[0005] Conventional methods for detecting CAR expression, such as anti-CD70 CAR expression (e.g., using soluble human CD70-Fc fusion protein) can lack specificity, have batch-to-batch variability, produce low intensity signals in analytical methods (e.g., flow cytometry), or be difficult to make in sufficient quantities, which can underestimate the true number of CAR expressing cells.

[0006] Thus, there is a need for robust reagents to accurately detect anti-CD70 CAR expression on engineered T cells or in *in vitro* assays. Provided herein are methods and compositions addressing this and other needs.

SUMMARY

[0007] Described herein are agents that specifically bind to antibodies, including antibody fragments such as single chain variable fragments (scFvs), and chimeric molecules containing the same, such as chimeric antigen receptors. Also among the examples described herein are uses and methods of using such agents, including for detection, use, manipulation, and/or stimulation of cells or therapies containing or suspected of containing the antibody or chimeric molecule, such as in the detection, stimulation or use of CAR-expressing cells.

[0008] In some examples, the antibody is or includes an anti-idiotype (anti-id) antibody or antigen-binding fragment thereof that specifically binds to a target antibody that is or contains variable region(s) of the antibody designated 4F11, and/or specifically binds to a chimeric molecule containing such an antibody fragment, such as a CAR with a binding domain containing antibody variable regions or portions thereof derived from 4F11, such as in the form of an scFv.

[0009] In some examples, the anti-idiotype antibody is a non-blocking anti-idiotype antibody, *e.g.*, the binding of the anti-idiotype antibody to the target antibody, *e.g.*, an anti-CD70 antibody, does not block the binding of the target antibody to the target antigen, *e.g.*, CD70.

[0010] In some examples, the anti-idiotype antibody is a blocking anti-idiotype antibody, *e.g.*, the binding of the anti-idiotype antibody to the target antibody, *e.g.*, an anti-CD70 antibody, blocks or reduces the binding of the target antibody to the target antigen, *e.g.*, CD70

[0010a] In one aspect, there is described an isolated antibody that specifically binds a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, said anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35, or said molecule comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, wherein the isolated antibody comprises (a) a heavy chain variable (VH) region comprising an amino acid sequence of SEQ ID NO: 2, and a light chain variable (VL) region comprising an amino acid sequence of SEQ ID NO: 4; or (b) a VH region comprising an amino acid sequence of SEQ ID NO: 21, and a VL region comprising an amino acid sequence of SEQ ID NO: 23.

[0010b] In another aspect, there is described an isolated antibody that specifically binds a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, said anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35, or said molecule comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, wherein the isolated antibody comprises: (a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, 7 or 8, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9 or 10, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 11, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 12, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 13, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14; or (b) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 25, 26 or 27, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 28 or 29, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 30, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 31, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 32, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 33.

[0011] In some examples, the anti-CD70 scFv derived from 4F11 comprises an amino acid sequence that is at least 80%, at least 90%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 35. In some examples, the isolated antibody does not bind to a framework region of 4F11. In some examples, the isolated antibody binds to an anti-CD70 scFv derived from 4F11 with a dissociation constant K_D of no more than 10 nM, no more than 5 nM, no more than 1 nM, no more than 100 pM, no more than 90 pM, no more than 80 pM, no more than 70 pM, no more than 60 pM,

no more than 50 pM, no more than 40 pM, no more than 30 pM, or no more than 20 pM, as determined by a Biacore assay at 25°C. In various examples an antigen binding molecule is selected from the group consisting of an antibody, an scFv, a Fab, a Fab', a Fv, a $F(ab')_2$, a dAb, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, an IgE antibody, an IgD antibody, an IgM antibody, an IgG1 antibody, an IgG1 antibody having at least one mutation in the hinge region, an IgG2 antibody an IgG2 antibody having at least one mutation in the hinge region, an IgG3 antibody, an IgG3 antibody having at least one mutation in the hinge region, an IgG4 antibody, an IgG4 antibody having at least one mutation in the hinge region, an antibody comprising at least one non-naturally occurring amino acid, and any combination thereof.

[0012] In further examples, an isolated anti-idiotypic antibody comprises a heavy chain (HC), and in specific examples the HC comprises a heavy chain variable region (VH) amino acid sequence of SEQ ID NO: 2. In further specific examples of an antibody described herein comprises a heavy chain CDR1 selected from the group consisting of SEQ ID NOs: 6, 7, and 8. In additional specific examples of an antibody described herein comprises a heavy chain CDR2 selected from the group consisting of SEQ ID NOs: 9 and 10. In yet other examples of an antibody described herein comprises a heavy chain CDR3 selected from the group consisting of SEQ ID NO: 11. In still further examples, a heavy chain of an antibody described herein comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, each CDR comprising an amino acid sequence shown in Table 1c.

[0013] In some examples, an anti-idiotypic antibody comprises a VH amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at

least about 98%, at least about 99%, or about 100% identical to a VH of an antigen binding molecule described herein.

[0014] In some examples, an isolated anti-idiotypic antibody described herein comprises a light chain (LC), and in various examples a LC comprises a light chain variable region (VL) sequence of SEQ ID NO: 4. In some examples a light chain variable region (VL) of an antibody described herein comprises one or more of (a) a CDR1, (b) a CDR2, and (c) a CDR3. In further specific examples, a light chain CDR1 of an antibody described herein can comprise SEQ ID NO: 12. In other examples, a light chain CDR2 of an antibody described herein can comprise SEQ ID NO: 13. In still further examples a light chain CDR3 of an antibody described herein can comprise SEQ ID NO: 14. In still further examples, a light chain of an antibody described herein comprises a light chain CDR1, a light chain CDR2 and a light chain CDR3, each CDR comprising an amino acid sequence in Table 1d.

[0015] In some examples, a VL amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VL of an antigen binding molecule are described herein.

[0016] In some examples, an anti-idiotypic antibody described herein comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 2; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 4. In another embodiment, an antibody described herein comprises (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO:6, 7 or 8; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 9 or 10; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 11; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 12; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 14.

[0017] In further examples, an isolated anti-idiotypic antibody comprises a heavy chain (HC), and in specific examples the HC comprises a heavy chain variable region (VH) amino acid sequence of SEQ ID NO: 21. In further specific examples of an antibody described herein comprises a heavy chain CDR1 selected from the group consisting of SEQ ID NOs: 25, 26, and 27. In additional specific examples of an

antibody described herein comprises a heavy chain CDR2 selected from the group consisting of SEQ ID NOs: 28 and 29. In yet other examples of an antibody described herein comprises a heavy chain CDR3 selected from the group consisting of SEQ ID NO: 30. In still further examples, a heavy chain of an antibody described herein comprises a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3, each CDR comprising an amino acid sequence shown in Table 1c.

[0018] In some examples, an anti-idiotypic antibody comprises a VH amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VH of an antigen binding molecule described herein.

[0019] In some examples, an isolated anti-idiotypic antibody described herein comprises a light chain (LC), and in various examples a LC comprises a light chain variable region (VL) sequence of SEQ ID NO: 23. In some examples a light chain variable region (VL) of an antibody described herein comprises one or more of (a) a CDR1, (b) a CDR2, and (c) a CDR3. In further specific examples, a light chain CDR1 of an antibody described herein can comprise SEQ ID NO: 31. In other examples, a light chain CDR2 of an antibody described herein can comprise SEQ ID NO: 32. In still further examples a light chain CDR3 of an antibody described herein can comprise SEQ ID NO: 33. In still further examples, a light chain of an antibody described herein comprises a light chain CDR1, a light chain CDR2 and a light chain CDR3, each CDR comprising an amino acid sequence in Table 1d.

[0020] In some examples, a VL amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VL of an antigen binding molecule are described herein.

[0021] In some examples, an anti-idiotypic antibody described herein comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 21; and (b) a VL comprising the amino acid sequence of SEQ ID NO: 23. In another example, an antibody described herein comprises (a) a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 25, 26 or 27; (b) a VH CDR2 comprising the amino acid sequence of SEQ ID

NO: 28 or 29; (c) a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 30; (d) a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 31; (e) a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 32; and (f) a VL CDR3 comprising the amino acid sequence of SEQ ID NO: 33.

[0022] In some examples, an anti-idiotypic antibody comprises a VH amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VH of an antigen binding molecule described herein.

[0023] In some examples, a VL amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VL of an antigen binding molecule are described herein.

[0024] In some examples, an anti-idiotypic antibody comprises a VH amino acid sequence that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a VH of an antigen binding molecule described herein.

[0025] In some of any such examples, the target antibody or antigen-binding fragment is a single chain fragment. In some examples, the fragment contains antibody variable regions joined by a flexible linker. In some of any such examples, the fragment contains an scFv.

[0026] In some of any such examples, the anti-idiotype antibody or antigen-binding fragment specifically binds to the same or an overlapping epitope of a target antibody or antigen-binding fragment thereof as the epitope specifically bound by the anti-id antibody or antigen-binding fragment according to any one of the examples described herein.

[0027] In some of any such examples, the target antibody or antigen-binding fragment is within or including in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor; and/or the anti-id antibody or antigen-binding fragment

specifically binds the target antibody or antigen-binding fragment contained within or included in the antigen-binding domain of the extracellular portion of a CAR. In some examples, the target antibody or antigen-binding fragment is an scFv and the anti-id antibody or antigen-binding fragment specifically binds to an epitope in the scFv of the CAR.

[0028] In some of any such examples, the antibody or fragment specifically binds to a scFv derived from antibody 4F11 contained in the extracellular portion of a chimeric antigen receptor, optionally wherein the scFv derived from antibody 4F11 contains a heavy chain variable region set forth in SEQ ID NO: 15 and/or a light chain variable region set forth in SEQ ID NO: 16 and/or contains the sequence of amino acids set forth in SEQ ID NO: 35.

[0029] In some of any such examples, the anti-idiotype antibody or antigen-binding fragment specifically binds to an epitope within or including all or a portion of a complementarity determining region (CDR) of the target antibody or antigen-binding fragment.

[0030] In some of any such examples, the anti-idiotype antibody or fragment is an antagonist antibody of a CAR containing the target antibody or antigen-binding fragment. In some of any such examples, the antibody or fragment is an antagonist of a CAR containing the target antibody or antigen-binding fragment.

[0031] In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is humanized. In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is recombinant. In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is monoclonal.

[0032] In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is an antigen-binding fragment. In some examples, the antigen-binding fragment is selected from among fragment antigen binding (Fab) fragments, F(ab') 2 fragments, Fab' fragments, Fv fragments, a single chain variable fragment (scFv) or a single domain antibody.

[0033] In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof contains at least a portion of an immunoglobulin constant region. In

some examples, the at least a portion of an immunoglobulin constant region contains an Fc region or a portion of the Fc containing the CH2 and CH3 domains. In some examples, the constant region is derived from human IgG. In some of any such examples, the anti-idiotype antibody or antigen-binding fragment is an intact antibody or full-length antibody.

[0034] Described herein is a vector containing the nucleic acid molecule according to any one of the examples described herein. Also described herein is a cell containing the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described herein or the nucleic acid molecule according to any one of the examples described herein.

[0035] Described herein is a method of producing an anti-idiotype antibody or antigen-binding fragment thereof, including expressing the heavy and/or light chain encoded by the nucleic acid molecule according to any one of the examples described herein or the vector according to any one of the examples described herein in a suitable host cell and recovering or isolating the antibody. In some examples, the method of producing an anti-idiotype antibody or antigen-binding fragment includes culturing the cell according to any one of the examples described herein under conditions in which the heavy chain and/or light chain is expressed and recovering or isolating the antibody. Also described herein is an anti-idiotype antibody or antigen-binding fragment thereof produced by the method according to any one of the examples described herein.

[0036] Also described herein is a method of making an isolated antibody disclosed herein that specifically binds a molecule comprising an anti-CD70 scFv comprising a VH comprising an amino acid sequence of SEQ ID NO: 15 and a VL comprising an amino acid sequence of SEQ ID NO: 16 (e.g. an anti-CD70 scFv comprising the amino acid sequence of SEQ ID NO:35), an anti-CD70 scFv comprising the amino acid sequence of SEQ ID NO: 35 or an anti-CD70 chimeric antigen receptor ("CAR") comprising the amino acid sequence of SEQ ID NO: 1 ("4F11 CAR") or 34 ("4F11-RSRQR" or "4F11-QR3," "R" referring to a rituximab recognition epitope and "Q" referring to a CD34 epitope recognized by QBEND10), the method comprising growing or culturing a cell comprising a polynucleotide encoding such an isolated antibody under suitable conditions.

[0037] Also described are methods of detection using any of the described agents, such as the anti-idiotype antibodies. In some examples, described is a method of detecting a target antibody or antigen-binding fragment thereof, such as a CAR containing the same, including (a) contacting a composition containing a target antibody (such as one with variable regions derived from an antibody 4F11, or from an antigen-binding fragment of 4F11) with the anti-idiotype antibody or antigen-binding fragment thereof; and (b) detecting the anti-idiotype antibody bound to the target antibody or antigen-binding fragment and/or detecting the presence or absence of the target antibody or agent.

[0038] In some examples, the described methods involve detecting a CAR containing a target antibody or antigen-binding fragment thereof of any of the examples, such as a CAR containing variable domains derived from 4F11. In some examples, the methods include (a) contacting a cell expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof with the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described or the conjugate according to any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof; and (b) detecting cells bound with the anti-idiotype antibody. In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is directly or indirectly labeled for detection.

[0039] In some examples, described is a method of selecting cells from a cell population, including (a) contacting a cell population expressing a chimeric antigen receptor (CAR) containing a target antibody or a cell bound to a target antibody with the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described herein or conjugate according to any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof, wherein the target antibody is the antibody 4F11 or an antigen-binding fragment thereof; and (b) selecting cells bound with the anti-idiotype antibody.

[0040] In some instances, the cells bound with the anti-idiotype antibody are selected by affinity-based separation. In some examples, the affinity-based separation is

immunoaffinity-based separation. In some of any such examples, the affinity-based separation is by flow cytometry.

[0041] Also among the described methods are methods for stimulating cells using the agents, such as stimulating cells containing a molecule such as a CAR that is or contains the target antibody recognized by the anti-idiotype antibody. In some examples, the methods involve incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof with the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described or the conjugate of any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof, thereby generating an output composition containing stimulated cells.

[0042] In some examples, the methods result in proliferation, activation, stimulation, cytokine release, or other functional outcome such as upregulation of an activation marker or cytokine release or production, of cells expressing the chimeric receptor such as the CAR recognized by the anti-id antibody.

[0043] In some examples, the CAR contains a target antibody that specifically binds to CD70. In some examples, the target antibody is the antibody 4F11 or an antigen-binding fragment thereof. In some of any such examples, the anti-idiotype antibody or antigen-binding fragment thereof is the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof.

[0044] In some of any such examples, the target antibody or antigen-binding fragment contains a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 15 and/or a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 16.

[0045] In some examples, described is a method of purifying an antibody or antigen-binding fragment thereof, including (a) contacting a composition or sample containing a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof with the anti-idiotype antibody or antigen-binding fragment thereof according to any one of the examples described herein or conjugate according to any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-

binding fragment thereof; and (b) isolating complexes containing the anti-idiotype antibody. In some examples, the method includes (a) contacting a composition or sample containing a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof with the anti-idiotype antibody or antigen-binding fragment thereof of any one of the examples described or the conjugate of any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof; and (b) isolating complexes comprising the anti-idiotype antibody.

[0046] In some of any such examples, the antigen-binding fragment contains the variable heavy chain region and/or variable light chain region of the target antibody. In some examples, the antigen-binding fragment is a single chain fragment. In some examples, the antigen-binding fragment is an scFv. In some of any such examples, the antigen-binding fragment is within or included in the antigen-binding domain of the extracellular portion of a chimeric antigen receptor (CAR).

[0047] In some of any such examples, the target antibody binds to CD70. In some examples, the antigen-binding fragment of the target antibody is derived from antibody 4F11, optionally wherein the antigen-binding fragment of the target antibody contains a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 15 and/or a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 16. In some of any such examples, the antigen-binding fragment of the target antibody is a single chain variable fragment (scFv) derived from antibody 4F11, optionally wherein the scFv contains the sequence of amino acids set forth in SEQ ID NO: 35.

[0048] In some examples, there is described a method of depleting cells, comprising administering, to a subject, a composition comprising the anti-idiotype antibody or antigen-binding fragment thereof of according to any one of the examples described herein or conjugate according to any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) comprising a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof. In some examples, the method includes administering, to a subject, a composition comprising the anti-idiotype antibody or antigen-binding

fragment thereof of any one of the examples described herein or conjugate of any one of the examples described that specifically binds to a target antibody that is antibody 4F11 or an antigen-binding fragment thereof, wherein the subject has been administered a cell expressing a chimeric antigen receptor (CAR) containing a target antibody that is the antibody 4F11 or an antigen-binding fragment thereof. In some examples, the depletion occurs via antibody-dependent cell-mediated cytotoxicity (ADCC).

[0049] In various examples, an antibody described herein further comprises a detectable label, and a detectable label can be selected from the group consisting of a fluorescent label, a photochromic compound, a proteinaceous fluorescent label, a magnetic label, a radiolabel, and a hapten. When a fluorescent label is desired, the fluorescent label can be selected from the group consisting of an Atto dye, an Alexafluor dye, quantum dots, Hydroxycoumarin, Aminocouramin, Methoxycourmarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer Yellow, NBD, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, PerCP, TruRed, FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhocamine B, Texas Red, Allophycocyanin (APC), APC-Cy7 conjugates, Indo-1, Fluo-3, Fluo-4, DCFH, DHR, SNARF, GFP (Y66H mutation), GFP (Y66F mutation), EBFP, EBFP2, Azurite, GFPuv, T-Sapphire, Cerulean, mCFP, mTurquoise2, ECFP, CyPet, GFP (Y66W mutation), mKeima-Red, TagCFP, AmCyan1, mTFP1, GFP (S65A mutation), Midorishi Cyan, Wild Type GFP, GFP (S65C mutation), TurboGFP, TagGFP, GFP (S65L mutation), Emerald, GFP (S65T mutation), EGFP, Azami Green, ZsGreen1, TagYFP, EYFP,Topaz, Venus, mCitrine, YPet, TurboYFP, ZsYellow1, Kusabira Orange, mOrange, Allophycocyanin (APC), mKO, TurboRFP, tdTomato, TagRFP, DsRed monomer, DsRed2 (“RFP”), mStrawberry, TurboFP602, AsRed2, mRFP1, J-Red, R-phycoerythrin (RPE), B-phycoeryhring (BPE), mCherry, HcRed1, Katusha, P3, Peridinin Chlorophyll (PerCP), mKate (TagFP635), TurboFP635, mPlum, and mRaspberry. In specific examples, a fluorescent label can be R-Phycoerythrin (PE) or Allophycocyanin (APC).

[0050] Also described herein is a composition comprising an antibody described herein and optionally a pharmaceutically acceptable carrier or vehicle.

[0051] Described herein are polynucleotides encoding the heavy chain of an isolated antibody of an antibody described herein. Further, polynucleotides encoding the light chain of an isolated antibody of an antibody described herein are also described. In one aspect, described herein is a polynucleotide encoding the isolated antibody described herein. Vectors comprising a polynucleotide encoding the heavy chain of an isolated antibody of an antibody described herein, and encoding the light chain of an isolated antibody of an antibody described herein are also described. In another aspect, described herein is a vector comprising the polynucleotide described herein. Cells comprising such vectors are also described, and in various examples, a cell comprises a cell selected from the group consisting of a CHO cell, a Sp2/0 cell, a rabbit cell and an *E. coli* cell. In yet another aspect, described herein is a cell comprising the polynucleotide described herein.

[0052] Methods of making an isolated antibody described herein are also described and can comprise incubating a cell described herein under suitable conditions.

[0053] A method of determining a number of cells expressing a 4F11 derived scFv is described and can comprise contacting a sample with an isolated antibody that specifically binds the 4F11 derived scFv conjugated to a detectable label and determining the number of cells expressing the 4F11 derived scFv in the sample. In some examples, the isolated antibody that specifically binds the 4F11 derived scFv is an antibody described herein or a humanized form thereof.

[0054] Also described is a method of determining a number of cells presenting a polypeptide comprising an anti-CD70 scFv derived from 4F11, wherein the method comprises: (a) providing a sample comprising cells known or suspected to be presenting a polypeptide comprising an anti-CD70 scFv derived from 4F11; (b) contacting the sample with the isolated antigen binding molecule described herein or a humanized form thereof under conditions that permit binding of the polypeptide and the antigen binding molecule; and (c) determining the number of cells presenting the polypeptide in the sample.

[0055] Described herein is a method of determining the presence or absence of a polypeptide comprising an anti-CD70 scFv derived from 4F11, wherein the method

comprises: (a) providing a sample known or suspected to comprise a polypeptide comprising an anti-CD70 scFv derived from 4F11; (b) contacting the sample with an isolated antigen binding molecule described herein or a humanized form thereof under conditions that permit binding of the polypeptide and the antigen binding molecule; and (c) detecting the presence or absence of a polypeptide:antigen binding molecule complex. In an example of the method, the sample is a formalin-fixed sample. In

another example, the 4F11 derived scFv is a component of a chimeric antigen receptor (CAR), and in further examples the cell expressing a 4F11 derived scFv CAR is an immune cell selected from the group consisting of CD8+ T cells, CD4+ T cells, tumor infiltrating lymphocytes (TILs), NK cells, TCR-expressing cells, dendritic cells, and NK-T cells. In some examples, the isolated antigen binding molecule is detectably labeled, and the detectable label can be selected from the group consisting of a fluorescent label, a photochromic compound, a proteinaceous fluorescent label, a magnetic label, a radiolabel, and a hapten. When the detectable label is a fluorescent label, the fluorescent label can be selected from the group consisting of an Atto dye, an Alexafluor dye, quantum dots, Hydroxycoumarin, Aminocouramin, Methoxycourmarin, Cascade Blue, Pacific Blue, Pacific Orange, Lucifer Yellow, NBD, R-Phycoerythrin (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, PerCP, TruRed, FluorX, Fluorescein, BODIPY-FL, Cy2, Cy3, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, TRITC, X-Rhodamine, Lissamine Rhocamine B, Texas Red, Allophycocyanin (APC), APC-Cy7 conjugates, Indo-1, Fluo-3, Fluo-4, DCFH, DHR, SNARF, GFP (Y66H mutation), GFP (Y66F mutation), EBFP, EBFP2, Azurite, GFPuv, T-Sapphire, Cerulean, mCFP, mTurquoise2, ECFP, CyPet, GFP (Y66W mutation), mKeima-Red, TagCFP, AmCyan1, mTFP1, GFP (S65A mutation), Midorishi Cyan, Wild Type GFP, GFP (S65C mutation), TurboGFP, TagGFP, GFP (S65L mutation), Emerald, GFP (S65T mutation), EGFP, Azami Green, ZsGreen1, TagYFP, EYFP, Topaz, Venus, mCitrine, YPet, TurboYFP, ZsYellow1, Kusabira Orange, mOrange, Allophycocyanin (APC), mKO, TurboRFP, tdTomato, TagRFP, DsRed monomer, DsRed2 ("RFP"), mStrawberry, TurboFP602, AsRed2, mRFP1, J-Red, R-phycoerythrin (RPE), B-phycoeryhring (BPE), mCherry, HcRed1, Katusha, P3, Peridinin Chlorophyll (PerCP), mKate (TagFP635), TurboFP635, mPlum, and mRaspberry. In a specific example, the fluorescent label is R-Phycoerythrin (PE) or Allophycocyanin (APC). In some examples, the cell expressing a 4F11 derived scFv CAR is an immune cell, in which the immune cell is a T cell, and which can be disposed *in vitro* or *in vivo*. In some examples, the T cell is disposed in blood, extracted tissue, tissue grown *ex vivo* or cell culture media. In one example, the T cell is an autologous T cell. In another example, the T cell is an allogenic T cell.

[0056] In further examples, described herein are methods of making and using the anti-idiotypic antibodies. In a related example, described herein are methods of quantifying or

determining a number of cells expressing an anti-CD70 antibody comprising amino acid sequences of SEQ ID NOs: 15 and 16, comprising contacting the cells with the anti-idiotypic antibodies disclosed herein and determining the number of cells expressing the anti-CD70 antibody in the cells.

[0057] In another example, the disclosure describes methods of determining a presence or absence of cells expressing an anti-CD70 antibody comprising amino acid sequences of SEQ ID NOs: 15 and/or 16, comprising contacting the cells with the isolated antibody disclosed herein and determining the presence or absence of cells expressing the anti-CD70 antibody in the cells. In yet another example, the instant disclosure provides methods of purifying an anti-CD70 antibody, or antigen-binding fragment thereof, wherein the anti-CD70 antibody comprises amino acid sequences of SEQ ID NOs: 15 and/or 16, the method comprising (a) contacting a sample comprising the anti-CD70 antibody with the anti-idiotypic antibodies disclosed herein to form a complex; and (b) separating or purifying the complex comprising the anti-CD70 antibody and the isolated antibody from the sample. In some examples, the anti-CD70 antibody is an scFv comprising an amino acid sequence of SEQ ID NO: 35. In some examples, the cells express an anti-CD70 chimeric antigen receptor (CAR) comprising an amino acid sequence of SEQ ID NO: 1 or of SEQ ID NO:34. In some examples, the cells are CAR T cells.

[0058] In one aspect, described herein methods of determining a presence or absence of cells expressing a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, the molecule comprising the amino acid sequence of SEQ ID NO: 1, 34, or 35, comprising contacting the cells with the isolated antibody described herein and determining the presence or absence of cells expressing the molecule comprising the anti-CD70 antibody. In another aspect, described herein are methods of selecting cells that express an anti-CD70 CAR comprising an amino acid sequence of SEQ ID NO: 1 or of SEQ ID NO:34, the method comprising contacting the cells with the anti-idiotypic antibodies described herein and selecting the cells bound with the anti-idiotypic antibody. In some examples, the anti-idiotypic antibodies further comprises a detectable label. In some examples, the cells bound with the anti-idiotypic antibodies are selected by affinity-based separation.

[0059] In one aspect, described herein are methods of purifying an anti-CD70 antibody, or antigen-binding fragment thereof, wherein the anti-CD70 antibody comprises the amino acid sequence of SEQ ID NO: 35, the method comprising the steps of: (a) contacting a sample comprising the anti-CD70 antibody with the isolated antibody described herein to form a complex; and (b) purifying the complex comprising the anti-CD70 antibody and the isolated antibody from the sample. In one example, described herein are methods for stimulating anti-CD70 CAR T cells comprising contacting the anti-CD70 CAR T cells with the antibodies described herein. In some examples, the antibodies are conjugated to beads or a solid surface. In certain examples, the anti-CD70 CAR T cells are present in a drug substance or drug product. In another aspect, described herein are methods of stimulating cells that express an anti-CD70 chimeric antigen receptor (anti-CD70 CAR T cells), the method comprising incubating the anti-CD70 CAR T cells with the isolated antibody described herein, wherein the anti-CD70 CAR T cells express an anti-CD70 antibody comprising amino acid sequences of SEQ ID NOs:15 and 16 or wherein the anti-CD70 CAR T cells express an anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35.

BRIEF DESCRIPTION OF THE DRAWINGS

WHAT IS CLAIMED IS

1. An isolated antibody that specifically binds a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, said anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35, or said molecule comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, wherein the isolated antibody comprises

(a) a heavy chain variable (VH) region comprising an amino acid sequence of SEQ ID NO: 2, and a light chain variable (VL) region comprising an amino acid sequence of SEQ ID NO: 4; or

(b) a VH region comprising an amino acid sequence of SEQ ID NO: 21, and a VL region comprising an amino acid sequence of SEQ ID NO: 23.

2. The isolated antibody of claim 1, wherein the isolated antibody comprises:

(a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, 7 or 8, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9 or 10, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 11, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 12, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 13, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14; or

(b) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 25, 26 or 27, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 28 or 29, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 30, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 31, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 32, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 33.

3. An isolated antibody that specifically binds a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, said anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35, or said molecule comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, wherein the isolated antibody comprises:

(a) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, 7 or 8, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 9 or 10, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 11, a light chain

CDR1 comprising an amino acid sequence of SEQ ID NO: 12, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 13, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 14; or

(b) a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO: 25, 26 or 27, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO: 28 or 29, a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 30, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 31, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 32, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO: 33.

4. The isolated antibody of claim 3, wherein (a) the heavy chain variable (VH) region comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 2, and the light chain variable (VL) region comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 4; or (b) the VH region comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 21, and the VL region comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 23.

5. The isolated antibody of claim 4, wherein (a) the VH region comprises the amino acid sequence of SEQ ID NO: 2, and the VL region comprises the amino acid sequence of SEQ ID NO: 4; or (b) the VH region comprises the amino acid sequence of SEQ ID NO: 21, and the VL region comprises the amino acid sequence of SEQ ID NO: 23.

6. The isolated antibody of any one of claims 1 to 5, wherein the isolated antibody further comprises a detectable label.

7. A polynucleotide encoding the isolated antibody of any one of claims 1 to 6.

8. A vector comprising the polynucleotide of claim 7.

9. A cell comprising the polynucleotide of claim 7.

10. A method of determining a presence or absence of cells expressing a molecule comprising an anti-CD70 antibody or an antigen-binding fragment thereof, the molecule comprising the amino acid sequence of SEQ ID NO: 1, 34, or 35, comprising contacting the cells with the isolated antibody of any one of claims 1 to 6 and determining the presence or absence of cells expressing the molecule comprising the anti-CD70 antibody.

11. The method of claim 10, further comprising determining a number of cells expressing the anti-CD70 antibody or antigen-binding fragment thereof, comprising contacting the cells with the isolated antibody of any one of claims 1 to 6 and determining the number of cells expressing the molecule comprising the anti-CD70 antibody.

12. The method of claim 10 or 11, wherein the anti-CD70 antibody is an scFv comprising the amino acid sequence of SEQ ID NO: 35.

13. The method of claim 10 or 11, wherein the cells express an anti-CD70 chimeric antigen receptor (CAR) comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, with or without a signal peptide.

14. A method of selecting cells that express an anti-CD70 CAR comprising an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 34, comprising

(a) contacting the cells with the isolated antibody of any one of claims 1 to 6; and

(b) selecting the cells bound with the antibody,

optionally wherein the isolated antibody further comprises a detectable label.

15. The method of claim 14, wherein the cells bound with the isolated antibody are selected by affinity-based separation or by flow cytometry.

16. A method of purifying an anti-CD70 antibody, or antigen-binding fragment thereof, wherein the anti-CD70 antibody comprises the amino acid sequence of SEQ ID NO: 35, the method comprising the steps of:

(a) contacting a sample comprising the anti-CD70 antibody with the isolated antibody of any one of claims 1 to 6 to form a complex; and

(b) purifying the complex comprising the anti-CD70 antibody and the isolated antibody from the sample.

17. A method of stimulating cells that express an anti-CD70 chimeric antigen receptor (anti-CD70 CAR T cells), the method comprising incubating the anti-CD70 CAR T cells with the isolated antibody of any one of claims 1 to 6, wherein the anti-CD70 CAR T cells express an anti-CD70 antibody comprising amino acid sequences of SEQ ID NOs:15 and 16 or wherein the anti-CD70 CAR T cells express an anti-CD70 antibody comprising the amino acid sequence of SEQ ID NO: 35.