

bispecific formats collectively increase the diversity of the antibodies that can be applied to the development of therapeutics for various indications.

[0006] It is also desirable to generate useful antibodies that are conditionally active. For example, antibodies virtually inactive at a normal physiological condition and significantly more active at a condition other than the normal physiological condition (e.g., an aberrant condition), antibodies that are activated or inactivated in certain microenvironments (e.g., activated in a tumor microenvironment), or antibodies that are activated or inactivated over time. Besides temperature, other trigger conditions for which the antibodies can be evolved or optimized include pH, osmotic pressure, osmolality, oxidative stress, oxygen concentration and electrolyte concentration. Besides the activity, other desirable properties of antibodies that can be optimized during evolution include stability, half-life, chemical resistance, and proteolytic resistance.

[0007] Many strategies for evolving or engineering a parent antibody to mutant antibodies having a desired property have been published. However, engineering or evolving a parent antibody to be inactive or virtually inactive (less than 10% activity and especially less than 5% activity) at the normal physiological condition, while having activity at an aberrant condition that is equivalent or better than the original activity of the parent antibody at the normal physiological condition, requires that destabilizing mutation(s) co-exist with activity increasing mutations that do not counter the destabilizing effect. It is expected that destabilizing mutations would reduce the antibody's activity by an amount greater than is predicted by standard rules such as the Q10 rule. Therefore the ability to evolve proteins that work efficiently (more active) at a specific aberrant condition, e.g. a lower temperature or pH, than the evolved protein at a normal physiological condition, or even being substantially inactive at the normal physiological condition, creates a surprising new class of antibodies referred to as conditionally active proteins. For example, the conditionally active proteins may have a ratio of activity at the aberrant condition to the activity at the normal physiological condition greater than the same ratio for the parent protein. In some cases, the ratio for the conditionally active proteins may be at least about 2:1, or at least about 4:1, or at least about 6:1, or at least about 10:1, or at least about 20:1, or at least about 50:1, or at least about 80:1, or at least about 100:1.

[0008] The present invention provides a new class of multi-specific antibodies that are conditionally active for at least one epitope or antigen. This new class of multi-specific antibodies can take advantage of the flexibility and versatility of traditional multi-specific antibodies, and while at the same time directing the activity, affinity and/or avidity of the multi-specific antibodies to locations, tissues or organs of a subject where the activity is desired.

SUMMARY

[0009] In one example, the disclosure provides a multi-specific antibody comprising at least one binding site for a cell antigen; and at least one binding site for a tumor-reactive lymphocyte antigen. The multi-specific antibody binds to at least one of the cell antigen and tumor-reactive lymphocyte antigen with a greater activity, affinity and/or avidity at a first physiological condition than at a second physiological condition.

[0009a] In one aspect, the present disclosure provides a multi-specific IgG antibody comprising:
 at least one binding site for a cancer cell specific antigen; and
 at least one binding site for a tumor-reactive lymphocyte antigen,
 wherein said multi-specific antibody reversibly binds to at least one of the cancer cell specific antigen and tumor-reactive lymphocyte antigen with a greater affinity at a first aberrant physiological pH of from 5.2 to 6.8 of a tumor microenvironment than at a second normal physiological pH of from 7.0 to 7.8.

[0010] In the previous example, the cell antigen may be a cancer cell associated antigen, or the cancer cell associated antigen may be a neoantigen.

[0011] In any one of the previous examples, the cell antigen may be a senescent cell associated antigen.

[0012] In any one of the previous examples, the first physiological condition may be an aberrant condition and the second physiological condition may be a normal physiological condition.

[0013] In the previous example, the aberrant condition may be a condition in a tumor microenvironment or a condition in a senescent cell microenvironment.

[0014] In any of the previous examples, the binding of the multi-specific antibody to at least one of the cell antigen and tumor-reactive lymphocyte antigen may be reversible.

[0015] In any one of the previous examples, the multi-specific antibody may be configured in a format selected from: (1) bispecific antibody conjugates; (2) hybrid bispecific IgG2; (3) variable domain only bispecific antibody molecules; (4) CH1/CL fusion proteins; (5) Fab fusion proteins; (6) non-immunoglobulin fusion proteins; (7) Fc-modified IgGs; (8) appended and Fc-modified IgGs; (9) modified Fc and CH3 fusion proteins; (10) appended IgGs-HC fusions; (11) appended IgGs-LC fusions; (12) appended IgGs-HC&LC fusions; (13) Fc fusions; (14) CH3 fusions; (15) IgE/IgM CH2 fusions; (16) F(ab')₂ fusion; (17) CH1/CL fusion proteins; (18) modified IgGs; and (19) non-immunoglobulin fusions.

[0016] In any one of the previous examples, the multi-specific antibody may be conjugated to a macromolecule. The macromolecule may be selected from at least one of a protein, a fatty acid and a polymer, or the macromolecule may be an albumin or polyethylene glycol.

[0017] In any one of the previous examples, the at least one binding site for a cell antigen may be an IgG antibody or fragment thereof and the cell antigen may be a tumor cell antigen.

[0018] In any one of the previous examples, the at least one binding site for a tumor-reactive lymphocyte antigen may be a single chain antibody. The single chain antibody may be an scFv antibody. The scFv antibody may be attached to a C terminus of at least one light chain of said IgG antibody or fragment thereof via a linker. In any one of the previous examples wherein the antibody is an scFv antibody, the scFv antibody may comprise a light chain variable region selected from light chain variable regions having the amino acid sequences of SEQ ID NOS: 1-10 and a heavy chain variable region selected from heavy variable regions having the amino acid sequences of SEQ ID NOS: 11-15. In any one of the previous examples, the scFv antibody may have an amino acid sequence selected from the amino acid sequences of SEQ ID NOS: 26-71.

[0019] In any one of the previous examples, the IgG antibody may comprise a light chain variable region selected from light chain variable regions binding to an antigen selected from Ax1, Her2, B7-H3 and EpCAM having the amino acid sequences of SEQ ID NOS: 16-17, 20, 22, and 88-95; and a heavy chain variable region selected from heavy chain variable regions binding to same antigen having the amino acid sequences of SEQ ID NOS: 18-19, 21, 23-25, and 80-87.

[0020] In any one of the previous examples, the tumor-reactive lymphocyte antigen may be a CD3 antigen.

[0021] In any one of the previous examples, the tumor-reactive lymphocyte antigen may be on a lymphocyte selected from T cells, macrophages, Jurkat cells, monocytes, NK cells, neutrophils, eosinophils, basophils, and lymphokine-activated killer cells.

[0022] In any one of the previous examples, the cell antigen may be selected from Axl, EpCAM, Ror2, Her2, and B7-H3.

[0023] In any one of the previous examples, the multi-specific antibody may bind to the cell antigen with a greater affinity at the first physiological condition than at the second physiological condition.

[0024] In any one of the previous examples, the multi-specific antibody may bind to the tumor-reactive lymphocyte antigen with a greater affinity at the first physiological condition than at the second physiological condition.

[0025] In any one of the previous examples, the multi-specific antibody may bind to both the cell antigen and the tumor-reactive lymphocyte antigen with a greater affinity at the first physiological condition than at the second physiological condition.

[0026] In any one of the previous examples, the multi-specific antibody may bind to a combination of the cell antigen and the tumor-reactive lymphocyte antigen with a greater avidity at the first physiological condition than at the second physiological condition.

[0027] In another example, the disclosure provides a second example of multi-specific antibody comprising an IgG antibody or fragment thereof that binds to a first antigen; and at least one scFv antibody that binds to a second antigen that is different from the first antigen and the at least one scFv antibody is linked to a C terminus of at least one light chain of said IgG antibody or fragment, and said multi-specific antibody reversibly binds to at least one of the first antigen and the second antigen with a greater affinity and/or avidity at an aberrant condition than at a normal physiological condition.

[0028] In the second example, the second antigen may be a CD3 antigen.

[0029] In any one of the previous second examples, the scFv antibody may comprise a light chain variable region selected from light chain variable regions having the amino acid sequences of SEQ ID NOS: 1-10 and a heavy chain variable region selected from heavy variable regions having the amino acid sequences of SEQ ID NOS: 11-15. In any one of the previous examples, the scFv antibody may have an amino acid sequence selected from the amino acid sequences of SEQ ID NOS: 26-71.

[0030] In any one of the previous second examples, the IgG antibody or fragment may comprise a light chain variable region selected from light chain variable regions binding to an antigen selected from Axl, Her2, B7-H3 and EpCAM having the amino acid sequences of SEQ ID NOS: 16-17, 20, 22, and 88-95; and a heavy chain variable region selected from heavy chain variable regions

binding to same antigen having the amino acid sequences of SEQ ID NOS: 18-19, 21, 23-25, and 80-87.

[0031] In any one of the previous second examples, the multi-specific antibody may bind to the first antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0032] In any one of the previous second examples, the multi-specific antibody may bind to the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0033] In any one of the previous second examples, the multi-specific antibody may bind to both of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0034] In any one of the previous second examples, the multi-specific antibody may bind to a combination of the first and second antigens with a greater avidity at the first physiological condition than at the second physiological condition.

[0035] In any one of the previous second examples, the first antigen may be a cell surface antigen and such cell surface antigen may be cancer cell surface antigen. In any one of the previous second examples, the first antigen may be a neoantigen. In any one of the previous second examples, the first antigen may be selected from Ax1, EpCAM, Ror2, Her2, and B7-H3.

[0036] In yet another example, the disclosure provides a method for making a multi-specific antibody, comprising: a) obtaining an IgG antibody or fragment thereof that binds to a first antigen; b) linking at least one scFv antibody that binds to a second antigen to a C-terminus of at least one light chain of said IgG antibody or fragment to produce one or more constructs; c) screening the one or more constructs of b) for binding to at least one of the first antigen and the second antigen under an aberrant condition and a normal physiological condition; and d) selecting a multi-specific antibody from the one or more constructs that reversibly binds to at least one of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0036a] In another aspect, the present disclosure refers to a method for making a multi-specific antibody, comprising steps of:

- a) obtaining an IgG antibody or fragment thereof that binds to a first antigen;
- b) linking at least one scFv antibody that binds to a second antigen to a C-terminus of at least one light chain of said IgG antibody or fragment to produce one or more constructs;
- c) screening the one or more constructs of step b) for binding to at least one of the first antigen and the second antigen under an aberrant pH of from 5.2 to 6.8 of a tumor microenvironment and a normal physiological pH of from 7.0 to 7.8; and
- d) selecting a multi-specific antibody from the one or more constructs that reversibly binds to at least one of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0036b] In another aspect, the present disclosure refers to a conjugated multi-specific antibody comprising the multi-specific antibody as disclosed herein conjugated to a macromolecule selected from at least one of a protein, a fatty acid, a polymer, albumin and polyethylene glycol.

[0037] In the previous method, the first antigen may be a tumor cell antigen which may be selected from Ax1, EpCAM, Ror2, Her2, and B7-H3, or a neoantigen.

[0038] In any one of the previous methods, the second antigen may be a tumor-reactive lymphocyte antigen such as CD3. The tumor-reactive lymphocyte antigen may be on a lymphocyte selected from T cells, macrophages, Jurkat cells, monocytes, NK cells, neutrophils, eosinophils, basophils, and lymphokine-activated killer cells.

[0039] In any one of the previous methods, the scFv antibody may comprise a light chain variable region selected from light chain variable regions having the amino acid sequences of SEQ ID NOS: 1-10 and a heavy chain variable region selected from heavy variable regions having the amino acid sequences of SEQ ID NOS: 11-15. In any one of the previous examples, the scFv antibody may have an amino acid sequence selected from the amino acid sequences of SEQ ID NOS: 26-71.

[0040] In any one of the previous methods, the IgG antibody may comprise a light chain variable region selected from light chain variable regions binding to an antigen selected from Axl, Her2, B7-H3, and EpCAM having the amino acid sequences of SEQ ID NOS: 16-17, 20, 22, and 88-95; and a heavy chain variable region selected from heavy chain variable regions binding to same antigen having the amino acid sequences of SEQ ID NOS: 18-19, 21, 23-25, and 80-87.

[0041] In any one of the previous methods, the multi-specific antibody may bind to the first antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0042] In any one of the previous methods, the multi-specific antibody may bind to the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0043] In any one of the previous methods, the multi-specific antibody may bind to both of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0044] In any one of the previous methods, the multi-specific antibody may bind to a combination of both of the first antigen and the second antigen with a greater avidity at the aberrant condition than at the normal physiological condition.

[0045] In yet another example, the disclosure provides a method of treating a tumor in a subject comprising administering the multi-specific antibody of any one of the previous examples.

[0046] In the treatment method, the multi-specific antibody may be administered in conjunction with a cancer neoantigen vaccine or the multi-specific antibody may be administered after administration of the cancer neoantigen vaccine.

[0047] In yet another example, the disclosure provides a method for making a multi-specific antibody, comprising steps of: a) obtaining an IgG antibody or fragment thereof that binds to a first antigen; b) obtaining an scFv antibody that binds to a second antigen; c) evolving one or both of the antibodies of a) and b) to produce one or more evolved antibodies; d) screening the one or more evolved antibodies of c) to select antibodies that bind to their respective antigens with greater affinity under an aberrant condition than under a normal physiological condition; e) linking an scFv antibody that binds to the second antigen to a C-terminus of at least one light chain of an IgG antibody or fragment that binds to the first antigen to produce one or more constructs, wherein at least one of the scFv antibody and the IgG antibody are selected in d), and, if present, the scFv antibody or the IgG antibody not selected in d) is from steps a) or b); f) screening the one or more constructs of step e) under the aberrant condition and the normal physiological condition for binding to at least one of the first antigen and the second antigen; and g) selecting a multi-specific antibody from the one or more constructs that binds to at least one of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

[0048] In yet another example, the disclosure provides a method of making a multi-specific antibody, comprising steps of: a) obtaining an IgG antibody or fragment thereof that binds to a first antigen; b) linking at least one scFv antibody that binds to a second antigen to a C-terminus of at least one light chain of said IgG or fragment thereof to produce one or more constructs, wherein said at least one scFv antibody binds to

said second antigen with greater affinity at an aberrant condition than at a normal physiological condition; c) screening the one or more constructs under the normal physiological condition and the aberrant condition for binding said first antigen and said second antigen; and d) selecting a multi-specific antibody that binds said first antigen and reversibly binds said second antigen with a greater affinity at said aberrant condition than at said normal physiological condition.

[0048a] In another aspect, the present disclosure refers to a method of making a multi-specific antibody comprising steps of:

- a) obtaining an IgG antibody or fragment thereof that binds to a cancer cell specific antigen;
- b) linking at least one scFv antibody that binds to a tumor reactive lymphocyte antigen to a C-terminus of at least one light chain of said IgG or fragment thereof to produce one or more constructs, wherein said at least one scFv antibody binds to said tumor reactive lymphocyte antigen with greater affinity at an aberrant pH of from 5.2 to 6.8 of a tumor microenvironment than at a normal physiological pH of from 7.0 to 7.8;

- c) screening the one or more constructs under the normal physiological condition and the aberrant condition for binding to said first antigen and to said second antigen; and

- d) selecting a multi-specific antibody that binds to said first antigen and reversibly binds to said second antigen with a greater affinity at said aberrant condition than at said normal physiological condition.

[0049] In yet another example, the disclosure provides a multi-specific antibody, comprising an IgG antibody or fragment thereof that binds to a cell-specific antigen and at least one scFv antibody that binds to a T-lymphocyte antigen linked to a C terminus of at least one light chain or at least one heavy chain of said IgG antibody or fragment thereof, wherein said at least one scFv antibody binds to said T-lymphocyte antigen with a greater affinity at an aberrant condition than at a normal physiological condition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] FIG. 1 is a schematic structure of a bi-valent multi-specific antibody that is a hetero-dimer with one arm for binding to an antigen (Ag) and the other arm for binding to CD3.

[0051] FIG. 2 is a schematic structure of a tetra-valent multi-specific antibody that is a homo-dimer with each arm having a binding site to an antigen (Ag) and a binding site to CD3.

[0052] FIG. 3A shows a representation of the general method used to measure the binding of the tetra-valent multi-specific antibody of FIG. 2 to an antigen and CD3 at the same time.

[0053] FIG. 3B shows non-conditional binding activity to CD3 by multi-specific antibodies to CD3/Axl at pH's of 6.0 and 7.4. Antibodies were added to CD3 coated ELISA plates and binding detected using an HRP labelled anti-IgG antibody (secondary antibody 1 of FIG. 3A)

[0054] FIG. 3C shows conditional binding activity to Axl by multi-specific antibodies at pH's of 6.0 and 7.4. Antibodies were added to CD3 coated ELISA plates and binding detected using AXL protein (Antigen in FIG. 3A) and an HRP labelled anti-AXL antibody (secondary antibody 2 in FIG. 3A).

[0055] FIG. 4A shows conditional and non-conditional binding activities to immobilized CD3 by multi-specific antibodies to CD3/Axl at pH's of 6.0 and 7.4.

[0056] FIG. 4B shows conditional and non-conditional binding activities to CD3 by some of the multi-specific antibodies of FIG. 4A at pH's of 6.0 and 7.4, when the Axl was immobilized.

[0057] FIG. 5A shows conditional and non-conditional binding activities to immobilized CD3 by multi-specific antibodies to CD3/Her2 at pH's of 6.0 and 7.4.

[0058] FIG. 5B shows conditional and non-conditional binding activities to CD3 by the multi-specific antibodies of FIG. 5A at pH's of 6.0 and 7.4, when the Her2 was immobilized.

[0059] FIG. 6 shows conditional binding activity to immobilized CD3 by multi-specific antibodies to CD3/B7-H3 at pH's of 6.0 and 7.4.

[0060] FIG. 7 shows a schematic of a working model for the multi-specific antibody of the present invention for binding to both CD3 on an engineered Jurkat effector cell and an antigen on a tumor target cell.

WHAT IS CLAIMED IS:

1. A multi-specific IgG antibody comprising:
 - at least one binding site for a cancer cell specific antigen; and
 - at least one binding site for a tumor-reactive lymphocyte antigen,wherein said multi-specific antibody reversibly binds to at least one of the cancer cell specific antigen and tumor-reactive lymphocyte antigen with a greater affinity at a first aberrant physiological pH of from 5.2 to 6.8 of a tumor microenvironment than at a second normal physiological pH of from 7.0 to 7.8.
2. The multi-specific antibody of claim 1,
 - wherein said at least one binding site for the cancer cell specific antigen is an IgG antibody or fragment thereof, and
 - said at least one binding site for the tumor-reactive lymphocyte antigen is a single chain antibody.
3. The multi-specific antibody of claim 2, wherein the single chain antibody is an scFv antibody and is attached to a C terminus of at least one light chain of said IgG antibody or fragment thereof via a linker.
4. The multi-specific antibody of claim 3, wherein the scFv antibody comprises a light chain variable region selected from light chain variable regions having amino acid sequences of SEQ ID NOS: 1-10 and a heavy chain variable region selected from heavy variable regions having amino acid sequences of SEQ ID NOS: 11-15.
5. The multi-specific antibody of claim 4, wherein the scFv antibody has an amino acid sequence selected from amino acid sequences of SEQ ID NOS: 26-71.
6. The multi-specific antibody of claim 2, wherein the multi-specific antibody comprises:
 - a light chain variable region selected from light chain variable regions binding to an antigen selected from Ax1, Her2, B7-H3, and EpCAM and being selected from light chain variable regions having amino acid sequences of SEQ ID NOS: 16-17, 20, 22, and 88-95; and
 - a heavy chain variable region selected from heavy chain variable regions binding to the same antigen as the light chain variable region and being selected from heavy chain variable regions having amino acid sequences of SEQ ID NOS: 18-19, 21, 23-25, and 80-87.
7. The multi-specific antibody of claim 1, wherein the tumor-reactive lymphocyte antigen is a CD3 antigen.

8. The multi-specific antibody of claim 1, wherein the tumor-reactive lymphocyte antigen is on a lymphocyte selected from T cells, macrophages, Jurkat cells, monocytes, NK cells, neutrophils, eosinophils, basophils, and lymphokine-activated killer cells.

9. The multi-specific antibody of claim 1, wherein the cancer cell specific antigen is selected from Ax1, EpCAM, Ror2, Her2, and B7-H3.

10. The multi-specific antibody of claim 1, wherein said multi-specific antibody binds to the cancer cell specific antigen with a greater affinity at the first physiological condition than at the second physiological condition and said multi-specific antibody binds to the tumor-reactive lymphocyte antigen with a greater affinity at the first physiological condition than at the second physiological condition.

11. The multi-specific antibody of claim 1, wherein said multi-specific antibody binds to a combination of the first antigen and the second antigen with a greater avidity at the aberrant condition than at the normal physiological condition.

12. A method for making a multi-specific antibody, comprising steps of:

- a) obtaining an IgG antibody or fragment thereof that binds to a first antigen;
- b) linking at least one scFv antibody that binds to a second antigen to a C-terminus of at least one light chain of said IgG antibody or fragment to produce one or more constructs;
- c) screening the one or more constructs of step b) for binding to at least one of the first antigen and the second antigen under an aberrant pH of from 5.2 to 6.8 of a tumor microenvironment and a normal physiological pH of from 7.0 to 7.8; and
- d) selecting a multi-specific antibody from the one or more constructs that reversibly binds to at least one of the first antigen and the second antigen with a greater affinity at the aberrant condition than at the normal physiological condition.

13. A conjugated multi-specific antibody comprising the multi-specific antibody of any one of claims 1-11 conjugated to a macromolecule selected from at least one of a protein, a fatty acid, a polymer, albumin and polyethylene glycol.

14. A method of making a multi-specific antibody comprising steps of:

- a) obtaining an IgG antibody or fragment thereof that binds to a cancer cell specific antigen;
- b) linking at least one scFv antibody that binds to a tumor reactive lymphocyte antigen to a C-terminus of at least one light chain of said IgG or fragment thereof to produce one or more constructs, wherein said at least one scFv antibody binds to said tumor reactive lymphocyte antigen with greater affinity

at an aberrant pH of from 5.2 to 6.8 of a tumor microenvironment than at a normal physiological pH of from 7.0 to 7.8;

c) screening the one or more constructs under the normal physiological condition and the aberrant condition for binding to said first antigen and to said second antigen; and

d) selecting a multi-specific antibody that binds to said first antigen and reversibly binds to said second antigen with a greater affinity at said aberrant condition than at said normal physiological condition.

15. The method of claim 14, wherein the tumor reactive lymphocyte antigen is CD3.