair of SEQ ID NO: 42/26.~~

~~26. — An isolated bispecific antigen-binding molecule comprising: (a) a first antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 44, 46 and 48 and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32; and (b) a second antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 4, 6, and 8, and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32.~~

~~27. — An isolated bispecific antigen-binding molecule comprising: (a) a first antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 44, 46 and 48 and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the first antigen-binding domain binds human CD3; and (b) a second antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 12, 14 and 16, and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the second antigen-binding domain binds human FcεR1α.~~

~~28. — An isolated bispecific antigen-binding molecule comprising: (a) a first antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 44, 46 and 48 and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the first antigen-binding domain binds human CD3; and (b) a second antigen-binding domain that comprises HCDR1, HCDR2 and HCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 20, 22 and 24, and LCDR1, LCDR2 and LCDR3 domains, respectively, comprising the amino acid sequences of SEQ ID NOs: 28, 30 and 32, wherein the second antigen-binding domain binds human FcεR1α.~~

~~29. — An isolated bispecific antigen-binding molecule that competes for binding to FcεR1α, or binds to the same epitope on FcεR1α as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR sequence pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR sequence pair comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 2/26, 10/26 and 18/26.~~

~~30. — An isolated bispecific antigen-binding molecule that competes for binding to human CD3, or binds to the same epitope on human CD3 as a reference antibody, wherein the reference antibody comprises a first antigen-binding domain comprising an HCVR/LCVR sequence pair comprising the amino acid sequences of SEQ ID NOs: 42/26, and a second antigen-binding domain comprising an HCVR/LCVR sequence pair comprising the amino acid sequences selected from the group consisting of SEQ ID NOs: 2/26, 10/26 and 18/26..~~

~~318.~~ The isolated antibody or antigen-binding fragment thereof of any one of claims ~~1-11~~ or the bispecific antigen-binding molecule of ~~any one of claims 12-30~~claim 1, wherein the antibody or antigen-binding fragment thereof or the bispecific antigen binding molecule binds to FcεR1α expressed on a cell surface in the presence of immunoglobulin E (IgE).

~~932.~~ The bispecific antigen-binding molecule of claim 1~~any one of claims 12-31~~ that is a bispecific antibody.

~~1033.~~ The bispecific antigen-binding molecule of claim ~~932~~, wherein the bispecific antibody comprises a human IgG heavy chain constant region.

~~1134.~~ The bispecific antigen-binding molecule of claim ~~1033~~, wherein the human IgG heavy chain constant region is isotype IgG4.

~~1235.~~ The bispecific antigen-binding molecule of claim ~~1033~~, wherein the human IgG heavy chain constant region is isotype IgG1.

~~36. — The bispecific antigen-binding molecule of any one of claims 32-34, wherein the bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 56, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 50, and a light chain comprising an amino acid sequence of SEQ ID NO: 40.~~

~~3713.~~ The bispecific antigen-binding molecule of ~~any one of claims 32-349~~, wherein the bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 56, a second heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, NO: 52, and 54, and a light chain comprising an amino acid sequence of SEQ ID NO: 40.

~~38. The bispecific antigen-binding molecule of any one of claims 32-34, wherein the bispecific antibody comprises a first heavy chain comprising an amino acid sequence of SEQ ID NO: 56, a second heavy chain comprising an amino acid sequence of SEQ ID NO: 54, and a light chain comprising an amino acid sequence of SEQ ID NO: 40.~~

~~3914. A nucleic acid molecule encoding the antibody, or antigen-binding fragment thereof, of any one of claims 1-11, or the bispecific antigen-binding molecule of any one of claims 12-38.~~

~~1540. A vector comprising the nucleic acid molecule of claim 1439.~~

~~1641. A host cell comprising the vector of claim 1540.~~

~~1742. A pharmaceutical composition comprising the bispecific antibody or antigen-binding molecule fragment thereof of claim any one of claims 1-11 or the bispecific antigen-binding molecule of any one of claims 12-38 and a pharmaceutically acceptable carrier or diluent.~~

~~43. A method of producing an antibody or antigen-binding fragment thereof, or a bispecific antigen-binding molecule, the method comprising culturing a host cell that expresses the antibody, or antigen-binding fragment thereof, of any one of claims 1-11, or the bispecific antigen-binding molecule of any one of claims 12-38 under conditions permitting production of the antibody, or antigen-binding fragment thereof, or bispecific antigen-binding molecule.~~

~~44. A method for treating a disease or disorder associated with FcεR1α expression and/or signaling in a subject, the method comprising administering to the subject the pharmaceutical composition of claim 42.~~

~~45. The method of claim 44, wherein the disease or disorder associated with FcεR1α expression and/or signaling is an allergy, a mast cell activation disorder, or mastocytosis.~~

~~46. The method of claim 45, wherein the allergy is selected from the group consisting of allergic asthma, hay fever, atopic dermatitis, chronic urticaria, food allergy, and pollen allergy.~~

~~47. The method of claim 45 or claim 46, wherein the allergy is an anaphylactic allergy.~~

~~48. The method of any one of claims 44-47, further comprising administering to the subject a second therapeutic agent.~~

49. — ~~The method of claim 48, wherein the second therapeutic agent is selected from the group consisting of an IgE antagonist, an anti-histamine, an anti-inflammatory agent, a corticosteroid, a leukotriene antagonist, a mast cell inhibitor, a bronchial dilator, a decongestant, epinephrine, an IL-4 inhibitor, an IL-4 receptor inhibitor, an IL-33 antagonist, an IL-25 antagonist, a plasma cell ablating agent, and a TSLP antagonist.~~

50. — ~~Use of the pharmaceutical composition of claim 42 in the treatment of a disease or disorder associated with FcεR1α expression and/or signaling.~~

51. — ~~The use of claim 50, wherein the disease or disorder is an allergy, a mast cell activation disorder, or mastocytosis.~~