

- ii. at least one preservative selected from benzyl alcohol, chlorobutanol, phenol, meta-cresol, methylparaben, phenoxyethanol, propylparaben and thiomerosal at a concentration effective to inhibit the growth of microbes,
- iii. a diluent, wherein the bispecific antibody construct is stable.

5 **[12a]** In one aspect, the present disclosure refers to a pharmaceutical composition comprising i. a bispecific antibody construct, binding to a target cell surface antigen via a first binding domain and to the T cell surface antigen CD3 via a second binding domain, wherein the chain antibody construct is present in a concentration in the range from 50 µg/ml to 5 mg/ml, ii. preservative benzyl alcohol at a concentration effective to
10 inhibit the growth of microbes, wherein the preservative serves to physically and microbiologically stabilize the pharmaceutical composition during storage and when administered, wherein the preservative is present in a concentration in the range 0.11 to 0.9% (w/v), and iii. a diluent, wherein the diluent is a buffer comprising a salt selected from the group consisting of phosphate, acetate, citrate, succinate and
15 tartrate, and/or wherein the buffer comprises histidine, glycine, TRIS glycine, Tris, or mixtures thereof, wherein the bispecific antibody construct shows reduction or prevention of the formation of protein aggregates (high molecular weight (HMW) species), and wherein the percentile HMW concentration is below 5% as determined by size exclusion chromatography (SEC-HPLC), and wherein said composition is to be
20 stored until use as solution, as solution in frozen state or as lyophilisate, and is then to be administered, without the need of adding further excipients selected from preservatives, stabilizers or surfactants.

[12b] In another aspect, the present disclosure refers to a method of preparing the pharmaceutical composition as described herein, wherein the bispecific antibody
25 construct is provided as a lyophilisate, the lyophilisate preferably comprising a lyoprotector, a buffer and/or a surfactant, and wherein the lyophilisate is reconstituted by a diluent, comprising a preservative and preferably comprising a buffer and/or an excipient as described herein.

[12c] In yet another aspect, the present disclosure refers to the use of the pharmaceutical
30 composition as described herein, in the manufacture of a medicament for treating of a malignant disease.

[12d] In yet another aspect, the present disclosure refers to a kit comprising the bispecific antibody construct as a lyophilisate, the lyophilisate preferably comprising a lyoprotector, a buffer and/or a surfactant, and a diluent, comprising a preservative and
35 preferably comprising a buffer and/or an excipient as described herein.

[12e] In another aspect, the present disclosure refers to a method for reducing or preventing the formation of protein aggregates (high molecular weight (HMW) species) of a bispecific antibody construct in a pharmaceutical composition, wherein the percentile HMW concentration is below 5%, the method comprising providing a pharmaceutical composition comprising i) a bispecific antibody construct, wherein the construct binds to a target cell surface antigen via a first binding domain and to the T cell surface antigen CD3 via a second binding domain, wherein the bispecific antibody construct is present in a concentration in the range from 50 µg/ml to 10 mg/ml; ii) preservative benzyl alcohol in a concentration in the range 0.11 to 0.9% (w/v); and iii) a diluent, wherein the diluent is a buffer comprising a salt selected from the group consisting of phosphate, acetate, citrate, succinate and tartrate, and/or wherein the buffer comprises histidine, glycine, TRIS glycine, Tris, or mixtures thereof, wherein the preservative serves to physically and microbiologically stabilize the pharmaceutical composition during storage and when administered, and wherein the composition is stored until use as a solution in a frozen state or as a lyophilisate without the need for adding an additional preservative, stabilizer or surfactant for administration.

20

25

30

35

40

Claims

1. A pharmaceutical composition comprising
 - i. a bispecific antibody construct, binding to a target cell surface antigen via a first binding domain and to the T cell surface antigen CD3 via a second binding domain, wherein the bispecific antibody construct is present in a concentration in the range from 50 µg/ml to 5 mg/ml,
 - ii. preservative benzyl alcohol, and methylparaben at a concentration effective to inhibit the growth of microbes, wherein the preservative serves to physically and microbiologically stabilize the pharmaceutical composition during storage and when administered, wherein the preservative is present in a concentration in the range 0.11 to 0.9% (w/v), and
 - iii. a diluent, wherein the diluent is a buffer comprising a salt selected from the group consisting of phosphate, acetate, citrate, succinate and tartrate, and/or wherein the buffer comprises histidine, glycine, TRIS glycine, Tris, or mixtures thereof,wherein the bispecific antibody construct shows reduction or prevention of the formation of protein aggregates (high molecular weight (HMW) species), and wherein the percentile HMW concentration is below 5% as determined by size exclusion chromatography (SEC-HPLC), and
wherein said composition is to be stored until use as solution, as solution in frozen state or as lyophilisate, and is then to be administered, without the need of adding further excipients selected from preservatives, stabilizers or surfactants.
2. The pharmaceutical composition according to claim 1, wherein the bispecific antibody construct is present at a concentration in the range selected from the group consisting of
 - (a) 50 to 200 µg/ml at a pH of 6.5 to 7.5, or
 - (b) 50 to 1000 µg/ml at a pH of 4.0 to 6.0, or
 - (c) 50 µg/ml to 2 mg/ml in the presence of a CD3 binding domain stabilizing agent, preferably citrate, at a pH of 4.0 to 7.5 or
 - (d) 50 µg/ml to 5 mg/ml, at a pH of 4.0 to 7.5, preferably 4.0 to 6.0, wherein the bispecific antibody comprises a third binding domain which comprises two polypeptide monomers, each comprising a hinge, a CH2 and a CH3 domain, wherein said two polypeptide monomers are fused to each other via a peptide linker, and wherein said third binding domain comprises in an amino to carboxyl order:

hinge-CH₂-CH₃-linker-hinge-CH₂-CH₃.

3. The pharmaceutical composition according to claim 1, wherein the bispecific antibody construct is a bispecific single chain antibody construct.
4. The pharmaceutical composition according to claim 1, wherein the diluent is a buffer selected from the group consisting of potassium phosphate, acetic acid/sodium acetate, citric acid/sodium citrate, succinic acid/sodium succinate, tartaric acid/sodium tartrate, and histidine/histidine HCl or mixtures thereof.
5. The pharmaceutical composition according to claim 1, wherein the diluent is a buffer present at a concentration in the range of 0.1 to 150 mM, preferably in the range of 0.25 to 50 mM.
6. The pharmaceutical composition according to claim 1, wherein the diluent is a buffer comprising citrate.
7. The pharmaceutical composition according to claim 1, wherein the diluent is a buffer comprising citrate at a concentration the range of 0.25 to 50 mM.
8. The pharmaceutical composition according to claim 1, wherein the pH of the composition is in the range of 4.0 to 8.0, preferably in the range of pH 4.0 to 5.0 or 6.0 to 7.5, preferably at pH 7.0.
9. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition further comprises one or more excipients selected from the group consisting of sucrose, trehalose, mannitol, sorbitol, arginine, lysine, polysorbate 20, polysorbate 80, poloxamer 188, pluronic and combinations thereof.
10. The pharmaceutical composition according to claim 9, further comprising polysorbate, preferably polysorbate 80, and/or lysine HCL.
11. The pharmaceutical composition according to claim 1, wherein the composition does not comprise polysorbate, preferably polysorbate 80, and/or lysine HCL if the concentration of bispecific antibody construct is at least 10, 15 or 20 µg/ml, preferably 15 µg/ml.

12. The pharmaceutical composition according to claim 1, wherein the first binding domain of the bispecific antibody construct binds to at least one target cell surface antigen selected from the group consisting of CD19, CD33, EGFRvIII, MSLN, CDH19, FLT3, DLL3, CDH3, BCMA and PSMA.

13. The pharmaceutical composition according to claim 1, wherein the first binding domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of:
 - (a) CDR-H1 as depicted in SEQ ID NO: 1, CDR-H2 as depicted in SEQ ID NO: 2, CDR-H3 as depicted in SEQ ID NO: 3, CDR-L1 as depicted in SEQ ID NO: 4, CDR-L2 as depicted in SEQ ID NO: 5 and CDR-L3 as depicted in SEQ ID NO: 6,
 - (b) CDR-H1 as depicted in SEQ ID NO: 29, CDR-H2 as depicted in SEQ ID NO: 30, CDR-H3 as depicted in SEQ ID NO: 31, CDR-L1 as depicted in SEQ ID NO: 34, CDR-L2 as depicted in SEQ ID NO: 35 and CDR-L3 as depicted in SEQ ID NO: 36,
 - (c) CDR-H1 as depicted in SEQ ID NO: 42, CDR-H2 as depicted in SEQ ID NO: 43, CDR-H3 as depicted in SEQ ID NO: 44, CDR-L1 as depicted in SEQ ID NO: 45, CDR-L2 as depicted in SEQ ID NO: 46 and CDR-L3 as depicted in SEQ ID NO: 47,
 - (d) CDR-H1 as depicted in SEQ ID NO: 53, CDR-H2 as depicted in SEQ ID NO: 54, CDR-H3 as depicted in SEQ ID NO: 55, CDR-L1 as depicted in SEQ ID NO: 56, CDR-L2 as depicted in SEQ ID NO: 57 and CDR-L3 as depicted in SEQ ID NO: 58,
 - (e) CDR-H1 as depicted in SEQ ID NO: 65, CDR-H2 as depicted in SEQ ID NO: 66, CDR-H3 as depicted in SEQ ID NO: 67, CDR-L1 as depicted in SEQ ID NO: 68, CDR-L2 as depicted in SEQ ID NO: 69 and CDR-L3 as depicted in SEQ ID NO: 70,
 - (f) CDR-H1 as depicted in SEQ ID NO: 83, CDR-H2 as depicted in SEQ ID NO: 84, CDR-H3 as depicted in SEQ ID NO: 85, CDR-L1 as depicted in SEQ ID NO: 86, CDR-L2 as depicted in SEQ ID NO: 87 and CDR-L3 as depicted in SEQ ID NO: 88,
 - (g) CDR-H1 as depicted in SEQ ID NO: 94, CDR-H2 as depicted in SEQ ID NO: 95, CDR-H3 as depicted in SEQ ID NO: 96, CDR-L1 as depicted in SEQ ID NO: 97, CDR-L2 as depicted in SEQ ID NO: 98 and CDR-L3 as depicted in SEQ ID NO: 99,
 - (h) CDR-H1 as depicted in SEQ ID NO: 105, CDR-H2 as depicted in SEQ ID NO: 106, CDR-H3 as depicted in SEQ ID NO: 107, CDR-L1 as depicted in SEQ ID NO: 109, CDR-L2 as depicted in SEQ ID NO: 110 and CDR-L3 as depicted in SEQ ID NO: 111,
 - (i) CDR-H1 as depicted in SEQ ID NO: 115, CDR-H2 as depicted in SEQ ID NO: 116, CDR-H3 as depicted in SEQ ID NO: 117, CDR-L1 as depicted in SEQ ID NO: 118, CDR-L2 as depicted in SEQ ID NO: 119 and CDR-L3 as depicted in SEQ ID NO: 120,

- (j) CDR-H1 as depicted in SEQ ID NO: 126, CDR-H2 as depicted in SEQ ID NO: 127, CDR-H3 as depicted in SEQ ID NO: 128, CDR-L1 as depicted in SEQ ID NO: 129, CDR-L2 as depicted in SEQ ID NO: 130 and CDR-L3 as depicted in SEQ ID NO: 131,
 - (k) CDR-H1 as depicted in SEQ ID NO: 137, CDR-H2 as depicted in SEQ ID NO: 138, CDR-H3 as depicted in SEQ ID NO: 139, CDR-L1 as depicted in SEQ ID NO: 140, CDR-L2 as depicted in SEQ ID NO: 141 and CDR-L3 as depicted in SEQ ID NO: 142,
 - (l) CDR-H1 as depicted in SEQ ID NO: 152, CDR-H2 as depicted in SEQ ID NO: 153, CDR-H3 as depicted in SEQ ID NO: 154, CDR-L1 as depicted in SEQ ID NO: 155, CDR-L2 as depicted in SEQ ID NO: 156 and CDR-L3 as depicted in SEQ ID NO: 157, and
 - (m) CDR-H1 as depicted in SEQ ID NO: 167, CDR-H2 as depicted in SEQ ID NO: 168, CDR-H3 as depicted in SEQ ID NO: 169, CDR-L1 as depicted in SEQ ID NO: 170, CDR-L2 as depicted in SEQ ID NO: 171 and CDR-L3 as depicted in SEQ ID NO: 172.
14. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is liquid.
 15. The pharmaceutical composition according to claim 1, wherein the composition comprises < 1% of high molecular weight (HMW) species of the bispecific antibody construct.
 16. The pharmaceutical composition according to claim 14, wherein the composition comprises < 1% of high molecular weight (HMW) species of the bispecific antibody construct, for a time period of at least 4 days, preferably at least 10 days, more preferably at least 14 days at room temperature.
 17. The pharmaceutical composition according to claim 1, wherein the pharmaceutical composition is filled in a plastic administration container preferably made of EVA, polyolefin, and/or PVC.
 18. The pharmaceutical composition according to claim 17, wherein the bispecific antibody construct is recovered by at least 90%, preferably at least 95, 96, 97, 98 or even at least 99% from the dilution from the plastic administration container comprising polysorbate 80.

19. The pharmaceutical composition according to claim 17, wherein the pharmaceutical composition comprises at least 0.25 mM citrate, at least 0.0125 mM lysine and/or at least 0.001% polysorbate 80 at a pH of 6.5 to 7.5.
20. The pharmaceutical composition according to claim 1, wherein the composition is a storage solution stored at -20°C.
21. The pharmaceutical composition according to claim 1, wherein the composition is provided as a lyophilisate for storage.
22. A method of preparing the pharmaceutical composition according to claim 1, wherein the bispecific antibody construct is provided as a lyophilisate, the lyophilisate preferably comprising a lyoprotector, a buffer and/or a surfactant, and wherein the lyophilisate is reconstituted by a diluent, comprising a preservative and preferably comprising a buffer and/or an excipient according to any of claims 1 or 4 to 10.
23. Use of the pharmaceutical composition according to claim 1, in the manufacture of a medicament for treating of a malignant disease.
24. The use of claim 23, wherein the medicament is to be administered intravenously continuously for 1 to 24 hours, preferably 1 to 10 or 2 to 5 hours, or is to be administered intravenously continuously for 2 to 14 days, preferably for 2 to 10 days, most preferably for 4 to 7 days.
25. A kit comprising the bispecific antibody construct as a lyophilisate, the lyophilisate preferably comprising a lyoprotector, a buffer and/or a surfactant, and a diluent, comprising a preservative and preferably comprising a buffer and/or an excipient according to any of claims 6 to 13.
26. A method for reducing or preventing the formation of protein aggregates (high molecular weight (HMW) species) of a bispecific antibody construct in a pharmaceutical composition, wherein the percentile HMW concentration is below 5%, the method comprising providing a pharmaceutical composition comprising
 - i) a bispecific antibody construct, wherein the construct binds to a target cell surface antigen via a first binding domain and to the T cell surface antigen CD3 via a

second binding domain, wherein the bispecific antibody construct is present in a concentration in the range from 50 µg/ml to 10 mg/ml;

ii) preservative benzyl alcohol in a concentration in the range 0.11 to 0.9% (w/v);
and

iii) a diluent, wherein the diluent is a buffer comprising a salt selected from the group consisting of phosphate, acetate, citrate, succinate and tartrate, and/or wherein the buffer comprises histidine, glycine, TRIS glycine, Tris, or mixtures thereof,

wherein the preservative serves to physically and microbiologically stabilize the pharmaceutical composition during storage and when administered, and wherein the composition is stored until use as a solution in a frozen state or as a lyophilisate without the need for adding an additional preservative, stabilizer or surfactant for administration.