[0005] Aggregation is the main type of physical instability observed in high concentration formulations. It is the assembly from initially native and folded proteins into high molecular weight species (HMW). Currently, protein aggregates are removed as impurities mainly in the polishing steps of downstream processing. However, in cases of high levels of HMW species, removing significant amount of HMW not only results in substantial yield loss but also makes the design of a robust downstream process challenging (Chi et al., Pharm Res, Vol. 20, No. 9, Sept 2003, pp. 1325-1336).

SUMMARY

[0006] The present disclosure provides stable formulations comprising a high concentration (i.e., greater than 10 mg/mL) of bispecific antibody constructs (e.g., BiTE® molecules), wherein less than approximately 3% of the bispecific antibody construct exists as high molecular weight (HMW) species in the formulation. Formulations comprising high concentration of antibodies, while desirable, are generally difficult to stabilize under storage conditions. It was surprisingly found that a formulation according to the present disclosure comprising a bispecific antibody construct at a higher concertation than expected is stable, without requiring the presence of a preservative or stabilizing agent.

[0007] In some examples, the formulation is a lyophilized formulation. In some examples, 2.5% or less (e.g., 2.5%, or 2.0%, or 1.9%, or 1.8%, or 1.7%, or 1.6%, or 1.5%, or 1.4%, or 1.3%, or 1.2%, or 1.1%, or 1.0%, or 0.5%) of the bispecific antibody construct exists as HMW species in the lyophilized formulation. In some examples, the amount of HMW species in the lyophilized formulation increases less than 1%, (e.g., 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%) upon storage at 4˚C for one month or more (e.g., for one month, for three months, or for six months). In some examples, upon storage at 4˚C for one month or more (e.g., for one month, for three months, or for six months), the amount of HMW species in the formulation increases approximately between 0.1% and 0.4% (e.g., 0.1%, 0.2%, 0.3%, or 0.4%). In some examples, the amount of HMW species in the lyophilized formulation increases less than 1%, (e.g., 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%) upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months). In some examples, upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months) the amount of HMW species in the

lyophilized formulation increases approximately between 0.1% and 0.7% (e.g., 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%. or 0.7%).

[0008] In some examples, less than 2%, (e.g., 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, or 0.5%) of the bispecific antibody construct exists as low molecular weight (LMW) species in the lyophilized formulation. In some examples, the amount of LMW species in the lyophilized formulation increases less than 2%, (e.g., 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, or 0.5%) upon storage at 4˚C for one month or more (e.g., for one month, for three months, or for six months). In some examples, upon storage at 4˚C for one month or more (e.g., for one month, for three months, or for six months), the amount of LMW species in the formulation increases approximately between 0.1% and 0.7% (e.g., 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%. or 0.7%). In some examples, the amount of LMW species in the lyophilized formulation increases less than 1%, (e.g., 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%) upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months). In some examples, upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months) the amount of LMW species in the lyophilized formulation increases approximately between 0.1% and 0.7% (e.g., 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%. or 0.7%).

[0009] In some examples, the percent of intact BiTE® molecule (i.e., main peak species) in the lyophilized formulation is greater than 95% of the total protein content in the formulation.

[0010] In some examples, the lyophilized formulation is stable upon storage at about 4˚C for one month, and the amount of HMW species in the formulation increases approximately between 0.1% to 0.7% (e.g., 0.1%, or 0.2%, or 0.3%, or 0.4%, or 0.5%, or 0.6%, or 0.7%) while in storage for at least one month. In some examples, the lyophilized formulation is stable upon storage at about 4˚C for three months, and the amount of HMW species in the formulation increases approximately between 0.0% to 0.2% (e.g., 0%, or 0.1%, or 0.2%), while in storage for at least three months. In some examples, the lyophilized formulation is stable upon storage at about 4˚C for six months, and the amount of HMW species in the formulation increases approximately between 0.0% to 0.4% (e.g., 0%, or 0.1%, or 0.2%, or 0.3%, or 0.4%), while in storage for at least six months.

[0011] In some examples, the lyophilized formulation is stable upon storage at about 4˚C for at least one month, three months and six months, and the percent of intact BiTE® molecule is above 95% of the total protein content during that time period in storage.

[0012] In some examples, the formulation is a liquid formulation. In some examples, less than 3% (e.g., 2.5% or 2%, or 1.5%, or 1%, or 0.5%) of the bispecific antibody construct exists as HMW species in the liquid formulation. In some examples, the amount of HMW species in the liquid formulation increases less than 3% (e.g., 3%, 2.5%, 2%, 1%, or 0.5%) upon storage at 4˚C for one month or more (e.g., for one month, for three months, for six months, or for one year). In some examples, upon storage at 4˚C for one month or more (e.g., for one month, for three months, for six months or for one year), the amount of HMW species in the formulation increases approximately between 0.1% and 1% (e.g., 0.1%, or 0.2%, or 0.3%, or 0.4%, or 0.5%, or 0.6%, or 0.7%, or 0.8%, or 0.9%, or 1%). In some examples, the amount of HMW species in the liquid formulation increases less than 5% (e.g., 4.5%, or 4%, or 3.5%, or 3%, or 2.5%, or 2%, or 1.5%, or 1%, or 0.5%) upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months). In some examples, upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months) the amount of HMW species in the liquid formulation increases approximately between 0.1% and 5% (e.g., 0.1%, or 0.2%, or 0.3%, or 0.4%, or 0.5%, or 0.6%, or 0.7%, or 0.8%, or 0.9%, or 1%, or 1.5%, or 2%, or 2.5%, or 3%, or 3.5%, or 4%, or 4.5% or 5%).

[0013] In some examples, less than 2%, (e.g., 1.9%, or 1.8%, or 1.7%, or 1.6%, or 1.5%, or 1.4%, or 1.3%, or 1.2%, or 1.1%, or 1%, or 0.9%, or 0.8%, or 0.7%, or 0.6%, or 0.5%) of the bispecific antibody construct exists as low molecular weight (LMW) species in the liquid formulation. In some examples, the amount of LMW species in the liquid formulation increases less than 2%, (e.g., 1.9%, or 1.8%, or 1.7%, or 1.6%, or 1.5%, or 1.4%, or 1.3%, or 1.2%, or 1.1%, or 1%, or 0.5%, 0.4%, or 0.3%, or 0.2% or 0.1%) upon storage at 4˚C for one month or more (e.g., for one month, for three months, for six months, or for twelve months). In some examples, upon storage at 4˚C for one month or more (e.g., for one month, for three months, for six months, or for twelve months), the amount of LMW species in the liquid formulation increases approximately between 0.1% and 0.7% (e.g., 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%. or 0.7%). In some examples, the amount of LMW species in the liquid formulation increases less than 7%, (e.g., 6%, or 5%, or 4%, or 3%, or 2%, or 1%, or 0.9%,

0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%) upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months). In some examples, upon storage at 40˚C for one week or more (e.g., for one week, for two weeks, for one month or for three months) the amount of LMW species in the lyophilized formulation increases approximately between 0.1% and 7% (e.g., 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%. or 0.7%, or 0.8%, or 0.9%, or 1%, or 1.5%, or 2%, or 3%, or 5%, or 6%, or 7%).

[0014] In some examples, the percent of intact BiTE® molecule (i.e., main peak species) in the liquid formulation is greater than 96% of the total protein content in the formulation.

[0015] In some examples, the liquid formulation is stable upon storage at about 4˚C for one month, and the amount of HMW species in the formulation increases approximately between 0.1% to 0.4% (e.g., 0.1%, or 0.2%, or 0.3%, or 0.4%). In some examples, the liquid formulation is stable upon storage at about 4˚C for three months, and the amount of HMW species in the formulation increases approximately between 0.0% to 0.3% (e.g., 0%, or 0.1%, or 0.2%, or 0.3%), while in storage for at least three months. In some examples, the liquid formulation is stable upon storage at about 4˚C for six months, and the amount of HMW species in the formulation increases approximately between 0.0% to 0.6% (e.g., 0%, or 0.1%, or 0.2%, or 0.3%, or 0.4%, or 0.5%, or 0.6%), while in storage for at least six months. In some examples, the liquid formulation is stable upon storage at about 4˚C for twelve months, and the amount of HMW species in the formulation increases approximately between 0.0% to 0.2% (e.g., 0%, or 0.1%, or 0.2%), while in storage for at least twelve months.

[0016] In some examples, the lyophilized formulation is stable upon storage at about 4˚C for one month, three months, six months, and twelve months, and the percent of intact BiTE® molecule is above 96% of the total protein content.

[0017] In some examples, the antibody binding protein is a bispecific antibody construct comprising 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.

[0018] In some examples, 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 examples, each of the first and second binding domains of the bispecific antibody construct comprises a VH region and a VL region.

[0019] In some examples, the bispecific antibody construct is a single chain antibody construct.

[0020] In some examples, 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.

[0020a] In one aspect, the present disclosure refs to a lyophilized pharmaceutical formulation that, when reconstituted, comprises a bispecific antibody construct in an amount of at least 10 mg/mL, a buffer, a saccharide at a concentration ranging from 1 to 15% (w/v), and a surfactant at a concentration ranging from 0.001% to 0.5% (w/v), wherein the formulation has a pH of 4-6, and wherein the bispecific antibody construct is in single-chain variable fragment (scFv) format and comprises a first binding domain that binds to a target cell surface antigen and a second binding domain that binds to human CD3 on the surface of a T cell.

[0021] In some examples, 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, SEQ ID NOs: 166-171, or SEQ ID NOs: 175-180, (l) SEQ ID NOs:132-137, or (m) SEQ ID NOs: 123-128.

[0022] In some examples, 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 or 17, 52, 94, 73**,** 62, 118, 154,163, 172, 181, 106, 138,143, or 129. In some examples, 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 or 17, 52, 94, 73, 62, 118, 154, 163, 172, 181, 106, 138, 143, or 129.

[0023] In some examples, 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 examples, 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.

[0024] In some examples, 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.

[0025] In some examples, the second binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in SEQ ID NOs: 1-6.

[0026] In some examples, 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 examples, the second binding domain of the bispecific antibody construct comprises a VH comprising the amino acid sequence set forth in SEQ ID NO: 7.

[0027] In some examples, 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 examples, the second binding domain of the bispecific antibody construct comprises a VL comprising the amino acid sequence set forth in SEQ ID NO: 8.

[0028] In some examples, 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.

[0029] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 87.

[0030] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 43, 44 or 45.

[0031] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 76 or 77.

[0032] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 65 or 66.

[0033] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 33.

[0034] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 98 or SEQ ID NO: 97.

[0035] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 121, 122, 109, 110 or 111.

[0036] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 20, 21, 22 or 23.

[0037] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 55.

[0038] In some examples, 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 examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 156, 165, 174 or 183.

[0039] In some examples, 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 example, 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 examples, the bispecific antibody construct comprises the amino acid sequence set forth in SEQ ID NO: 141, 142, 146 or 147.

[0040] In some examples, 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 examples, the bispecific antibody construct comprises an amino acid sequence set forth in SEQ ID NO: 131.

[0041] The pharmaceutical formulation of the disclosure comprises a buffer. In some examples, the buffer is acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, 2-(N-morpholino)ethanesulfonate or a combination thereof. In some examples, 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).

[0042] The pharmaceutical formulation of the disclosure comprises a saccharide. In some examples, the saccharide is monosaccharide or a disaccharide. In some examples, the saccharide is a sugar alcohol (e.g., sorbitol). In some examples, the saccharide is sucrose, trehalose, mannitol, sorbitol or a combination thereof. In some examples, 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)).

[0043] The pharmaceutical formulation of the disclosure comprises a surfactant. In some examples, 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 examples, 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)).

[0044] In some examples, the formulation has an osmolarity in the range of about 150 to about 500 mOsm. In some examples, the formulation has an osmolarity of no greater than 500 mOsm/L, 450 mOsm/L, 400 mOsm/L, or 350 mOsm/L. In some examples, the formulation is close to isotonic, e.g. 250-350 mOsm/L.

[0045] The pharmaceutical formulation, in some examples, comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, and wherein the pH of the pharmaceutical formulation is 4.2. In some examples, the bispecific antibody construct is present in the formulation at a concentration ranging from about 10 mg/mL to about 100 mg/mL. In some examples, the bispecific antibody construct is present in the formulation at a concentration of

10 mg/mL, 11 mg/mL, 12 mg/mL, 13 mg/mL, 14 mg/mL, 15 mg/mL, 16 mg/mL, 17 mg/mL, 18 mg/mL, 19 mg/mL, 20 mg/mL, 21 mg/mL, 22 mg/mL, 23 mg/mL, 24 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, or 50 mg/mL. In some examples, the bispecific antibody is present in the formulation in an amount ranging from about 1000 µg to about 200 mg.

[0046] In some examples, the pharmaceutical formulation of the disclosure is a liquid formulation.

[0047] In another aspect, the present disclosure refers to a use of the formulation described herein in the manufacture of a medicament for treating cancer in a subject in need thereof. In yet another aspect, the present disclosure refers to a formulation described herein for use in therapy.

[0048] It should be understood that while various examples in the specification are presented using "comprising" language, under various circumstances, a related example may also be described using "consisting of" or "consisting essentially of" language. The disclosure contemplates examples described as "comprising" a feature to include examples 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.

[0049] 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 to about pH 6," could be, but is not limited to, pH 4, 4.2, 4.6, 5.1, 5.5 etc. and any value in between such values. Additionally, "a pH from about pH 4 to about pH 6," should not be construed to mean that the pH of a formulation in question varies 2 pH units in the range from pH 4 to pH 6 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.

[0050] 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.

[0051] 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 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 examples of the disclosure. Likewise, features of the disclosure described herein can be re-combined into additional examples that also are intended as examples of the disclosure, irrespective of whether the combination of features is specifically mentioned above as an aspect or example of the disclosure. Also, only such limitations which are described herein as critical to the disclosure should be viewed as such; variations lacking limitations which have not been described herein as critical are intended as examples of the disclosure.

[0052] All references cited herein are hereby incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE FIGURES

[0053] Figure 1 provides graphs showing the percent high molecular weight (HMW) species of BiTE®-I, BiTE®-C, and BiTE®-G, each at 20 mg/mL in a lyophilized formulation comprising 10 mM glutamate, 9% w/v sucrose, 0.01% w/v Polysorbate 80, pH 4.2, when assessed by size-exclusion ultra high-performance liquid chromatography (SE-UHPLC) under storage conditions at 4˚C t time points 0, one month and three months.

[0054] Figure 2 provides graphs showing the percent high molecular weight (HMW) species of BiTE®-I , BiTE®-C, and BiTE®-G, each at 20 mg/mL in a liquid formulation comprising 10 mM glutamate, 9% w/v sucrose, 0.01% w/v Polysorbate 80, pH 4.2, when assessed by size-exclusion ultra high-performance liquid chromatography (SE-UHPLC) under storage conditions at 4˚C t time points 0, one month and three months.

DETAILED DESCRIPTION

[0055] 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. There is a critical need in the art to increase stability and reduce aggregation of therapeutic proteins; and optimized pharmaceutical formulations can aid in doing so.

[0056] Normally, antibody constructs as described herein are stored and/or employed in a lyophilized formulation at a concentration of about 1 mg/ml (International Publication No. WO

What is claimed is:

1. A lyophilized pharmaceutical formulation that, when reconstituted, comprises a bispecific antibody construct in an amount of at least 10 mg/mL, a buffer, a saccharide at a concentration ranging from 1 to 15% (w/v), and a surfactant at a concentration ranging from 0.001% to 0.5% (w/v), wherein the formulation has a pH of 4-6, and wherein the bispecific antibody construct is in single-chain variable fragment (scFv) format and comprises a first binding domain that binds to a target cell surface antigen and a second binding domain that binds to human CD3 on the surface of a T cell.

2. The formulation of claim 1, that is stable for up to 3 months at 4°C.

3. The formulation of claim 1 or claim 2, wherein the formulation comprises less than 2% high molecular weight species after 3 months at 4°C.

4. The formulation of any one of claims 1-3, wherein the formulation has a pH of 4-5.

5. The formulation of any one of claims 1-3, wherein the formulation has a pH of 4.2.

6. The formulation of any one of claims 1-5, wherein the buffer is an acetate buffer, a glutamate buffer, a citrate buffer, a lactic buffer, a succinate buffer, a tartrate buffer, a fumarate buffer, a maleate buffer, a histidine buffer, or a phosphate buffer.

7. The formulation of any one of claims 1-6, that when reconstituted, comprises the buffer at a concentration ranging from 5 to 200 mM.

8. The formulation of claim 7, wherein the buffer is present at a concentration ranging from 10 to 50 mM.

9. The formulation of any one of claims 1-8, wherein the saccharide is a monosaccharide or a disaccharide.

10. The formulation of any one of claims 1-9, wherein the saccharide is glucose, galactose, fructose, xylose, sucrose, lactose, maltose, trehalose, sorbitol, mannitol or xylitol.

11. The formulation of any one of claims 1-10, that when reconstituted, comprises the saccharide at a concentration ranging from 5 to 12% (w/v).

12. The formulation of claim 11, wherein the saccharide is present in a concentration ranging from 7 to 12% (w/v).

13. The formulation of any one of claims 1-12 wherein 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.

14. The formulation of any one of claims 1-13, that when reconstituted, comprises the surfactant at a concentration ranging from 0.001% to 0.01% (w/v).

15. The formulation of any one of claims 1-14, that when reconstituted, comprises the bispecific antibody construct at a concentration ranging from 10 mg/mL to 20 mg/mL.

16. The formulation of any one of claims 1-15, wherein the bispecific antibody construct is present in an amount of 20 mg/mL.

17. The formulation of any one of claims 1-16, that when reconstituted, comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, wherein the formulation has a pH of 4.2.

18. The formulation of any one of claims 1-17, wherein 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.

19. The formulation of any one of claims 1-17, wherein each of the first and second binding domains comprise a VH region and a VL region.

20. The formulation of claim 18, wherein the bispecific antibody construct is a single chain antibody construct.

21. The formulation of claim 18 or claim 19, wherein the target cell surface antigen is CDH19, MSLN, DLL3, FLT3, EGFR, EGFRvlll, BCMA, PSMA, CD33, CD19, CD70, MUC17 or CLDN18.2.

22. The formulation of any one of claims 18-21, wherein 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, SEQ ID NOs: 166-171, or SEQ ID NOs: 175-180, (l) SEQ ID NOs:132-137, or (m) SEQ ID NOs: 123-128.

23. The formulation of any one of claims 18-22, wherein the second binding domain of the bispecific antibody construct comprises a set of 6 CDRs set forth in SEQ ID NOs: 1-6.

24. The formulation of any one of claims 18-23, 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.

25. The formulation of any one of claims 18-24, wherein the second binding domain comprises 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.

26. The formulation of any one of claims 18-25, wherein the bispecific antibody construct comprises amino acid sequence set forth in SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 33, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 55, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 87, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 131, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 156, SEQ ID NO: 165, SEQ ID NO: 174, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, or SEQ ID NO: 188.

27. Use of the formulation of any one of claims 1-26 in the manufacture of a medicament for treating cancer in a subject in need.

28. A formulation of any one of claims 1-26 for use in therapy.