compositions that provide for enhanced stabilization of antibodies or therapeutic proteins and reduce aggregation and denaturation or degradation during formulation, filling, shipping, storage and administration, thereby preventing loss-of-function and adverse immunogenic reactions.

SUMMARY

[0010] The present disclosure provides pharmaceutical compositions and/or formulations useful to minimize or reduce aggregation and/or high-molecular weight species of masked antigen binding proteins. In one aspect, the present disclosure refers to a -pharmaceutical composition comprising: (a) a masked antigen binding protein selected from a Probody and a ProTIA prodrug; (b) at least one buffer agent; and (c) at least one polyol, wherein the pH of the pharmaceutical composition is between 3.5 and 7.0. In one example~~aspect~~, the present disclosure provides a pharmaceutical composition or formulation comprising: (A) a masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug) comprising: (i) an antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and (ii) a masking domain (MD1) coupled to AB1, wherein the masking domain comprises: (a) a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and (b) a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen; (B) at least one buffer agent; and (C) at least one polyol, wherein the pH of the pharmaceutical composition or formulation is below the isoelectric point (pI) of the AB1 and MD1 regions of the masked antigen binding protein. In another aspect, the present disclosure provides use of the composition as described herein in the manufacture of a medicament for treating cancer in a subject.

[0011] In some other examples, the present disclosure also provides a pharmaceutical composition or formulation comprising: (A) a masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug) comprising: (i) a first antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and (ii) a first masking domain (MD1) coupled to AB1, wherein the masking domain comprises: (a) a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and (b) a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen; (iii) a second antibody or antigen binding fragment thereof (AB2) that binds to a second antigen; and (vi) a second masking domain (MD2) coupled to AB2, wherein the second masking domain comprises: (a) a masking peptide or masking polypeptide (MP2) that inhibits or reduces the binding of AB2 to the antigen; and (b) a second protein recognition site (PR2), wherein binding to or cleavage of the PR2 by a protein or a protease

CLAIMS

1. A pharmaceutical composition comprising:

(a) a masked antigen binding protein selected from a Probody and a ProTIA prodrug; ~~comprising:~~

~~(i) an antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and~~

~~(ii) a masking domain (MD1) coupled to AB1, wherein the masking domain comprises:~~

~~a. a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and~~

~~b. a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen;~~

(b) at least one buffer agent; and

(c) at least one polyol,

wherein the pH of the pharmaceutical composition is between 3.5 and 7.0~~below the isoelectric point (pI) of the AB1 and MD1 regions of the masked antigen binding protein~~.

~~2. The pharmaceutical composition of claim 1, wherein the masked antigen binding protein further comprises:~~

~~(iii) a second antibody or antigen binding fragment thereof (AB2) that binds to a second antigen; and~~

~~(iv) a second masking domain (MD2) coupled to AB2, wherein the second masking domain comprises:~~

~~a. a masking peptide or masking polypeptide (MP2) that inhibits or reduces the binding of AB2 to the antigen; and~~

~~b. a protein recognition site (PR2), wherein binding to or cleavage of the PR2 by a protein or a protease increases AB2 binding to the second antigen,~~

~~wherein the pH of the pharmaceutical composition is below the isoelectric points (pI) of the AB1, AB2, MD1 and MD2 regions of the masked antigen binding protein.~~

~~3. The pharmaceutical composition of claim 1 or 2, wherein the masked antigen binding protein is a Probody or a ProTIA prodrug.~~

~~4. The pharmaceutical composition of any one of claims 1-3, wherein AB1 and/or AB2 is an scFv or a Fab.~~

~~5~~2. The pharmaceutical composition ~~of any one~~ of claim~~s~~ 1-~~4~~, wherein the masked antigen binding protein ~~AB1 and/or AB2~~ binds an antigen expressed on the surface of a cell.

~~3~~6. The pharmaceutical composition of ~~any one of claims 1-5~~claim 1 or claim 2, wherein the at least one buffer agent is selected from the group consisting of acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, ~~and~~ 2-(N-morpholino) ethanesulfonate, and ~~or~~ a combination thereof.

~~7~~4. The pharmaceutical composition of claim ~~6~~3, wherein the at least one buffer agent is present at a concentration range of 5 mM to 200 mM, optionally, 10 mM to 50 mM.

~~8~~5. The pharmaceutical composition of any one of claims 1-~~7~~4, wherein the at least one polyol is selected from the group consisting of a saccharide, a cyclic polysaccharide, a sugar alcohol, a linear branched dextran, ~~and~~ a linear non-branched dextran, ~~or~~ and combinations thereof, optionally, wherein the saccharide is a monosaccharide or a disaccharide.

~~9~~6. The pharmaceutical composition of any one claims of 1-~~8~~5, wherein the polyol is selected from the group consisting of sucrose, trehalose, mannitol, sorbitol, xylitol, erythitol, isomalt, glycerol, a cyclodextrin, captisol, and ~~or~~ a combination thereof.

~~10~~7. The pharmaceutical composition of any one of claims 1-~~9~~6, wherein the at least one polyol is present at a concentration in the range of 1 to 20% (w/V), optionally, 9 to 12% (w/V).

~~11~~8. The pharmaceutical composition of any one of claims 1-~~10~~7, further comprising at least one surfactant, optionally, wherein the at least one surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, pluronic F68, triton X-100, polyoxyethylen3, ~~and~~ PEG 3350, PEG 4000, ~~and~~ PEG 8000, and ~~or~~ a combination thereof.

~~12~~9. The pharmaceutical composition of claim ~~11~~8, wherein the at least one surfactant is present at a concentration in the range of 0.001 to 0.5 % (w/V), optionally, 0.001 to 0.01% (w/V).

~~13 The pharmaceutical composition of any one of claims 1-12, wherein the pH of the composition is about 2 pH units lower than the pI of AB1, AB2, MD1, and/or MD2.~~

~~14~~10. The pharmaceutical composition of any one of claims 1-~~13~~9, wherein the pH of the composition is (a) ~~greater than about 3.5; (b) between 7.0 and 3.6;(c)~~ between 4.0 and 5.0;(~~d~~b) between 3.5 and 5.5; (~~e~~c) about 4.2 or (d) about 3.6~~; or (f) a combination thereof~~.

~~15~~11. The pharmaceutical composition of any one of claims 1-~~14~~10, having an osmolarity in the range of 150 to 500 mOsm.

~~16~~12. The pharmaceutical composition of any one of claims 1-~~15~~11, further comprising an amino acid excipient.

~~17~~13. The pharmaceutical composition of claim ~~16~~12, wherein the amino acid excipient is present in the concentration range of 0.1 to 20 mM.

~~18~~14. The pharmaceutical composition of any one of claims 1-~~17~~13, wherein the composition comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, and wherein the pH of the pharmaceutical composition is 4.2 or 3.6.

~~19~~15. The pharmaceutical composition of any one of claims 1-~~18~~14, wherein the masked antigen binding protein is present in a concentration range of 0.1 to 30 mg/ml.

~~20~~16. The pharmaceutical composition of any one of claims 1-~~19~~15, wherein the masked antigen binding protein is present in an amount ranging from 50 µg to 200 mg.

~~21~~17. The pharmaceutical composition of any one of claims 1-~~20~~16, wherein the pharmaceutical composition is a lyophilized composition.

~~22~~18. The pharmaceutical composition of any one of claims 1-~~21~~16, wherein the pharmaceutical composition is a liquid composition.

~~23~~19. The pharmaceutical composition of claim ~~22~~18, wherein the pharmaceutical composition is a reconstituted lyophilized composition.

~~24~~20. The pharmaceutical composition of any one of claims 1-~~23~~19, wherein the composition is a liquid composition and is stable after storage at 2-8°C, 4°C and/or 25°C for 0 weeks,

1 week, 2, weeks, 4 weeks, 2 months, 6 months, 1 year and/or 2 years; or wherein the composition is a lyophilized composition and is stable after storage at 2-8°C, 4°C and/or 25°C for 0 weeks, 1 week, 2, weeks, 4 weeks, 2 months, 6 months, 1 year, 2 years, 3 years, 4 years and/or 5 years.

~~25~~21. The pharmaceutical composition of any one of claims 1-~~24~~20, wherein (a) the amount of antibody aggregation is reduced;(b) the amount of high-molecular weight antibody species is reduced as measured by ultra-high performance liquid chromatography (SE-UHPLC); (c) the amount of antibody aggregation and/or high-molecular weight antibody species is reduced by about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold compared to a reference pharmaceutical composition at a higher pH than the pharmaceutical composition;(d) the pI is measured or determined using the Bjellqvist algorithm; or (e) a combination thereof.

~~26~~22. Use of the composition of any one of claims 1-~~25~~ 21 in the manufacture of a medicament for treating cancer in a subject.