PHARMACEUTICAL FORMULATION

FIELD OF THE INVENTION

[0001] The present disclosure is in the field of stable bispecific antibody construct formulations.

INCORPORATION BY REFERENCE

[0002] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: ASCII (text) file named "54910_Seqlisting.txt", 345,249 bytes created April 28, 2020.

BACKGROUND

[0003] Protein-based pharmaceuticals are among the fastest growing therapeutic agents in (pre)clinical development and as commercial products. In comparison with small chemical drugs, protein pharmaceuticals have high specificity and activity at relatively low concentrations, and typically provide for therapy of high impact diseases such as various cancers, auto-immune diseases, and metabolic disorders (Roberts, Trends Biotechnol. 2014 Jul;32(7):372-80, Wang, Int J Pharm. 1999 Aug 20;185(2):129-88).

[0004] Protein-based pharmaceuticals, such as recombinant proteins, can now be obtained in high purity when first manufactured due to advances in commercial scale purification processes. However, proteins are only marginally stable and are highly susceptible to degradation, both chemical and physical. Chemical degradation refers to modifications involving covalent bonds, such as deamidation, oxidation, cleavage, clipping/fragmentation, formation of new disulfide bridges, hydrolysis, isomerization, or deglycosylation. Physical degradation includes protein unfolding, undesirable adsorption to surfaces, and aggregation. Dealing with these physical and chemical instabilities is one of the most challenging tasks in the development of protein pharmaceuticals (Chi et al., Pharm Res, Vol. 20, No. 9, Sept 2003, pp. 1325-1336, Roberts, Trends Biotechnol. 2014 Jul;32(7):372-80).

[0005] Clipping (fragmentation) of protein-based pharmaceuticals can occur during manufacturing process or storage due to chemical and enzymatic causes. Clipping is undesirable because it represents degradation of the parental polypeptide, and in the case of therapeutic polypeptides, a possible reduction in potency. Preserving protein stability and activity in biological and biotechnological applications poses serious challenges. There is a need in the art for optimized pharmaceutical formulations that provide for enhanced

stabilization of therapeutic proteins and reduce clipping, aggregation, denaturation or degradation during formulation, filling, shipping, storage and administration, thereby preventing loss-of-function and adverse immunogenic reactions.

SUMMARY

[0006] Protein-based pharmaceuticals including bispecific (and/or multispecific) antibodies that bind to two (or more) different antigens simultaneously, such as bispecific T cell engaging (BiTE®) antibody constructs, are prone to protein instability. This extends to those antibody constructs comprising half-life extending formats (HLE formats) which include the single chain Fc format (designated scFc), the hetero Fc (also designated as hetFc or heterodimeric Fc, hFc) format and the fusion of human serum albumin (also designated as HSA or hALB).

[0007] Protein instability, and in particular clipping (fragmentation), is an increasing challenge in the biotechnology industry. All bispecific antibody constructs are susceptible to clipping in a liquid formulation. Clipping generates low molecular weight (LMW) species of the bispecific antibody construct and leads to inactive polypeptides. In lower (i.e., more acidic) pH liquid formulations, clipping of the bispecific antibody construct tends to increase but aggregation tends to decrease. In higher (i.e., more basic) pH liquid formulations, aggregation of the bispecific antibody construct tends to increase but clipping tends to decrease.

[0008] Unexpectedly, the present disclosure identifies an optimized pH range for liquid bispecific antibody construct formulations that allows for the liquid formulation to be stable under various storage conditions. For example, the Examples provided herein identify a particular pH range (i.e., pH 4.8-5.5) of a liquid formulation in which both the clipping of the bispecific antibody construct (i.e., amount of % low molecular weight species) and aggregation of the bispecific antibody construct (i.e., % high molecular weight species) in a liquid formulation is decreased, when stored, for example at either 2-8°C or 40°C. As shown in the Examples described herein, the levels of clipping and aggregation of the bispecific antibody construct in a liquid formulation having a pH ranging from 4.8-5.5 were compared to the levels of clipping and aggregation in a liquid formulation at pH outside of the pH 4.8-5.5 range (i.e., pH 4.2 or pH 7.4). Thus, liquid pharmaceutical formulations comprising a bispecific antibody construct and having a pH range of about pH 4.8 to about 5.5 (e.g., pH 5.2) are more stable and demonstrate improved structural integrity compared to liquid

formulations comprising the bispecific antibody construct at a pH outside of this range (e.g., pH 4.2 or pH 7.4), when under the same storage conditions.

[0009] In one aspect, described herein is a stable, aqueous pharmaceutical formulation comprising a bispecific antibody construct described herein, a buffer, a saccharide, and a surfactant, wherein the pH of the pharmaceutical formulation ranges from 4.8 to 5.5. In some embodiments, the pH of the formulation ranges from 4.5 to 5.3. In some embodiments, the pH of the formulation is 5.2.

[0010] In some embodiments, the formulation comprises less than about 10% (e.g., 9%, 8%, 7%, 6%, 5%, 4%, 3% or less) of low molecule weight (LMW) species of the bispecific antibody construct after storage at about 40˚C for one month.

[0011] In some embodiments, the formulation comprises less than about 10% (e.g., 9%, 8%, 7%, 6%, 5%, 4%, 3% or less) of low molecule weight (LMW) species of the bispecific antibody construct after storage at about 2-8˚C for one month.

[0012] In some embodiments, the formulation comprises less than about 5% (e.g., 4%, 3%, 2%, 1% or less) of high molecule weight (HMW) species of the bispecific antibody construct after storage at about 40˚C for one month.

[0013] In some embodiments, the formulation comprises less than about 5% (e.g., 4%, 3%, 2%, 1% or less) of high molecule weight (HMW) species of the bispecific antibody construct after storage at about 2-8˚C for one month.

[0014] In some embodiments, the relative potency of the bispecific antibody construct in the formulation is greater than about 30% (e.g., about 35%, 40%, 50%, 55%, 60% or more after storage at one month at about 40˚C.

[0015] In some embodiments, the percent of intact BiTE® molecule (i.e., main peak species) in the formulation is greater than 95% of the total protein content in the formulation.

[0016] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds to a target cell surface antigen, a second binding domain that binds to human CD3 on the surface of a T cell. In some embodiments, the bispecific antibody construct further comprises a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain. In some embodiments, each of the first and second binding domains of the bispecific antibody construct comprises a VH region and a VL region.

[0017] In some embodiments, the bispecific antibody construct is a single chain antibody construct.

[0018] In some embodiments, the bispecific antibody construct binds to a target cell surface antigen, such as, CDH19, MSLN, DLL3, FLT3, EGFRvlll, BCMA, PSMA, CD33, CD19, CD70, CLDN18.2 or MUC17.

[0019] In some embodiments, the first binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in (a) SEQ ID NOs: 24-29, (b) SEQ ID NOs: 34-39, (c) SEQ ID NOs: 78-83, (d) SEQ ID NOs: 10-15, (e) SEQ ID NOs: 46-51, (f) SEQ ID NOs: 88-93, (g) SEQ ID NOs: 67-72, (h) SEQ ID NOs: 56-61, (i) SEQ ID NOs: 112-117, (j) SEQ ID NOs: 100-105, (k) SEQ ID NOs:148-153, SEQ ID NOs: 157-162, or SEQ ID NOs: 166-171, or SEQ ID NOs: 175-180, (l) SEQ ID NOs:132-137, or (m) SEQ ID NOs: 123-128.

[0020] In some embodiments, the first binding domain of the bispecific antibody construct comprises a VH region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 30, 40, 84, 16, 17, 52, 94, 73, 62, 118, 154, 163, 172, 181, 106, 138, 143, or 129. In some embodiments, the first binding domain of the bispecific antibody construct comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 30, 40, 84, 16, 17, 52, 94, 73, 62, 118, 154, 163, 172, 181, 106, 138 or 143, or 129.

[0021] In some embodiments, the first binding domain of the bispecific antibody construct comprises a VL region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 31, 41, 85, 18, 19, 53, 95, 74, 63, 119, 155, 164, 173, 182, 107, 139, 144, or 130. In some embodiments, the first binding domain of the bispecific antibody construct comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 31, 41, 85, 18, 19, 53, 95, 74, 63, 119, 155, 164, 173, 182, 107, 139, 144, or 130.

[0022] In some embodiments, wherein the first binding domain comprises (a) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 30 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 31; (b) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 40 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 41; (c) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 84 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 85; (d) a VH region comprising an amino acid sequence set forth in SEQ ID

NO: 16 or 17 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 18 or 19; (e) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 52 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 53; (f) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 94 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 95; (g) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 73 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 74; (h) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 62 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 63; (i) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 118 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 119; (j) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 154, 163, 172 or 181, and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 155, 164,173, or 182 **;** (k) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 106 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 107; (l) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 138 or 143, and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 139 or 144; or (m) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 129 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 130.

[0023] In some embodiments, the second binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in SEQ ID NOs: 1-6.

[0024] In some embodiments, the second binding domain of the bispecific antibody construct comprises a VH region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the second binding domain of the bispecific antibody construct comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 7.

[0025] In some embodiments, the second binding domain of the bispecific antibody construct comprises a VL region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the second binding domain of the bispecific antibody construct comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 8.

[0026] In some embodiments, wherein the second binding domain comprises (a) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 7 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 8.

[0027] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD19 comprising an anti-CD19 variable light domain comprising the amino acid sequence of SEQ ID NO: 85 and an anti-CD19 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 84, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 86 a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 87.

[0028] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds MSLN comprising an anti-MSLN variable light domain comprising the amino acid sequence of SEQ ID NO: 41 and an anti-MSLN variable heavy domain comprising the amino acid sequence of SEQ ID NO: 40, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 42, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 43, 44 or 45.

[0029] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds DLL3 comprising an anti-DLL3 variable light domain comprising the amino acid sequence of SEQ ID NO: 74 and an anti-DLL3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 73, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 75, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the

bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 76 or 77.

[0030] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds FLT3 comprising an anti-FLT3 variable light domain comprising the amino acid sequence of SEQ ID NO: 63 and an anti-FLT3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 62, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 64, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 65 or 66.

[0031] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds EGFRvIII comprising an anti-EGFRvIII variable light domain comprising the amino acid sequence of SEQ ID NO: 31 and an anti-EGFRvIII variable heavy domain comprising the amino acid sequence of SEQ ID NO: 30, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 32, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 33.

[0032] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds BCMA comprising an anti-BCMA variable light domain comprising the amino acid sequence of SEQ ID NO: 95 and an anti-BCMA variable heavy domain comprising the amino acid sequence of SEQ ID NO: 94, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 96, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 98 or SEQ ID NO: 97.

[0033] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds PSMA comprising an anti-PSMA variable light domain comprising the amino acid sequence of SEQ ID NO: 119 or 107 and an anti-PSMA variable heavy domain comprising the amino acid sequence of SEQ ID NO: 118 or 106, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 120 or 108, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 121, 122, 109, 110 or 111.

[0034] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD33 comprising an anti-CD33 variable light domain comprising the amino acid sequence of SEQ ID NO: 18 or 19 and an anti-CD33 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 16 or 17, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 189 or 190, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, 21, 22 or 23.

[0035] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CDH19 comprising an anti-CDH19 variable light domain comprising the amino acid sequence of SEQ ID NO: 53 and an anti-CDH19 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 52, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 54, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 55.

[0036] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds MUC17 comprising an anti-MUC17 variable light domain comprising the amino acid sequence of SEQ ID NO: 155, 164, 173, or 182 and an anti-MUC17 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 154, 163, 172, or 181 a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 156, 165, 174 or 183.

[0037] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds cldn18.2 comprising an anti-cldn18.2 variable light domain comprising the amino acid sequence of SEQ ID NO: 139 or 144 and an anti-cldn18.2 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 138 or 143, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 140 or 145, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 141, 142, 146 or 147.

[0038] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD70 comprising an anti-CD70 variable light domain comprising the amino acid sequence of SEQ ID NO: 130 and an anti-CD70 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 129, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 131.

[0039] The pharmaceutical formulation of the disclosure comprises a buffer. In some embodiments, the buffer acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, and 2-(N-morpholino)ethanesulfonate or a combination thereof. In some embodiments, the buffer is present in the formulation at a concentration ranging from about 5 mM to about 200 mM (or about 10 mM to about 50 mM). In some embodiments, the buffer is present in the formulation at a concentration of 10 mM.

[0040] The pharmaceutical formulation of the disclosure comprises a saccharide. In some embodiments, the saccharide is monosaccharide or a disaccharide. In some embodiments, the saccharide is a sugar alcohol (e.g., sorbitol). In some embodiments, the saccharide is sucrose, trehalose, mannitol, sorbitol or a combination thereof. In some embodiments, the saccharide is present in the formulation at a concentration ranging from about 1 to about 15% (w/V) (or about 9 to about 12% (w/V) or about 5% to about 12% (w/V) or about 7% to about 12% (w/V)).

[0041] The pharmaceutical formulation of the disclosure comprises a surfactant. In some embodiments, the surfactant is polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, poloxamer 407, triton X-100, polyoxyethylene, PEG 3350, PEG 4000, or a combination thereof. In some embodiments, the surfactant is present in the formulation at a concentration ranging from 0.004 to about 0.5% (w/V) (or about 0.001 to about 0.01% (w/V), or about 0.001 to about 0.5% (w/V) or about 0.004 to about 0.01% (w/V)).

[0042] In some embodiments, the formulation has an osmolarity in the range of about 150 to about 500 mOsm. In some embodiments, the formulation has an osmolarity of no greater than 500 mOsm/L, 450 mOsm/L, 400 mOsm/L, or 350 mOsm/L. In some embodiments, the formulation is close to isotonic, e.g. 250-350 mOsm/L.

[0043] The pharmaceutical formulation, in some embodiments, comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, and wherein the pH of the liquid pharmaceutical formulation is 5.2. In some embodiments, the bispecific antibody construct is present in the formulation at a concentration ranging from about 0.1 mg/mL to about 20 mg/mL. In some embodiments, the bispecific antibody construct is present in the formulation at a concentration of 1 mg/mL, 5 mg/mL, 10 mg/mL or 20 mg/mL. In some embodiments, the bispecific antibody is present in the formulation in an amount ranging from about 1000 μg to about 200 mg.

[0044] In some embodiments, the pharmaceutical formulation of the disclosure is a liquid formulation.

[0045] In another aspect, described herein is a method of treating cancer in a subject in need thereof comprising administering a formulation of the disclosure to the subject.

[0046] It should be understood that while various embodiments in the specification are presented using "comprising" language, under various circumstances, a related embodiment may also be described using "consisting of" or "consisting essentially of" language. The

disclosure contemplates embodiments described as "comprising" a feature to include embodiments which "consist of" the feature. It is to be noted that the term "a" or "an" refers to one or more, for example, "an immunoglobulin molecule," is understood to represent one or more immunoglobulin molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0047] It should also be understood that when describing a range of values, the characteristic being described could be an individual value found within the range. For example, "a pH from about pH 4.8 to about pH 5.5," could be, but is not limited to, pH 4.6, 5.2, 5.5 etc. and any value in between such values. Additionally, "a pH from about pH 4.8 to about pH 5.5," should not be construed to mean that the pH of a formulation in question varies 2 pH units in the range from pH 4.8 to pH 5.5 during storage, but rather a value may be picked in that range for the pH of the solution, and the pH remains buffered at about that pH.

[0048] When the term "about" is used, it means the recited number plus or minus 5%, 10%, 15% or more of that recited number. The actual variation intended is determinable from the context.

[0049] 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. Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the drawing and detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

[0050] All references cited herein are hereby incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE FIGURES

[0051] Figure 1 is a graph showing the % low molecular weight (LMW) by reduced Capillary electrophoresis sodium dodecyl sulfate (rCE-SDS), for BiTE-I, BiTE-C, BiTE-D, and BiTE-G) from accelerated stability studies at higher pH formulations.

[0052] Figure 2 provides a graph showing the clipping rates for BiTE-G in pH 4.2 and pH 4.8 liquid formulations under accelerated and recommended storage conditions.

[0053] Figures 3A-3D provides graphs showing BiTE-D and BiTE-G) %LMW (as assessed by rCE) (Figures 3A and 3B) and %HMW (as assessed by SEC) (Figure 3C and 3D) in formulations at pH 3.8, pH 4.8, pH, 5.2 and pH 7.4.

DETAILED DESCRIPTION

[0054] Despite the high quality of current therapeutic biotech products and the resemblance of recombinant human proteins and antibodies to endogenous human proteins, protein instability remains an important concern. The process to generate recombinant proteins (e.g. antibodies) can result in loss of structural integrity due to fragmentation or clipping (Vlasak et al., MAbs, 3:253-263, 2011; Wu et al., FEBS Lett., 588:935-941, 2014). There is a critical need in the art to increase stability and structural integrity of therapeutic proteins; and optimized pharmaceutical formulations can aid in doing so.

[0055] Specific protein-based pharmaceuticals such as BiTE® antibody constructs molecules are not stable in liquid formulations over a longer period of time and especially not at accelerated temperatures, e.g. refrigeration temperature 4°C and above. A general concept underlying the present disclosure is the finding that the structural integrity of a bispecific antibody construct in a liquid pharmaceutical formulation described herein is improved at a pH ranging from 4.8 to 5.5 compared to liquid formulations having a more acidic pH (i.e., less than 4.8) or a more basic pH (i.e., higher than 5.5). As shown in the Examples provide herein, liquid formulations having a pH ranging from 4.8 to 5.5 results in minimal to no clipping (as well as in minimal to no aggregation) of the bispecific antibody construct in the liquid formulation.

[0056] Previously, formulations comprising bispecific antibody constructs would need to be lyophilized in order to exhibit the stability observed herein in the liquid formulations having a pH 4.8-5.5 (see, for example International Publication Nos. WO 2018/114190 and WO 2018/204907). The lyophilized formulations described in International Publication No. WO 2018/204907 include a preservative (such as chlorobutanol or methylparaben, or benzyl alcohol) that attributed to the stabilization of the bispecific antibody construct. In some instances, stability is also achieved by additional means, such as by lowering the pH of the formulation. For example, International Publication No. WO 2018/141910 discloses that lyophilized formulations comprising a bispecific antibody construct and having a low pH

(i.e., pH 4.0) were more stable than lyophilized formulations having the same antibody concentration at a more basic pH (e.g., pH 6 or higher). In contrast, the identification of the optimized pH range described in the Examples of the present disclosure allows for storage of the pharmaceutical formulations comprising bispecific antibody constructs as a liquid formulation at 4°C and higher (up to 40°C) at various time points.

[0057] Within the present disclosure, the term "stability," "stable" or "stabilization" relates to the structural integrity of the pharmaceutical formulation in total and in particular to the structural integrity of the active ingredient (e.g. the bispecific antibody construct) itself, specifically during formulation, filling, shipment, storage and administration. For example, the stability of an active ingredient in the context of the pharmaceutical formulations of the disclosure is determined by the amount of fragmentation and/or clipping of the active ingredient. A "stable aqueous formulation" as used herein refers to a liquid (i.e., non-lyophilized) pharmaceutical formulation comprising a bispecific antibody construct of the disclosure that has minimal to no fragmentation or clipping of the bispecific antibody construct when stored at, for example 4°C for at least 4 weeks. Exemplary stable aqueous formulations have a pH 4.8-5.5 (e.g., pH 5.2). "Formulation" and "composition" as used herein are synonymous.

[0058] The terms "clipping" or "fragmentation" as used herein refers to the partial cleaving of expressed protein, usually by proteolysis. Fragmentation in the CDRs of the bispecific antibody construct within the disclosed pharmaceutical formulations is likely to have an effect on the binding of the bispecific antibody construct to the target and, consequently, have an effect on its potency. There are few, if any reports in the literature to describe the altered binding affinity to a target for a bispecific antibody construct with fragmentation occurring in the CDR; it is probable that, unless the CDR-clipped species is purified and tested, the low levels of clipping in CDRs may preclude any detectable changes by potency assays. Fragmentation in the hinge region may have more implications on the function of a bispecific antibody construct: the Fab fragment generated will be devoid of any Fc-mediated effector function and have a reduced circulation half-time; the Fc-Fab fragment may not be potent at all if interaction with the target receptor requires both Fab arms. See Vlasak et al., mAbs, 3:3, 253-263, 2011.

[0059] The term "low molecular weight (LMW)"species as used herein refers to fragments of the bispecific antibody construct as a result of clipping.

[0060] The term "(protein) aggregate" as used herein generally encompasses protein species of higher molecular weight such as ''oligomers'' or ''multimers'' instead of the desired defined species (e.g., a monomer). The term is used interchangeably herein with the terms "high molecular weight species" and "HMW". Protein aggregates may generally differ in size (ranging from small (dimers) to large assemblies (subvisible or even visible particles) and from the nanometer to micrometer range in diameter), morphology (approximately spherical to fibrillar), protein structure (native vs. non-native/denatured), type of intermolecular bonding (covalent vs. non-covalent), reversibility and solubility. Soluble aggregates cover the size range of roughly 1 to 100 nm, and protein particulates cover subvisible (~0.1–100 .m) and visible (>100 .m) ranges. All of the aforementioned types protein of aggregates are generally encompassed by the term. The term "(protein) aggregate" thus refers to all kinds physically-associated or chemically linked non-native species of two or more protein monomers.

[0061] The term "aggregation" as used herein refers to the direct mutual attraction between molecules, e.g. via van der Waals forces or chemical bonding. In particular, the term "aggregation" is understood as proteins accumulating and clumping together. Aggregates may include amorphous aggregates, oligomers, and are typically referred to as high molecular weight (HMW) species, i.e. molecules having a higher molecular weight than pure product molecules.

[0062] In some embodiments, the disclosure describes formulations comprising a bispecific antibody construct that binds CD3 on human T cells in an amount of 0.1-20 mg/mL, a buffer, a saccharide, and a surfactant, wherein the formulation has a pH ranging from 4.8-5.5 (e.g., pH 5.2). In some embodiments, the bispecific antibody construct co-engages CD3 and one of human CDH19, human MSLN, human DLL3, human FLT3, human EGFRvlll, human BCMA, human PSMA, human CD33, human CD19, human CD70, human CLDN18.2 or human MUC17 in such a manner so as to transiently connect malignant cells with T cells, thereby inducing T cell mediated killing of the bound malignant cell.

[0063] Various aspects of the formulations are described below. The use of section headings are merely for the convenience of reading, and not intended to be limiting per se. The entire document is intended to be viewed as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated.

[0064] *Antigen-Binding Proteins*

[0065] An “antigen-binding protein” is a protein comprising a domain that binds a specified target antigen (such as CD3 and/or CDH19, MSLN, DLL3, FLT3, EGFRvlll, BCMA, PSMA, CD33, CD19, CD70, CLDN18.2 or MUC17). An antigen-binding protein comprises a scaffold or framework portion that allows the antigen binding domain to adopt a conformation that promotes binding of the antigen-binding protein to the antigen. In exemplary aspects, the antigen-binding protein is an antibody or immunoglobulin, or an antigen-binding antibody fragment.

[0066] The term "antibody" refers to an intact antigen-binding immunoglobulin. An "antibody" is a type of an antigen-binding protein. The antibody can be an IgA, IgD, IgE, IgG, or IgM antibody, including any one of IgG1, IgG2, IgG3 or IgG4. In various embodiments, an intact antibody comprises two full-length heavy chains and two full-length light chains. An antibody has a variable region and a constant region. In IgG formats, a variable region is generally about 100-110 or more amino acids, comprises three complementarity determining regions (CDRs), is primarily responsible for antigen recognition, and substantially varies among other antibodies that bind to different antigens. A variable region typically comprises at least three heavy or light chain CDRs (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, Public Health Service N.I.H., Bethesda, Md.; see also Chothia and Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat et al., 1991; see also Chothia and Lesk, 1987, supra). The constant region allows the antibody to recruit cells and molecules of the immune system.

[0067] In some embodiments, the antibody of the formulation is a bispecific antibody, i.e., an antibody that binds two different targets (e.g., CD3 and a second, different target).

[0068] The term “bispecific” as used herein refers to an antibody construct that binds to two different target antigens, *i.e.*, it comprises a first binding domain and a second binding domain, wherein the first binding domain binds to one antigen or target (e.g., the target cell surface antigen), and the second binding domain binds to another antigen or target (e.g. CD3). Accordingly, antibody constructs according to the disclosure comprise specificities for two different antigens or targets. The term “target cell surface antigen” refers to an antigenic structure expressed by a cell and which is present at the cell surface such that it is accessible for an antibody construct as described herein. It may be a protein, preferably the extracellular portion of a protein, or a carbohydrate structure, preferably a carbohydrate structure of a protein, such as a glycoprotein. It is preferably a tumor antigen. The invention also

encompasses multispecific antibody constructs such as trispecific antibody constructs, the latter ones including three binding domains, or constructs having more than three (e.g. four, five…) specificities.

[0069] Bispecific antibodies and/or antibody constructs as understood herein include, but are not limited to, traditional bispecific immunoglobulins (e.g., BsIgG), IgG comprising an appended antigen-binding domain (e.g., the amino or carboxy termini of light or heavy chains are connected to additional antigen-binding domains, such as single domain antibodies or paired antibody variable domains (e.g., Fv or scFv)), BsAb fragments (e.g., bispecific single chain antibodies), bispecific fusion proteins (e.g., antigen binding domains fused to an effector moiety), and BsAb conjugates. See, e.g., Spiess et al., Molecular Immunology 67(2) Part A: 97-106 (2015), which describes various bispecific formats and is hereby incorporated by reference. Examples of bispecific constructs include, but are not limited to, diabodies, single chain diabodies, tandem scFvs, bispecific T cell engager (BiTE®) format (a fusion protein consisting of two single-chain variable fragments (scFvs) joined by a linker), and Fab2 bispecifics, as well as engineered constructs comprising full length antibodies. See, e.g., Chames & Baty, 2009, mAbs 1[6]:1-9; and Holliger & Hudson, 2005, Nature Biotechnology 23[9]:1126-1136; Wu et al., 2007, Nature Biotechnology 25[11]:1290-1297;Michaelson et al., 2009, mAbs 1[2]:128-141; International Patent Publication No. 2009032782 and 2006020258; Zuo et al., 2000, Protein Engineering 13[5]:361-367; U.S. Patent Application Publication No. 20020103345; Shen et al., 2006, J Biol Chem 281[16]:10706-10714; Lu et al., 2005, J Biol Chem 280[20]:19665-19672; and Kontermann, 2012 MAbs 4(2):182, all of which are expressly incorporated herein.

[0070] In some embodiments, the formulations described herein comprise a bispecific antibody construct comprises a first binding domain that binds to a target cell surface antigen, a second binding domain that binds to human CD3 on the surface of a T cell, and optionally a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain. In some embodiments, each of the first and second binding domains comprise a VH region and a VL region.

[0071] The term "binding domain" as used herein refers to a domain which (specifically) binds to / interacts with / recognizes a given target epitope or a given target site on the target molecules (antigens), e.g. CDH19, MSLN, DLL3, FLT3, EGFRvlll, BCMA, PSMA, CD33, CD19, CD70, CLDN18.2 or MUC17 and CD3, respectively. The structure and function of the first binding domain (recognizing e.g. CDH19, MSLN, DLL3, FLT3, EGFRvlll, BCMA,

PSMA, CD33, CD19, CD70, CLDN18.2 or MUC17) and preferably also the structure and/or function of the second binding domain (recognizing CD3), is/are based on the structure and/or function of an antibody, e.g. of a full-length or whole immunoglobulin molecule and/or is/are drawn from the variable heavy chain (VH) and/or variable light chain (VL) domains of an antibody or fragment thereof. Preferably, the first binding domain is characterized by the presence of three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region). The second binding domain preferably also comprises the minimum structural requirements of an antibody which allow for the target binding. More preferably, the second binding domain comprises at least three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region). It is envisaged that the first and/or second binding domain is produced by or obtainable by phage-display or library screening methods rather than by grafting CDR sequences from a pre-existing (monoclonal) antibody into a scaffold.

[0072] In some embodiments, the first binding domain which binds to the target cell surface antigen and/or the second binding domain which binds to CD3ε is/are human binding domains. Antibodies and antibody constructs comprising at least one human binding domain avoid some of the problems associated with antibodies or antibody constructs that possess non-human such as rodent (*e.g.* murine, rat, hamster or rabbit) variable and/or constant regions. The presence of such rodent derived proteins can lead to the rapid clearance of the antibodies or antibody constructs or can lead to the generation of an immune response against the antibody or antibody construct by a patient. In order to avoid the use of rodent derived antibodies or antibody constructs, human or fully human antibodies / antibody constructs can be generated through the introduction of human antibody function into a rodent so that the rodent produces fully human antibodies.

[0073] In some embodiments, the antigen binding protein comprises a single chain antibody construct. An scFv comprises a variable heavy chain, an scFv linker, and a variable light domain. Optionally, the C-terminus of the variable light chain is attached to the N-terminus of the scFv linker, the C-terminus of which is attached to the N-terminus of a variable heavy chain (N-vh-linker-vl-C), although the configuration can be switched (N-vl-linker-vh-C). Alternatively, the C-terminus of the variable heavy chain is attached to the N-terminus of the scFv linker, the C-terminus of which is attached to the N-terminus of a variable light chain (N-vl-linker-vh-C), although the configuration can be switched (N-vh-

linker-v-C). Thus, specifically included in the depiction and description of scFvs are the scFvs in either orientation.

[0074] The at least two binding domains and the variable domains (VH/VL) of the antibody construct of the present disclosure may or may not comprise peptide linkers (spacer peptides). The term "peptide linker" comprises in accordance with the present invention an amino acid sequence by which the amino acid sequences of one (variable and/or binding) domain and another (variable and/or binding) domain of the antibody construct of the disclosure are linked with each other. The peptide linkers can also be used to fuse the third domain to the other domains of the antibody construct of the invention. An essential technical feature of such peptide linker is that it does not comprise any polymerization activity. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233 or WO 88/09344, the disclosure of which are incorporated herein by reference in their entireties. The peptide linkers can also be used to attach other domains or modules or regions (such as half-life extending domains) to the bispecific antibody construct described herein.

[0075] In some embodiments, the third domain comprises a "Fc" or "Fc region" or "Fc domain," which refers to the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, "Fc domain" refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). The bispecific antibody construct is preferably an IgG antibody (which includes several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, amino acid modifications are made to the Fc region, for example, to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0076] In some embodiments, the formulations described herein comprise a bispecific antibody construct which binds human CD3 and human CDH19, or human CD3 and human MSLN, or human CD3 and human DLL3, or human CD3 and human FLT3, or human CD3 and human EGFRvIII, or human CD3 and human BCMA, or human CD3 and PSMA, or

human CD3 and human CD33, or human CD3 and human CD19, human CD3 and human CD70, or human CD3 and human MUC17, or human CD3 and human CLDN18.2.

[0077] In some embodiments, the first binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in (a) SEQ ID NOs: 24-29, (b) SEQ ID NOs: 34-39, (c) SEQ ID NOs: 78-83, (d) SEQ ID NOs: 10-15, (e) SEQ ID NOs: 46-51, (f) SEQ ID NOs: 88-93, (g) SEQ ID NOs: 67-72, (h) SEQ ID NOs: 56-61, (i) SEQ ID NOs: 112-117, (j) SEQ ID NOs: 100-105, (k) SEQ ID NOs:148-153, SEQ ID NOs: 157-162, or SEQ ID NOs: 166-171, or SEQ ID NOs: 175-180, (l) SEQ ID NOs:132-137, or (m) SEQ ID NOs: 123-128.

[0078] In some embodiments, the first binding domain of the bispecific antibody construct comprises a VH region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 30, 40, 84, 16, 17, 52, 94, 73, 62, 118, 154, 163, 172, 181, 106, 138, 143, or 129. In some embodiments, the first binding domain of the bispecific antibody construct comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 30, 40, 84, 16, 17, 52, 94, 73, 62, 118, 154, 163, 172, 181, 106, 138, 143, or 129.

[0079] In some embodiments, the first binding domain of the bispecific antibody construct comprises a VL region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 31, 41, 85, 18, 19, 53, 95, 74, 63, 119, 155, 164, 173, 182, 107, 139, 144, or 130. In some embodiments, the first binding domain of the bispecific antibody construct comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 31, 41, 85, 18, 19, 53, 95, 74, 63, 119, 155, 164, 173, 182, 107, 139, 144, or 130.

[0080] In some embodiments, wherein the first binding domain comprises (a) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 30 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 31; (b) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 40 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 41; (c) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 84 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 85; (d) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 16 or 17 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 18 or 19; (e) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 52 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 53; (f) a VH

region comprising an amino acid sequence set forth in SEQ ID NO: 94 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 95; (g) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 73 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 74; (h) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 62 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 63; (i) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 118 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 119; (j) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 154, 163, 172 or 181, and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 155, 164, 173 or 182; (k) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 106 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 107; (l) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 138 or 143, and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 139 or 144; or (m) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 129 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 130.

[0081] In some embodiments, the second binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in SEQ ID NOs: 1-6.

[0082] In some embodiments, the second binding domain of the bispecific antibody construct comprises a VH region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, the second binding domain of the bispecific antibody construct comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 7.

[0083] In some embodiments, the second binding domain of the bispecific antibody construct comprises a VL region comprising an amino acid sequence at least 90% identical (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 8. In some embodiments, the second binding domain of the bispecific antibody construct comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 8.

[0084] In some embodiments, wherein the second binding domain comprises (a) a VH region comprising an amino acid sequence set forth in SEQ ID NO: 7 and a VL region comprising an amino acid sequence set forth in SEQ ID NO: 8.

[0085] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD19 comprising an anti-CD19 variable light domain comprising the amino acid sequence of SEQ ID NO: 85 and an anti-CD19 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 84, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 86 a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 87.

[0086] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds MSLN comprising an anti-MSLN variable light domain comprising the amino acid sequence of SEQ ID NO: 41 and an anti-MSLN variable heavy domain comprising the amino acid sequence of SEQ ID NO: 40, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 42, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 43, 44 or 45.

[0087] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds DLL3 comprising an anti-DLL3 variable light domain comprising the amino acid sequence of SEQ ID NO: 74 and an anti-DLL3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 73, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 75, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 76 or 77.

[0088] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds FLT3 comprising an anti-FLT3 variable light domain comprising the amino acid sequence of SEQ ID NO: 63 and an anti-FLT3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 62, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 64, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 65 or 66.

[0089] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds EGFRvIII comprising an anti-EGFRvIII variable light domain comprising the amino acid sequence of SEQ ID NO: 31 and an anti-EGFRvIII variable heavy domain comprising the amino acid sequence of SEQ ID NO: 30, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 32, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 33.

[0090] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds BCMA comprising an anti-BCMA variable light domain comprising the amino acid sequence of SEQ ID NO: 95 and an anti-BCMA variable heavy domain comprising the amino acid sequence of SEQ ID NO: 94, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 96, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 98 or SEQ ID NO: 97.

[0091] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds PSMA comprising an anti-PSMA variable light domain comprising the

amino acid sequence of SEQ ID NO: 119 or 107 and an anti-PSMA variable heavy domain comprising the amino acid sequence of SEQ ID NO: 118 or 106, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 120 or 108, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 121, 122, 109, 110 or 111.

[0092] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD33 comprising an anti-CD33 variable light domain comprising the amino acid sequence of SEQ ID NO: 18 or 19 and an anti-CD33 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 16 or 17, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 189 or 190, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, 21, 22 or 23.

[0093] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CDH19 comprising an anti-CDH19 variable light domain comprising the amino acid sequence of SEQ ID NO: 53 and an anti-CDH19 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 52, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 54, a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 55.

[0094] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds MUC17 comprising an anti-MUC17 variable light domain comprising the amino acid sequence of SEQ ID NO: 155, 164, 173, or 182 and an anti-MUC17 variable

heavy domain comprising the amino acid sequence of SEQ ID NO: 154, 163, 172, or 181 a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 156, 165, 174 or 183.

[0095] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds cldn18.2 comprising an anti-cldn18.2 variable light domain comprising the amino acid sequence of SEQ ID NO: 139 or 144 and an anti-cldn18.2 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 138 or 143, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. For example, in one embodiment, the bispecific antibody construct comprises a first binding domain comprising the amino acid sequence of SEQ ID NO: 140 or 145, and a second binding domain comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 141, 142, 146 or 147.

[0096] In some embodiments, the bispecific antibody construct comprises a first binding domain that binds CD70 comprising an anti-CD70 variable light domain comprising the amino acid sequence of SEQ ID NO: 130 and an anti-CD70 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 129, a second binding domain comprising an anti-CD3 variable heavy domain comprising the amino acid sequence of SEQ ID NO: 7, and an anti-CD3 variable light domain comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 131.

[0097] In some embodiments, the formulations comprises an antigen-binding protein (e.g., bispecific antibody construct) described herein in an amount ranging from about 10 mg to about 50 mg (or from about 10 mg to about 20 mg, or about 20 mg to about 50 mg, or about 15 mg to about 20 mg, or about 20 mg to about 55 mg). In some embodiments, the formulation comprises a bispecific antibody construct in an amount of about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, or about 50 mg.

[0098] In some embodiments, the formulation comprises an antigen-binding protein (e.g., bispecific antibody construct) in a concentration ranging from about 1 mg/mL to about 20 mg/mL (or from about 1 mg/mL to about 8 mg/mL or from about 1 mg/mL to about 5 mg/mL). In some embodiments, the formulation comprises a bispecific antibody construct n a concentration of about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, about 16 mg/mL, about 17 mg/mL, about 18 mg/mL, about 19 mg/mL or about 20 mg/mL.

[0099] In some embodiments, the formulation comprises a bispecific antibody construct in a concentration ranging from about 0.1 to about 8 mg/mL (or from about 0.5 to about 5 mg/mL or from about 1 to about 5 mg/mL, or from about 3 to about 6 mg/mL). In some embodiments, the formulation comprises a bispecific antibody construct in a concentration of about 0.1 mg/mL, about 0.5 mg/mL, about 1 mg/mL, about 2 mg/mL, about 3 mg/mL, about 4 mg/mL, about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, or about 8 mg/mL.

[00100] *Buffers*

[00101] The pharmaceutical formulation of the invention comprises a buffer, which optionally may be acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, 2-(N-morpholino)ethanesulfonate or combinations thereof.

[00102] Buffering agents are often employed to control pH in the formulation. In some embodiments, the buffer is added in a concentration that maintains pH of the liquid formulation to one that reduces the rate of clipping and aggregating of the bispecific antibody construct in the formulation. In some embodiments, the pH of the liquid formulation is about pH 4.8 to pH 5.5, or about pH 4 to pH 5.5, or about pH 5.2. The effect of pH on formulations may be characterized using any one or more of several approaches such as accelerated stability studies and calorimetric screening studies (Remmele R.L. Jr., et al., Biochemistry, 38(16): 5241-7 (1999)).

[00103] The buffer system present in the formulation is selected to be physiologically compatible and to maintain a desired pH (e.g., between 4.8 to 5.5, or 5.2).

[00104] The buffer may be present in any amount suitable to maintain the pH of the formulation at a predetermined level. The buffer may be present at a concentration between about 0.1 mM and about 1000 mM (1 M), or between about 5 mM and about 200 mM, or between about 5 mM to about 100 mM, or between about 10 mM and about 50 mM. Suitable

buffer concentrations encompass concentrations of about 200 mM or less. In some embodiments, the buffer in the formulation is present in a concentration of about 190 mM, about 180 mM, about 170 mM, about 160 mM, about 150 mM, about 140 mM, about 130 mM, about 120 mM, about 110 mM, about 100 mM, about 80 mM, about 70 mM, about 60 mM, about 50 mM, about 40 mM, about 30 mM, about 20 mM, about 10 mM or about 5 mM. In some embodiments, the concentration of the buffer is at least 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 700, or 900 mM. In some embodiments, the concentration of the buffer is between 1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, or 90 mM and 100 mM. In some embodiments, the concentration of the buffer is between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 mM and 50 mM. In some embodiments, the concentration of the buffer is about 10 mM.

[00105] *Surfactants*

[00106] The pharmaceutical formulations described here comprise a surfactant. Exemplary surfactants include but are not limited to, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, poloxamer 407, triton X-100, polyoxyethylene, PEG 3350, PEG 4000, or a combination thereof.

[00107] Pharmaceutical formulations described herein comprise at least one surfactant, either individually or as a mixture in different ratios. In some embodiments, the formulation comprises a surfactant at a concentration of about 0.001% to about 5% w/v (or about 0.001% to about 0.5%, or about 0.004 to about 0.5% w/v or about 0.001 to about 0.01% w/v or about 0.004 to about 0.01% w/v). In some embodiments, the formulation comprises a surfactant at a concentration of at least 0.001, at least 0.002, at least 0.003, at least 0.004, at least 0.005, at least 0.007, at least 0.01, at least 0.05, at least 0.1, at least 0.2, at least 0.3, at least 0.4, at least 0.5, at least 0.6, at least 0.7, at least 0.8, at least 0.9, at least 1.0, at least 1.5, at least 2.0, at least 2.5, at least 3.0, at least 3.5, at least 4.0, or at least 4.5% w/v. In some embodiments, the formulation comprises a surfactant at a concentration of about 0.001% to about 0.5% w/v. In some embodiments, the formulation comprises a surfactant at a concentration of about 0.001 to about 0.01% w/v. In some embodiments, the formulation comprises a surfactant at a concentration of about 0.001 to about 0.01% w/v. In some embodiments, the formulation comprises a surfactant at a concentration of about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, to about 0.5%

w/v. In some embodiments, the formulation comprises a surfactant incorporated in a concentration of about 0.001% to about 0.01% w/v. In some embodiments, the surfactant is polysorbate 80 and the polysorbate 80 is present in a concentration of about 0.01% w/v.

[00108] *Saccharides*

[00109] The pharmaceutical formulations described herein comprise a saccharide. In some embodiments, the saccharide is a monosaccharide or a disaccharide. In some embodiments, the saccharide is glucose, galactose, fructose, xylose, sucrose, lactose, maltose, trehalose, sorbitol, mannitol or xylitol or a combination thereof.

[00110] In some embodiments, the pharmaceutical formulation comprises a saccharide at a concentration of about 0.01% to about 40% w/v, or about 00.1% to about 20% w/v, or about 1% to about 15%, or about 5% to about 12 %, or about 7% to about 12% w/v. In some embodiments, the pharmaceutical formulation comprises at least one saccharide at a concentration of at least 0.5%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 30%, or at least 40% w/v. In some embodiments, the pharmaceutical formulation comprises at least one saccharide at a concentration of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% w/v. In some embodiments, the pharmaceutical formulation comprises at least one saccharide at a concentration of about 1% to about 15% w/v. In a yet further embodiment, the pharmaceutical formulation comprises at least one saccharide at a concentration of about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, about 10%, about 10.5%, about 11%, about 11.5%, or about 12% w/v. In some embodiments, the pharmaceutical formulation comprises at least one saccharide at a concentration of about 7% to about 12% w/v. In some embodiments, the at least one saccharide is in the formulation at a concentration of about 9% w/v. In some embodiments, the saccharide is sucrose and is present in the formulation ranging from about 9% to about 12% w/v.

[00111] In some embodiments, the pharmaceutical formulation comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, wherein the pH of the pharmaceutical formulation is 5.2.

[00112] In some embodiments, the pharmaceutical formulation comprises 10 mM acetate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, wherein the pH of the pharmaceutical formulation is 5.2.

[00113] *Stability*

[00114] As described in the Examples, the identification of the optimized pH range (pH 4.8- pH 5.2) allows for storage of the pharmaceutical formulations comprising bispecific antibody constructs as a liquid formulation at both 4˚C and 40˚C at various time points. The stability of a bispecific antibody construct formulation can be quantified in several ways. In some embodiments, stability of an antibody formulation is characterized by size exclusion high performance liquid chromatography (SE-HPLC), size exclusion ultra high performance liquid chromatography (SE-UHPLC), cation exchange high performance liquid chromatography (CE-HPLC), dynamic light scattering, analytical ultracentrifugation (AUC), field flow fractionation (FFF), isoelectric focusing and ion exchange chromatography (IEX). In some embodiments, stability of an antibody formulation is characterized by partial dissociation as measured by sodium-dodecyl sulfate capillary electrophoresis (CE-SDS) and/or sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In some embodiments, stability of the formulation is assessed by reduced capillary electrophoresis-sodium dodecyl sulfate (rCE-SDS). The rCE-SDS method separates the heavy chain (HC), light chain (LC), non-glycosylated HC (NGHC), and other minor peak species and groups under reducing conditions.

[00115] In some embodiments, stability of the formulation is characterized by the amount of high molecular (HMW) species of a bispecific antibody construct or by the rate of increase of the amount of HMW species of the bispecific antibody construct under storage conditions at various time points. In some embodiments, the amount of HMW species is determined at one week, two weeks, one months, three months, six months, twelve months, eighteen months or two years in storage at approximately 2-8˚C (e.g., 4˚C) or 40˚C. In some embodiments, the rate of increase of HMW species is determined at one week, two weeks, one months, three months, six months, twelve months, eighteen months or two years in storage at approximately 2-8˚C (e.g., 4˚C) or 40˚C. In some embodiments, the HMW species of a bispecific antibody construct in the formulation is measured by Size Exclusion Chromatography (SEC). In some embodiments, the HMW species of a bispecific antibody construct in the formulation is measured by SE-UHPLC.

[00116] The stability of a bispecific antibody construct, and the capability of the formulation to maintain stability of the bispecific antibody construct, may be assessed over extended periods of time (e.g., weeks or months). In the context of a formulation, a stable formulation is one in which the bispecific antibody construct therein essentially retains its physical and/or chemical integrity and/or biological activity upon storage and during processes such as freeze/thaw, mechanical mixing and lyophilization. Bispecific antibody construct stability can be assessed, for example, by measuring the level and/or rate of formation of high molecular weight (HMW) aggregates, shift of charge profiles, and change in particle size.

[00117] In some embodiments, the relative values of any particular species of the bispecific antibody construct, as described herein, such as the intact BiTE® molecule or main species, or the high molecular weight (HMW) species (i.e., aggregates), or the low molecular weight (LMW) species (i.e., fragments), are expressed in relation to the respective values of the total product. For example, in some embodiments, 10% or less (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less) of the bispecific antibody construct exists as HMW species in the formulation. In some embodiments, the amount of HMW species in the formulation increases less than 10% (e.g., 9%, 8%, 7%, 6%, 5%, 4%, 3% or less) upon storage at 2-8°C for one month or more (e.g., for one month, for three months, for six months, for 12 months, for eighteen months or for two years). In some embodiments, the amount of HMW species in the formulation increases less than 10% or less (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less) upon storage at 40°C for one month or more (e.g., for one week, for two weeks, for one month, for three months, for six months, or for a year). In some embodiments, the HMW species of a bispecific antibody construct in the formulation is measured by Size Exclusion Chromatography (SEC). In some embodiments, the HMW species of a bispecific antibody construct in the formulation is measured by SE-UHPLC.

[00118] In some embodiments, stability of the formulation is characterized by the amount of low molecular (LMW) species of a bispecific antibody construct or by the rate of increase of the amount of LMW species of the bispecific antibody construct under storage conditions at various time points. In some embodiments, the amount of LMW species is determined at one month, three months, six months, twelve months, eighteen months or two years in storage at approximately 2-8°C. In some embodiments, the amount of LMW species is determined at one week, two weeks, one month, three months, six months, or a year in storage or 40°C. In some embodiments, the rate of increase of LMW species is determined at one month, three

months, six months, twelve months, eighteen months or two years in storage at approximately 2-8°C. In some embodiments, the rate of increase of LMW species is determined at one week, two weeks, one month, three months, six months, or a year in storage or 40°C. In some embodiments, the LMW species of a bispecific antibody construct in the formulation is measured by rCE-SDS.

[00119] In some embodiments, less 10% or less (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less) of the bispecific antibody construct exists as LMW species in the formulation. In some embodiments, the amount of LMW species in the formulation increases less than 10% (e.g., 9%, 8%, 7%, 6%, 5%, 4%, 3% or less) upon storage at 2-8°C for one month or more (e.g., for one month, for three months, for six months, for 12 months, for eighteen months or for two years). In some embodiments, the amount of LMW species in the formulation increases less than 10% or less (e.g., 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less) upon storage at 40°C for one month or more (e.g., for one week, for two weeks, for one month, for three months, for six months, or for a year). In some embodiments, the LMW species of a bispecific antibody construct in the formulation is measured by rCE-SDS.

[00120] In some embodiments, the percent of intact BiTE® molecule (i.e., main peak species) in the formulation is greater than 90% (90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) of the total protein content in the formulation.

[00121] In one embodiment, the liquid formulation is stable upon storage at about 2-8°C for one month, and the percent of intact BiTE® molecule (i.e., main peak species) is greater than 95% of the total protein content in the formulation. In another embodiment, the liquid formulation is stable upon storage at about 40°C for one month, and the percent of intact BiTE® molecule (i.e., main peak species) is greater than 95% of the total protein content in the formulation.

[00122] The stability of a formulation described herein can also be characterized by charge distribution, e.g., a change in the amount of the charge variant peaks of the antibody. For example, in some embodiments, the amount of acidic peak (e.g., deamidation, charge variants having a relatively lower isolectric point (pI)) in the formulation increases by less than 2% (e.g., 2%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, or less) when stored at 4°C for at least one month (e.g., for one month, three months, six months or twelve months). In some embodiments, the amount of basic peak (e.g., charge variants having a relatively higher pI) in the formulation increases by less than 6%

(e.g., 6%, 5%, 4%, 3%, 2% or 1%) when stored at 4°C for at least one month (e.g., for one month, three months, six months or twelve months).

[00123] In some embodiments, the amount of acidic peak in the formulation increases by less than 30% (e.g., 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 4%, 4%, 3%, 2%, 1% or less) when stored at 40°C for at least one week (e.g., for one week, two weeks, one month or three months). In some embodiments, the amount of basic peak (e.g., charge variants having a relatively higher pI) in the formulation increases by less than 15% (e.g., 15%, 10%, 9%, 8%, 7%, 6%, 4%, 4%, 3%, 2%, 1% or less), when stored at 40°C for at least one week (e.g., for one week, two weeks, one month or three months).

[00124] The stability of a liquid formulation described herein can also be assessed by determining the level of relative potency of the bispecific antibody construct in the liquid formulation under storage conditions. Relative potency of a bispecific antibody construct can be determined, for example, by a cell-based bioassay that measures cell death by the loss of luminescence in a carcinoma cell line. The biological activity of the test sample is determined by comparing the test sample response to that of the reference standard (relative potency). In some embodiments, the relative potency of the bispecific antibody construct in the formulation is greater than about 30% (e.g., about 35%, 40%, 50%, 55%, 60%) or more after storage at one month at about 40°C as assessed as described in Example 3.

[00125] *Therapeutic Use of the Formulation*

[00126] The formulations described herein are useful as pharmaceutical formulations in the treatment, amelioration of cancer in a subject in need thereof. The terms “subject in need” or those “in need of treatment" includes those already with the disorder, as well as those in which the disorder is to be prevented. The “subject in need” or "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment. The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Treatment includes the application or administration of the formulation to the body, an isolated tissue, or cell from a patient who has a disease/disorder, a symptom of a disease/disorder, or a predisposition toward a disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptom of the disease, or the predisposition toward the disease.

[00127] The term “amelioration” as used herein refers to any improvement of the disease state of a patient having a tumor or cancer or a metastatic cancer as specified herein below,

by the administration of formulation comprising an antigen-binding protein described herein to a subject in need thereof. Such an improvement may also be seen as a slowing or stopping of the progression of the tumor or cancer or metastatic cancer of the patient. The term "prevention" as used herein means the avoidance of the occurrence or re-occurrence of a patient having a tumor or cancer or a metastatic cancer as specified herein below, by the administration of the formulation comprising an antigen-binding protein (i.e., bispecific antibody construct) described herein to a subject in need thereof.

[00128] The disclosure provides a method of treating cancer, comprising administering to a subject in need thereof a therapeutically effective amount of a recombinant protein or a pharmaceutical formulation described herein. In certain embodiments, the subject is a human. In certain embodiments, the cancer is a solid tumor.

[00129] In some embodiments, the cancer is brain cancer, bladder cancer, breast cancer, clear cell kidney cancer, cervical cancer, colon and rectal cancer, endometrial cancer, gastric cancer, head/neck squamous cell carcinoma, lip and oral cancer, liver cancer, lung squamous cell carcinoma, melanoma, mesothelioma, non-small-cell lung cancer (NSCLC), non-melanoma skin cancer, ovarian cancer, oral cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, sarcoma, small-cell lung cancer (SCLC), Squamous Cell Carcinoma of the Head and Neck (SCCHN), triple negative breast cancer, or thyroid cancer.

[00130] In some embodiments, the cancer is adrenocortical tumor, alveolar soft part sarcoma, carcinoma, chondrosarcoma, colorectal carcinoma, desmoid tumors, desmoplastic small round cell tumor, endocrine tumors, endodermal sinus tumor, epithelioid hemangioendothelioma, Ewing sarcoma, germ cell tumor, hepatoblastoma, hepatocellular carcinoma, melanoma, nephroma, neuroblastoma, non-rhabdomyosarcoma soft tissue sarcoma (NRSTS), osteosarcoma, paraspinal sarcoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, synovial sarcoma, or Wilms tumor.

[00131] In some embodiments, the cancer is acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), or chronic myeloid leukemia (CML).

[00132] In some embodiments, the cancer is diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or small lymphocytic lymphoma (SLL).

[00133] Indeed, cancers that can be treated include, but are not limited to, alveolar rhabdomyosarcoma, bone cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, esophageal cancer, gastrointestinal carcinoid tumor, hypopharynx cancer, larynx cancer, nasopharynx cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, ureter cancer, and urinary bladder cancer.

[00134] *Routes of Administration*

[00135] Preferably, the pharmaceutical formulation is administered parenterally, e.g., intravenously, subcutaneously, or intramuscularly. Parenteral administration may be achieved by injection, such as bolus injection, or by infusion, such as continuous infusion. Administration may be achieved via depot for long-term release. In some embodiments, the formulation is administered intravenously by an initial bolus followed by a continuous infusion to maintain therapeutic circulating levels of drug product. In some embodiments, the formulation is administered as a one-time dose. Pharmaceutical formulations may be administered using a medical device. Examples of medical devices for administering pharmaceutical formulations are described in U.S. Patent Nos. 4,475,196; 4,439,196; 4,447,224; 4,447, 233; 4,486,194; 4,487,603; 4,596,556; 4,790,824; 4,941,880; 5,064,413; 5,312,335; 5,312,335; 5,383,851; and 5,399,163.

[00136] The disclosure also contemplates uninterrupted administration of the suitable formulation. As a non-limiting example, uninterrupted or substantially uninterrupted, i.e., continuous administration may be realized by a small pump system worn by the patient for metering the influx of therapeutic agent into the body of the patient. The pharmaceutical formulation can be administered by using said pump systems. Such pump systems are generally known in the art, and commonly rely on periodic exchange of cartridges containing the therapeutic agent to be infused. When exchanging the cartridge in such a pump system, a temporary interruption of the otherwise uninterrupted flow of therapeutic agent into the body of the patient may ensue. In such a case, the phase of administration prior to cartridge replacement and the phase of administration following cartridge replacement would still be considered within the meaning of the pharmaceutical means and methods of the invention together make up one “uninterrupted administration” of such therapeutic agent.

[00137] The continuous or uninterrupted administration of the formulation may be intravenous or subcutaneous by way of a fluid delivery device or small pump system including a fluid driving mechanism for driving fluid out of a reservoir and an actuating mechanism for actuating the driving mechanism. Pump systems for subcutaneous administration may include a needle or a cannula for penetrating the skin of a patient and delivering the suitable formulation into the patient's body. Said pump systems may be directly fixed or attached to the skin of the patient independently of a vein, artery or blood vessel, thereby allowing a direct contact between the pump system and the skin of the patient. The pump system can be attached to the skin of the patient for 24 hours up to several days. The pump system may be of small size with a reservoir for small volumes. As a non-limiting example, the volume of the reservoir for the suitable pharmaceutical formulation to be administered can be between 0.1 and 50 ml.

[00138] *Kits*

[00139] As an additional aspect, the described herein are kits which comprise one or more pharmaceutical formulations described herein packaged in a manner which facilitates their use for administration to subjects. In one embodiment, such a kit includes a formulation described herein (e.g., a formulation comprising an antibody described therein), packaged in a container such as a sealed bottle, vessel, single-use or multi-use vial, prefilled syringe, or prefilled injection device, optionally with a label affixed to the container or included in the package that describes use of the compound or formulation in practicing the method. In one aspect, the formulation is packaged in a unit dosage form. The kit may further include a device suitable for administering the formulation according to a specific route of administration. Preferably, the kit contains a label that describes use of an antibody described herein or formulation described herein.

[00140] The pharmaceutical formulations described herein can be formulated in various forms, e.g., in solid, liquid, frozen, gaseous or lyophilized form and may be, inter alia, in the form of an ointment, a cream, transdermal patches, a gel, powder, a tablet, solution, an aerosol, granules, pills, suspensions, emulsions, capsules, syrups, liquids, elixirs, extracts, tincture or fluid extracts.

[00141] Generally, various storage and/or dosage forms are conceivable for the pharmaceutical formulation of the invention, depending, i.e., on the intended route of administration, delivery format and desired dosage (see, for example, Remington's

Pharmaceutical Sciences, 22nd edition, Oslo, A., Ed., (2012)). The skilled person will be aware that such choice of a particular dosage form may for example influence the physical state, stability, rate of *in vivo* release and rate of in vivo clearance of an antibody.

[00142] For instance, the primary vehicle or carrier in a pharmaceutical formulation may be either aqueous or non-aqueous in nature. A suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in formulations for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles.

EXAMPLES

[00143] Materials and Methods

[00144] Stability samples were analyzed using a variety of techniques to monitor critical quality attributes of the drug product. The following stability indicating assays were performed at accelerated conditions: SE- UHPLC (Size Exclusion Ultra High-Performance Liquid Chromatography) to monitor aggregation, rCE-SDS and Multi-Attribute Method (MAM) to monitor clipping, and visual appearance for visible particle analysis respectively.

[00145] Size Exclusion Ultra High-Performance Liquid Chromatography (SE-UHPLC) separates proteins based on differences in their hydrodynamic volumes. Molecules with higher hydrodynamic volumes elute earlier than molecules with smaller volumes. The samples are loaded onto an SE-UHPLC column (BEH200, 4.6 x 300 mm, (Waters Corporation, 186005226)), separated isocratically and the eluent is monitored by UV absorbance. Purity is determined by calculating the percentage of each separated component as compared to the total integrated area. SE-UHPLC settings are as follows: Flow rate: 0.4 mL/min, Run time: 12 min, UV detection: 280 nm, Column temperature: Ambient, Target protein load: 6 µg, Protein compatible flow cell: 5 mm.

[00146] Reduced capillary electrophoresis- sodium dodecyl sulfate (rCE-SDS) separates proteins based on differences in their hydrodynamic size under reducing and denaturing conditions. The protein species are bound to SDS, an anionic detergent, and electokinetically injected into a bare fused silica capillary filled with SDS gel buffer. An electric voltage is applied across the capillary, under which the SDS coated proteins are separated by their difference in migration in a hydrophilic polymer-based solution. Proteins are detected by a photodiode array (PDA) detector as they pass through a UV detection window. Purity is evaluated by determining the percent corrected peak area of reach component. The rCE-SDS

method separates the heavy chain (HC), light chain (LC), non-glycosylated HC (NGHC), and other minor peak species and groups under reducing conditions.

Example 1 –Increased pH reduced percentage of LMW species as assessed by CE-SDS

[00147] The stability of each of BiTE®-I, BiTE®-C, BiTE®-D and BiTE®-G in the following formulation at various pH (pH 4.2, 4.8 and 5.5) was assessed in an accelerated stability study. The formulations were stored at 40°C for one month, and the level of LMW species was then analyzed by reduced capillary electrophoresis sodium dodecyl sulfate (rCE-SDS):

[00148] Liquid formulation tested: 1 mg/mL BiTE molecule, 10 mM L-glutamic acid, 9% (w/v) sucrose, 0.01% (w/v) Polysorbate 80.

[00149] As shown in Figure 1 and in Table 1 below, liquid formulations at pH 4.8 and pH 5.2 had a reduced percentage of LMW species after storage at 40°C for 1 month when compared to liquid formulations at pH 4.2 stored under the same storage conditions.

[00150] Table 1. % LMW after 1-month storage at 40°C (rCE-SDS)

	% LMW at pH 4.2	% LMW at pH 4.8	% LMW at pH 5.2
BiTE®-I	6	4.2	2.6
BiTE®-C	6.1	4	4.5
BiTE®-D	6.4	4.6	3
BiTE®-G	5.3	3.2	3

Example 2 – Assessment of LMW Species by MAM

[00151] The percent clipping of BiTE-G in the liquid formulation (described in Example 1) at pH 4.2, pH 4.8 and pH 7.4 was assessed by MAM (multi-attribute methodology) after storage for one month at 40°C. Briefly, samples were captured on a 30 kDa MWCO spin filter and denatured, reduced, and then alkylated on the filter. Subsequently, the samples were buffer exchanged into digest buffer, and then digested with trypsin. Digested samples were centrifuged, and tryptic peptides were captured. A sequential on-filter digestion with human neutrophil elastase was then performed, and resulting peptides were collected and combined with the tryptic peptides. Digestion was quenched by addition of 8M guanidine in acetate buffer (pH 4.8). Peptides were then analyzed with LC-MS using an Agilent 1290 UPLC (Waters BEH C18 column) and a Thermo Q Exactive Biopharma using data-dependent acquisition. Resulting data were searched with MassAnalyzer to determine

sequence coverage, and quantitation was performed using Skyline workbooks. Observed chemical modifications fell within the commercially acceptable ranges (data not shown).

Example 3 – BiTE molecule® Retains Potency at a pH Greater than 4.2

[00152] The relative potency of BiTE®-G in the liquid formulation (described in Example 1) at pH 4.2 and pH 4.8 was also assessed after storage at 2-8˚C for two years. Briefly, the potency of BiTE®-G was measured by a cell-based bioassay that measures cell death by the loss of luminescence in a carcinoma cell line. The biological activity of the test sample was determined by comparing the test sample response to that of the reference standard (relative potency). This assay demonstrated that BiTE®-G in the liquid formulation at both pH 4.2 and pH 4.8 exhibited an acceptable level potency after storage at 2-8˚C for two years.

Example 4 – Clipping rates of a BiTE® molecule vary at different pH

[00153] The clipping rates for BiTE®-G in the liquid formulation (described in Example 1) at pH 4.2 and pH 4.8 was assessed by rCE-SDS. As shown in Figure 2, and Table 2 below, the liquid formulation at pH 4.8 showed a 27% decrease in clipping levels compared to the liquid formulation at pH 4.2 when stored at 40˚C for one month. A 29% decrease in clipping levels was also observed in the liquid formulation at pH 4.8 compared to the liquid formulation at pH 4.2 when stored at 2-8˚C for two years.

[00154] Table 2. Decrease in clip levels at pH 4.8 compared to pH 4.2 formulation under accelerated and recommended storage conditions.

	% LMW after 40°C for 1 month	% LMW after 2-8°C for 2 years
pH 4.2	10	2.1
pH 4.8	7.3	1.5

Example 5 - Assessment of HMW species by SE-UHLPC

[00155] The percent high molecular weight (HMW) species was assessed by size-exclusion UltraPerformance liquid chromatography(SE-UHLPC) in liquid formulations described in Example 1, comprising BiTE®-G at pH 4.2 and pH 4.8 after storage at either 2-8˚C or -30˚C for one month, three months, six months, twelve months, eighteen months and two years. The analysis demonstrated that the observed percent HMW for BiTE®-G in both the pH 4.2 formulation and the pH 4.8 formulation was less than 0.35% under storage conditions at 2-8˚C at all tested time points (data not shown). The analysis also demonstrated that the observed percent HMW for BiTE®-G in both the pH 4.2 formulation and the pH 4.8

formulation was less than 1.3% under storage conditions at -30°C at all tested time points (data not shown).

Example 6 – Assessment of both LMW and low HMW under various storage conditions

[00156] The percent LMW and HMW species of BiTE®-G and BiTE®-D in various liquid formulations was assessed in each of the formulations is set out below. The %LMW was determined by reduced capillary electrophoresis (rCE) and the % HMW was determined by size-exclusion chromatograph (SEC). The liquid formulations tested were as follows:

[00157] Formulation A: 1 mg/mL BiTE® molecule, 10 mM L-glutamic acid, 9% (w/v) sucrose, 0.01% (w/v) Polysorbate 80, pH 3.8;

[00158] Formulation B: 1 mg/mL BiTE® molecule, 10 mM L-glutamic acid, 9% (w/v) sucrose, 0.01% (w/v) Polysorbate 80, pH 4.8;

[00159] Formulation C: 1 mg/mL BiTE® molecule, 10 mM L-glutamic acid, 9% (w/v) sucrose, 0.01% (w/v) Polysorbate 80, pH 5.2;

[00160] Formulation D: 1 mg/mL BiTE® molecule, 10 mM acetate, 9% (w/v) sucrose, 0.01% (w/v) polysorbate 80, pH 5.2;

[00161] Formulation E: 1 mg/mL BiTE® molecule, 10 mM phosphate, 9% (w/v) sucrose, 0.01% (w/v) polysorbate 80, pH 7.4.

[00162] The results as shown in Figures 3A-3D and Tables 3-6 below:

[00163] Table 3. Percent LMW of BiTE®-G as determined by rCE

Formulation	Time Point	Temperature	%LMW
	T=0	4C	2.9
A	1M	4C	3.2
	1M	40C	28.0
	T=0	4C	3.1
B	1M	4C	1.4
	1M	40C	15.4
	T=0	4C	2.8
C	1M	4C	1.3
	1M	40C	14.3
	T=0	4C	3.1
D	1M	4C	1.5
	1M	40C	15.1
	T=0	4C	2.8
E	1M	4C	3.4
	1M	40C	40.0

[00164] Table 4. Percent LMW of BiTE®-D as determined by rCE

Formulation	Time Point	Temperature	%LMW	%Main
	T=0	4C	0.8	99.2
A	1M	4C	1.3	98.7
	1M	40C	34.4	65.6
	T=0	4C	0.8	99.2
B	1M	4C	0.9	99.1
	1M	40C	17.0	83.0
	T=0	4C	0.9	99.1
C	1M	4C	2.2	97.8
	1M	40C	16.0	84.0
	T=0	4C	1.0	99.0
D	1M	4C	1.5	98.5
	1M	40C	15.4	84.6
	T=0	4C	1.1	98.9
E	1M	4C	1.0	99.0
	1M	40C	39.0	61.0

[00165] Table 5. Percent HMW of BiTE®-G as determined by SEC.

Formulation	Time point	Temperature	%HMW
	T=0	4C	0.1
A	1M	4C	0.1
	1M	40C	0
	T=0	4C	0.7
B	1M	4C	0.7
	1M	40C	0.6
	T=0	4C	1.6
C	1M	4C	0.6
	1M	40C	1.7
	T=0	4C	1.1
D	1M	4C	0.1
	1M	40C	1.3
	T=0	4C	15.7
E	1M	4C	16.4
	1M	40C	19.2

[00166] Table 6. Percent HMW of BiTE®-D as determined by SEC.

Formulation	Time point	Temperature	%HMW
	T=0	4C	0.2
A	1M	4C	0.3
	1M	40C	0.1
	T=0	4C	0.4
B	1M	4C	0.4
	1M	40C	1

	T=0	4C	0.6
C	1M	4C	0.7
	1M	40C	1.3
	T=0	4C	0.6
D	1M	4C	0.7
	1M	40C	1.6
	T=0	4C	3.3
E	1M	4C	3.4
	1M	40C	7.1

[00167] As shown in Figures 3A-3D, Formulation B (pH 4.8) and Formulation C (pH 5.2) showed the lowest percentage of both LMW and HMW species compared to Formulation A (pH 3.8) and Formulation E (pH 7.4) after storage at both 4°C and 40°C for four weeks. Formulation D (pH 5.2) also showed a low percentage of both LMW and HMW, demonstrating that buffer selection in the formulation does not affect the fragmentation of the BiTE® molecule. The data provided in this Example demonstrates, that regardless of the buffer within the formulation, identification of the optimized pH range (pH 4.8- pH 5.5) allows for storage of the pharmaceutical formulations comprising bispecific antibody constructs as a liquid formulation at both 4°C and 40°C at various time points.