

[0008] The present disclosure is based, at least in part, on the surprising discovery that methionine reduces the formation of bispecific antibody construct HMW species when frozen and stored at -30°C. As shown in the Example described herein, methionine reduced aggregation (i.e., the appearance of HMW species) by about 25% to about 85% in frozen formulations comprising bispecific antibody constructs stored at -30°C; a similar protective effect was not detected in liquid formulations stored at 4°C or 40°C for a similar time frame. The materials and methods described herein provide a significant technical advantage by, e.g., simplifying the equipment and procedures required to store and transport bispecific antibody constructs while minimizing aggregation.

[0009] In various ~~aspect~~examples, the disclosure provides a pharmaceutical formulation comprising a bispecific antibody construct, a saccharide, a surfactant, a buffer, and methionine present at a molar ratio of methionine to bispecific antibody construct of about 10X to about 5000X (e.g., a molar ratio of methionine to bispecific antibody construct of about 50X to about 5000X). Optionally, the formulation may comprise about 10 mM to about 200 mM methionine. The pH of the formulation is from about 4 to about 7 (e.g., about 4 to about 6, such as about 4.2). Optionally, the saccharide is sucrose, the surfactant is polysorbate 80, and/or the buffer is a glutamate buffer. In various ~~aspect~~examples, the bispecific antibody construct is present in the formulation at a concentration of from about 1 mg/ml to about 20 mg/ml. In various ~~embodiment~~examples, the formulation is frozen. For example, the disclosure provides a frozen pharmaceutical formulation comprising about 1 mg/mL to about 20 mg/mL bispecific antibody construct, sucrose, glutamic acid, polysorbate 80, and about 10 mM to about 200 mM methionine, wherein the pH of the formulation is from about 4 to about 7 (e.g., about 4 to about 6). In alternative ~~embodiment~~examples, the formulation is a thawed formulation or is lyophilized. In various ~~aspect~~examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 131, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 156, SEQ ID NO: 165, SEQ ID NO: 174, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, or SEQ ID NO: 188.

[0010] The disclosure further provides a method of treating cancer in a subject in need thereof comprising administering a formulation of the disclosure to the subject. The use of the formulation in any of the methods disclosed herein or for the preparation of medicaments for administration according to any of the methods disclosed herein is specifically contemplated. The disclosure also provides a formulation as described herein for use in treating cancer.

[0010a] In another aspect, the present disclosure provides use of the formulation as disclosed herein in the manufacture of a medicament for treating cancer in a subject.

[0011] Additionally, the disclosure provides a method comprising (a) preparing a formulation comprising a bispecific antibody construct, methionine, and a buffer, wherein the methionine present at a molar ratio of methionine to bispecific antibody construct of about 10X to about 5000X; (b) freezing the formulation of (a); and (c) storing the formulation of (b) at a temperature of about -10°C to about -40°C. In various ~~aspect~~examples of the disclosure, steps (b) and (c) are performed at a temperature of about -20°C to about -35°C (e.g., 30°C) and/or step (c) comprises storing the formulation for at least one month. Optionally, the method further comprises (d) thawing the formulation of (c); and (e) lyophilizing the formulation of (d). In some ~~embodiment~~examples, the formulation comprises methionine at molar ratio of methionine to bispecific antibody construct of about 50X to about 5000X, such as in an amount of about 10 mM to about 200 mM methionine, and/or the bispecific antibody construct is present at a concentration of from about 1 mg/ml to about 20 mg/ml.

[0012] It should be understood that, while various ~~embodiment~~examples in the specification are presented using “comprising” language, under various circumstances, a related ~~embodiment~~example may also be described using “consisting of” or “consisting

essentially of" language. The disclosure contemplates ~~embodiment~~examples described as "comprising" a feature to include ~~embodiment~~examples which "consist of" or "consist essentially of" the feature. The term "a" or "an" refers to one or more. For example, "a bispecific antibody construct" is understood to represent one or more bispecific antibody constructs. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. The term "or" should be understood to encompass items in the alternative or together, unless context unambiguously requires otherwise.

[0013] It should also be understood that when describing a range of values, the disclosure contemplates individual values found within the range. For example, "a pH from about pH 4 to about pH 6," could be, but is not limited to, pH 4.2, 4.6, 5.2, 5.5, etc., and any value in between such values. In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded. When the term "about" is used, it means the recited number plus or minus 5%, 10%, or more of that recited number. The actual variation intended is determinable from the context.

[0014] Additional features and variations of the ~~invention~~disclosure will be apparent to those skilled in the art from the entirety of this application, including the figures and detailed description, and all such features are intended as ~~aspect~~examples of the ~~invention~~disclosure. Likewise, features of the ~~invention~~disclosure described herein can be re-combined into additional ~~embodiment~~examples that also are intended as ~~aspect~~examples of the ~~invention~~disclosure, irrespective of whether the combination of features is specified as an ~~aspect~~example or ~~embodiment~~ of the ~~invention~~disclosure. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein (even if described in separate sections) are contemplated, even if the combination of features is not found together in the same sentence, or paragraph, or section of this document. Also, only such limitations which are described herein as critical to the ~~invention~~disclosure should be viewed as such; variations of the ~~invention~~disclosure lacking limitations which have not been described herein as critical are intended as aspects of the ~~invention~~disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1 is a graph showing the increase in percent (%) high molecular weight (HMW) species of BiTE®-1, BiTE®-2, BiTE®-3, BiTE®-4, BiTE®-5, BiTE®-6, BiTE®-7, and BiTE®-8 in a formulation comprising 10 mM glutamate, 9% sucrose, 0.01% polysorbate 80 (PS80) (pH 4.2), with (gray bar on left) and without (black bar on right) 50 mM methionine, after one month storage at -15°C. Storage at -15°C represents accelerated stress conditions for -30°C storage. HMW species were detected using SE-UHPLC.

[0016] Figure 2 is a graph showing the increase in percent (%) high molecular weight (HMW) species of BiTE®-1, BiTE®-2, BiTE®-3, BiTE®-4, BiTE®-5, BiTE®-6, BiTE®-7, and BiTE®-8 in a formulation comprising 10 mM glutamate, 9% sucrose, 0.01% polysorbate 80 (PS80) (pH 4.2), with and without 50 mM methionine, after one month liquid storage at 4°C. HMW species were detected using SE-UHPLC. Measurements illustrated in the graph for each bispecific antibody construct include (from left to right): %HMW detected at time 0 in the formulation without methionine, %HMW detected at four weeks in the formulation without methionine, %HMW detected at time 0 in the formulation with methionine, and %HMW detected at four weeks in the formulation with methionine.

[0017] Figure 3 is a graph showing the increase in percent (%) high molecular weight (HMW) species of BiTE®-1, BiTE®-2, BiTE®-3, BiTE®-4, BiTE®-5, BiTE®-6, BiTE®-7, and BiTE®-8 in a formulation comprising 10 mM glutamate, 9% sucrose, 0.01% polysorbate 80 (PS80) (pH 4.2), with and without 50 mM methionine, after one month liquid storage at 40°C. HMW species were detected using SE-UHPLC. Measurements illustrated in the graph for each bispecific antibody construct include (from left to right): %HMW detected at time 0 in the formulation without methionine, %HMW detected at four weeks

What is claimed is:

1. A pharmaceutical formulation comprising
 - (a) a bispecific antibody construct,
 - (b) a saccharide,
 - (c) a surfactant,
 - (d) a buffer, and
 - (e) methionine present at a molar ratio of methionine to bispecific antibody construct of about 10X to about 5000X;
wherein the pH of the formulation is from about 4 to about 7.
2. The formulation of claim 1, wherein the pH of the formulation is about 4.2.
3. The formulation of claim 1 or claim 2, wherein the saccharide is a monosaccharide or a disaccharide.
4. The formulation of any one of claims 1-3, wherein the saccharide is glucose, galactose, fructose, xylose, sucrose, lactose, maltose, trehalose, sorbitol, mannitol, or xylitol.
5. The formulation of claim 4, wherein the saccharide is sucrose.
6. The formulation of any one of claims 1-5, wherein the surfactant is a nonionic surfactant.
7. The formulation of any one of claims 1-6, wherein the surfactant is polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, poloxamer 407, or Triton™ x-100.
8. The formulation of claim 7, wherein the surfactant is polysorbate 20, polysorbate 40, polysorbate 60, or polysorbate 80.
9. The formulation of claim 7, wherein the surfactant is polysorbate 80.
10. The formulation of any one of claims 1-9, wherein the buffer is an acetate buffer, a glutamate buffer, a citrate buffer, a lactic buffer, a succinate buffer, a tartrate buffer, a fumarate buffer, a maleate buffer, a histidine buffer, or a phosphate buffer.
11. The formulation of claim 10, wherein the buffer is a glutamate buffer.
12. The formulation of any one of claims 1-11, wherein the formulation comprises the bispecific antibody construct at a concentration of from about 1 mg/ml to about 20 mg/ml.
13. The formulation of any one of claims 1-12, wherein the formulation comprises methionine at molar ratio of methionine to bispecific antibody construct of about 50X to about 5000X.
14. The formulation of any one of claims 1-12, wherein the formulation comprises about 10 mM to about 200 mM methionine.
15. The formulation of any one of claims 1-14, wherein the formulation is frozen.
16. The formulation of any one of claims 1-14, wherein the formulation is a thawed formulation.

17. The formulation of any one of claims 1-14, wherein the formulation is lyophilized.
18. The formulation of any one of claims 1-17, which comprises at least about 10% less high molecule weight (HMW) species compared to a matched formulation not comprising methionine when stored for four weeks at -15°C.
19. The formulation of claim 18, which comprises at least about 25% to about 85% less high molecule weight (HMW) species compared to a matched formulation not comprising methionine when stored for four weeks at -15°C.
20. A frozen pharmaceutical formulation comprising about 1 mg/mL to about 20 mg/mL bispecific antibody construct, sucrose, glutamic acid, polysorbate 80, and about 10 mM to about 200 mM methionine, wherein the pH of the formulation is from about 4 to about 7.
21. The formulation of any one of claims 1-20, wherein the bispecific antibody construct comprises a first binding domain that binds to a target cell surface antigen and a second binding domain that binds to human CD3 on the surface of a T cell.
22. The formulation of claim 21, further comprising a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain.
23. The formulation of claim 22, wherein each of the first and second binding domains comprise a VH region and a VL region.
24. The formulation of claim 22 or claim 23, wherein the bispecific antibody construct is a single chain antibody construct.
25. The formulation of any one of claims 21-24, wherein the target cell surface antigen is CDH19, MSLN, DLL3, FLT3, EGFR, EGFRvIII, BCMA, PSMA, CD33, CD19, CD70, MUC17, or CLDN18.2.
26. The formulation of any one of claims 21-25, wherein the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 131, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 156, SEQ ID NO: 165, SEQ ID NO: 174, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, or SEQ ID NO: 188.
27. The formulation of any one of claims 1-26, wherein the formulation comprises methionine at molar ratio of methionine to bispecific antibody construct of about 105X to about 5000X, and the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 77.
28. ~~A method of treating cancer in a subject in need thereof comprising administering~~ Use of the formulation of any one of claims 1-26 ~~to the~~ in the manufacture of a medicament for treating cancer in a subject.
29. The ~~method-use~~ of claim 28, wherein the formulation is frozen and the ~~method-use~~ further comprises thawing the formulation prior to administration to the subject.
30. The ~~method-use~~ of claim 27 or 28, wherein the formulation is prepared for intravenous administration. ~~the method comprises intravenously administering the formulation to the subject.~~

31. The ~~method-use~~ of any one of claims 28-30, wherein the formulation comprises methionine at molar ratio of methionine to bispecific antibody construct of about 105X to about 5000X, and the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 77.

32. A method comprising

(a) preparing a formulation comprising a bispecific antibody construct, methionine, and a buffer, wherein the methionine is present at a molar ratio of methionine to bispecific antibody construct of about 10X to about 5000X;

(b) freezing the formulation of (a); and

(c) storing the formulation of (b) at a temperature of about -10°C to about -40°C.

~~30~~33. The method of claim ~~29~~32, wherein the method further comprises

(d) thawing the formulation of (c); and

(e) lyophilizing the formulation of (d).

~~33~~34. The method of claim 32, wherein the formulation of (a) further comprises a saccharide, and comprises pH of from about 4 to about 7.

~~34~~35. The method of claim 33, wherein between step (d) and (e) a buffer exchange step is performed to produce a pharmaceutical formulation comprising the bispecific antibody construct, a saccharide, a surfactant, a buffer, and methionine present at a molar ratio of methionine to bispecific antibody construct of about 10X to about 5000X; wherein the pH of the formulation is from about 4 to about 7.

~~35~~36. The method of any one of claims 32-~~34~~35, wherein steps (b) and (c) are performed at a temperature of about -20°C to about -35°C.

~~36~~37. The method of any one of claims 32-~~35~~36, wherein step (c) comprises storing the formulation for at least one month.

~~37~~38. The method of any one of claims 32-~~36~~37, wherein the formulation comprises methionine present at molar ratio of methionine to bispecific antibody construct of about 50X to about 5000X

~~38~~39. The method of any one of claims 32-~~37~~38, wherein the formulation comprises about 10 mM to about 200 mM methionine.

~~39~~40. The method of any one of claims 33-~~38~~39, wherein the saccharide is a monosaccharide or a disaccharide.

~~40~~41. The method of any one of claims 33-~~39~~40, wherein the saccharide is glucose, galactose, fructose, xylose, sucrose, lactose, maltose, trehalose, sorbitol, mannitol, or xylitol.

~~41~~42. The method of claim ~~40~~41, wherein the saccharide is sucrose.

~~42~~43. The method of any one of claims ~~34~~-~~41~~35-42, wherein the surfactant is a nonionic surfactant.

~~43~~44. The method of any one of claims ~~34~~-~~42~~35-43, wherein the surfactant is polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, poloxamer 407, or Triton™ x-100.

~~44~~45. The method of claim ~~43~~44, wherein the surfactant is polysorbate 80.

4546. The method of any one of claims 32-~~44~~45, wherein the buffer is an acetate buffer, a glutamate buffer, a citrate buffer, a lactic buffer, a succinate buffer, a tartrate buffer, a fumarate buffer, a maleate buffer, a histidine buffer, or a phosphate buffer.

4647. The method of claim ~~45~~46, wherein the buffer is a glutamate buffer.

~~47~~48. The method of any one of claims 31-~~46~~47, wherein the formulation comprises the bispecific antibody construct at a concentration of from about 1 mg/ml to about 20 mg/ml.

4849. The method of any one of claims 31-~~47~~48, wherein the bispecific antibody construct comprises a first binding domain that binds to a target cell surface antigen and a second binding domain that binds to human CD3 on the surface of a T cell.

4950. The method of claim ~~48~~49, further comprising a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain.

~~50~~51. The method of claim ~~49~~50, wherein each of the first and second binding domains comprise a VH region and a VL region.

~~51~~52. The method of ~~claim 49 or claim 50 or claim 51~~, wherein the bispecific antibody construct is a single chain antibody construct.

5253. The method of any one of claims ~~48-51~~49-52, wherein the target cell surface antigen is CDH19, MSLN, DLL3, FLT3, EGFR, EGFRvIII, BCMA, PSMA, CD33, CD19, CD70, MUC17, or CLDN18.2.

5453. The method of any one of claims ~~48-52~~49-53, wherein the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 131, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 156, SEQ ID NO: 165, SEQ ID NO: 174, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, or SEQ ID NO: 188.

~~53~~55. The method of claim ~~53~~54, wherein the formulation comprises methionine at molar ratio of methionine to bispecific antibody construct of about 105X to about 5000X, and the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 77.