

CLAIMS

- 1) A framework binding interface comprising a framework region of a chimeric murine-human antibody or functional fragment thereof that binds a mediotope having the sequence CQFDLSTRRLKC (SEQ ID NO:1), CQYNLSSRALKC (SEQ ID NO:2) or a variant thereof.
- 2) The framework binding interface of claim 1, wherein mediotope is cyclic.
- 3) The framework binding interface of claim 1, wherein mediotope is linear.
- 4) The framework binding interface of claim 1, wherein the binding interface is located between Fab light and Fab heavy chains of the chimeric murine-human antibody or functional fragment thereof.
- 5) The framework binding interface of claim 1, wherein the framework region is comprised of a Fab portion.
- 6) The framework binding interface of claim 1, wherein the antibody is cetuximab or functional fragment thereof.
- 7) A multivalent mediotope tethering entity comprising two or more mediotopes coupled using one of a long linker, a multivalent scaffold, a small chemical scaffold, a biotin-streptavidin, or an IgG Fc domain.
- 8) The multivalent mediotope tethering entity of claim 7, wherein the two or more mediotopes are selected from CQFDLSTRRLKC (SEQ ID NO:1), CQYNLSSRALKC (SEQ ID NO:2), or a variant thereof.
- 9) The multivalent mediotope tethering entity of claim 7, wherein the multivalent mediotope tethering entity is a bivalent, trivalent, or tetravalent mediotope tethering entity.
- 10) The multivalent mediotope tethering entity of claim 7, wherein the multivalent mediotope tethering entity is used to tether two or more mediotope-enabled therapeutic antibodies or functional fragments thereof.

- 11) The multivalent meditope tethering entity of claim 7, wherein the multivalent meditope tethering entity enhances binding stability and/or efficacy of a meditope-enabled monoclonal antibody or functional fragment thereof.
- 12) The multivalent meditope tethering entity of claim 11, wherein the meditope-enabled monoclonal antibody is a meditope-enabled therapeutic antibody selected from abagovomab, abciximab, adalimumab, adecatumumab, alemtuzumab, atilizumab, altumumab pentetate, anatumumab mafenatox, arcitumumab, basiliximab, bectumumab, benralizumab, capromab pendetide, catumaxomab, certolizumab, cetuximab, clivatuzumab tetraxetan, daclizumab, denosumab, eculizumab, efalizumab, etaracizumab, etrumaxomab, fanolesomab, FBT05, fontolizumab, gemtuzumab, girentuximab, golimumab, ibritumomab, igovomab, infliximab, ipilimumab, labetuzumab, mepolizumab, muromonab-CD3, natalizumab, nimotuzumab, ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, ranibizumab, rituximab, tiuzetan, tositumomab, trastuzumab, TRBS07, ustekinumab, visilizumab, votumumab, zalutumumab or functional fragment thereof.
- 13) The multivalent meditope tethering entity of claim 7, further comprising an imaging agent, therapeutic agent or both.
- 14) The multivalent meditope tethering entity of claim 13, wherein the imaging agent, therapeutic agent or both is a metal chelator bound to a metal ion.
- 15) A composition comprising a meditope associated with one or more of a therapeutic substance, imaging substance or a combination thereof.
- 16) The composition of claim 15, wherein the meditope comprises the sequence CQFDLSTRRLKC (SEQ ID NO:1) or CQYNLSSRALKC (SEQ ID NO:2).
- 17) The composition of claim 15, wherein the therapeutic substance is one or more of a small molecule, a chemotherapeutic agent, a therapeutic antibody or functional fragment, a toxin, a radioisotope, an enzyme, a nuclease, a hormone, an immunomodulator, an oligonucleotide, a nanoparticle, an RNAi molecule, a chelator, a boron compound, a photoactive agent, a dye or a combination thereof.

- 18) The composition of claim 15, wherein the imaging substance is one or more of a fluorescent or luminescent substance, an enzyme, an enhancing agent, a radioactive substance, or a nanoparticle.
- 19) The composition of claim 15, wherein the therapeutic substance is associated with a CovX-Body™.
- 20) A method for modifying a therapeutic human or humanized antibody comprising substituting one or more human framework residues located within a human framework region of central Fab cavity with corresponding one or more murine residues such that a mediotope having the sequence CQFDLSTRRLKC (SEQ ID NO:1), CQYNLSSRALKC (SEQ ID NO:2) or a variant thereof binds the one or more human framework residues.
- 21) The method of claim 20, wherein the one or more substitutions are framework residues at positions P8, V9, I10, S14, E17, Q38, R39, T40, N41, G42, S43, P44, D82, I83, A84, D85, Y86, Y87, G99, A100, G101, T102, K103, L104, E105, K107, R142, S162, V163, T164, E165, Q166, D167, S168, or Y173 of the light chain; or Q6, P9, R38, Q39, S40, P41, G42, K43, G44, L45, S87, D89, T90, A91, I92, Y93, Y94, W109, G110, Q111, G112, T113, L114, V115, T116, Y151, E154, P155, V156, T171, F172, P173, A174, V175, Y182, S183, or L184 of the heavy chain.
- 22) The method of claim 20, wherein the one or more substitutions are framework residues S9I, S10L, K39R, P40T, P40S, G41N, K42G, A43S, F83I, F83V, T85N, T85D, or Q100A of the light chain; or A36S or V89I of the heavy chain of cetuximab.
- 23) The method of claim 20, wherein the substitution allows binding of a multivalent mediotope tethering entity that enhances binding and/or therapeutic effect of a therapeutic human antibody or functional fragment thereof.
- 24) The method of claim 20, wherein binding to the framework involves at least one disulfide bond or at least one bond between an unnatural amino acid between the mediotope and the framework.

25) A meditope-enabled monoclonal antibody comprising a therapeutic antibody having a framework binding interface of a chimeric murine antibody or functional fragment thereof that binds a meditope having the sequence CQFDLSTRRLKC (SEQ ID NO:1), CQYNLSSRALKC (SEQ ID NO:2) or a variant thereof.

26) The meditope-enabled monoclonal antibody of claim 25, wherein the binding interface is located between a Fab light chain and a Fab heavy chain of the chimeric murine antibody or functional fragment thereof.

27) The meditope-enabled monoclonal antibody of claim 25, wherein the framework region is comprised of a Fab portion.

28) The meditope-enabled monoclonal antibody of claim 25, wherein the antibody is abagovomab, abciximab, adalimumab, adecatumumab, alemtuzumab, atlizumab, altumumab pentetate, anatumumab mafenatox, arcitumumab, basiliximab, bectumumab, benralizumab, capromab pendetide, catumaxomab, certolizumab, clivatuzumab tetraxetan, daclizumab, denosumab, eculizumab, efalizumab, etaracizumab, etrumaxomab, fanolesomab, FBT A05, fontolizumab, gemtuzumab, girentuximab, golimumab, ibritumomab, igovomab, infliximab, ipilimumab, labetuzumab, mepolizumab, muromonab-CD3, natalizumab, nimotuzumab, ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, ranibizumab, rituximab, tiuzetan, tositumomab, trastuzumab, TRBS07, ustekinumab, visilizumab, votumumab, zalutumumab or functional fragment thereof.

29) A method of purifying a monoclonal antibody or functional fragment thereof, comprising:

coupling a meditope or a meditope variant to a solid support;

contacting the coupled meditope or meditope variant with a solution containing or suspected of containing the monoclonal antibody or functional fragment thereof such that the monoclonal antibody or functional fragment thereof binds the meditope.

30) The method of claim 29, wherein the monoclonal antibody is a meditope-enabled therapeutic antibody selected from abagovomab, abciximab, adalimumab, adecatumumab, alemtuzumab, atlizumab, altumumab pentetate, anatumumab mafenatox, arcitumumab, basiliximab, bectumumab, benralizumab, capromab pendetide, catumaxomab, certolizumab, cetuximab, clivatuzumab tetraxetan, daclizumab, denosumab, eculizumab, efalizumab, etaracizumab, etrumaxomab, fanolesomab, FBT A05, fontolizumab, gemtuzumab, girentuximab, golimumab, ibritumomab, igovomab, infliximab, ipilimumab, labetuzumab, mepolizumab, muromonab-CD3, natalizumab, nimotuzumab, ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, ranibizumab, rituximab, tiuzetan, tositumomab, trastuzumab, TRBS07, ustekinumab, visilizumab, votumumab, zalutumumab or functional fragment thereof.

31) A method of treating, imaging or diagnosing a disease or condition comprising administering a therapeutically effective amount of a pharmaceutical composition to a subject, the pharmaceutical composition comprising an antibody-meditope complex; a multivalent tethering agent in combination with a monoclonal antibody or functional fragment thereof; or a combination thereof.

32) The method of claim 31, wherein the antibody-meditope complex; the multivalent tethering agent in combination with a monoclonal antibody or functional fragment thereof; or a combination thereof is conjugated to one or more diagnostic or imaging agents for diagnosing or imaging the disease or condition, one or more therapeutic agents for treating the disease, or a combination thereof.

33) The method of claim 32, wherein the therapeutic agent is one or more of a small molecule, a chemotherapeutic agent, a therapeutic antibody or functional fragment, a toxin, a radioisotope, an enzyme, a nuclease, a hormone, an immunomodulator, an oligonucleotide, a nanoparticle, an RNAi molecule, a chelator, a boron compound, a photoactive agent, a dye or a combination thereof.

34) The method of claim 32, wherein the diagnostic or imaging agent is one or more of a fluorescent or luminescent substance, an enzyme, an enhancing agent, a radioactive substance, or a nanoparticle.

35) The method of claim 31, wherein the antibody-meditope complex; the multivalent tethering agent in combination with a monoclonal antibody or functional fragment thereof; or a combination thereof enhances the treatment, diagnostic or imaging efficacy of a therapeutic antibody alone.

36) The method of claim 31, wherein the antibody-meditope complex comprises a mediotope and a mediotope-enabled monoclonal antibody, wherein the mediotope-enabled monoclonal antibody or functional fragment thereof is a therapeutic antibody selected from abagovomab, abciximab, adalimumab, adecatumumab, alemtuzumab, atlizumab, altumumab pentetate, anatumumab mafenatox, arcitumumab, basiliximab, bectumumab, benralizumab, capromab pendetide, catumaxomab, certolizumab, cetuximab, clivatuzumab tetraxetan, daclizumab, denosumab, eculizumab, efalizumab, etaracizumab, etrumaxomab, fanolesomab, FBT A05, fontolizumab, gemtuzumab, girentuximab, golimumab, ibritumomab, igovomab, infliximab, ipilimumab, labetuzumab, mepolizumab, muromonab-CD3, natalizumab, nimotuzumab, ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, ranibizumab, rituximab, tiuzetan, tositumomab, trastuzumab, TRBS07, ustekinumab, visilizumab, votumumab, zalutumumab or functional fragment thereof.

37) The method of claim 31, wherein the multivalent tethering agent is administered after administering a therapeutically effective amount of cetuximab to the subject or at the same time as administering a therapeutically effective amount of cetuximab to the subject.

38) A method of targeting cells overexpressing a cancer biomarker, comprising administering a therapeutic amount of a mediotope-enabled monoclonal antibody or a functional fragment thereof in combination with a mediotope, mediotope variant or a multivalent tethering agent to a population of cells overexpressing or suspected of overexpressing the biomarker.

39) The method of 38, wherein the cancer biomarker is EGFR and the meditope-enabled monoclonal antibody is cetuximab.

40) A method of altering binding affinity of a meditope variant, comprising modifying the meditope variant at one or more positions resulting in an increase or decrease in binding affinity of the meditope at a low pH level.

41) The method of claim 40, wherein the meditope variant has a decrease in binding affinity at a low pH level for lysosomal drug delivery.

42) The method of claim 40, wherein the meditope variant has an increase in binding affinity at a low pH and hypoxic environment for specifically binding tumor cells.

43) A method of screening for meditopes or small molecules with framework binding functionality similar to that of a meditope comprising:

contacting a library of putative meditopes or small molecules with a meditope-enabled antibody;

determining whether the putative meditopes or small molecules bind the meditope-enabled antibody at a framework binding interface;

identifying one or more candidate meditopes or small molecules with framework binding functionality similar to that of a meditope;

determining binding affinity of the one or more candidates; and

identifying one or more of the candidates as a meditope or small molecule with framework binding functionality similar to that of a meditope when the binding dissociation constant is at least 0.70 μM .

CLAIMS

1. A method of purifying a monoclonal antibody or antigen-binding fragment thereof, comprising
contacting a solution containing the monoclonal antibody or antigen-binding fragment thereof with a peptide coupled to a solid support, such that a mediotope binding interface of the monoclonal antibody or fragment thereof binds to the peptide, and isolating the antibody or fragment, thereby purifying the antibody or fragment,
wherein the monoclonal antibody is cetuximab and
wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 5, 6, 7, 8, 21, 22, 26, 32, and 33.
2. The method of claim 1, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 7 or 8.
3. The method of claim 1, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 6, and 22.
4. The method of claim 1, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 7, 8, 22, and 26.
5. The method of claim 1, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 21, 22, 26, 32, and 33.
6. The method of claim 1, wherein the peptide comprises the amino acid sequence of SEQ ID NO: 32.
7. A method of purifying a monoclonal antibody or antigen-binding fragment thereof, comprising:
contacting a solution containing the monoclonal antibody or antigen-binding fragment thereof with a peptide coupled to a solid support, such that a mediotope binding

interface of the monoclonal antibody or fragment thereof binds the peptide, and isolating the antibody or fragment, thereby purifying the antibody or fragment,

wherein the monoclonal antibody is cetuximab, and

wherein the peptide comprises the sequence of SEQ ID NO: 32.

8. A method of purifying a monoclonal antibody or antigen-binding fragment thereof, comprising

contacting a solution containing the monoclonal antibody or antigen-binding fragment thereof with a peptide coupled to a solid support, such that a mediotope binding interface of the monoclonal antibody or fragment thereof binds to the peptide, and isolating the antibody or fragment, thereby purifying the antibody or fragment,

wherein the monoclonal antibody is cetuximab, and

wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 6, 7, 8, 21, 22, 26, 32, and 33.