

FORMULATIONS OF DR5 BINDING POLYPEPTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 63/151,131, filed February 19, 2021, which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present invention relates to formulations of DR5-binding proteins, and methods of using the formulations. Such methods include, but are not limited to, methods of treating cancer.

BACKGROUND

[0003] DR5 is a member of the TNF receptor superfamily (TNFRSF) and a cell surface receptor of the TNF-receptor superfamily that binds TNF-related apoptosis-inducing ligand (TRAIL). TRAIL evolved to play critical roles in mammalian development and host defense by selectively eradicating unwanted, infected and malignant cells from healthy cell populations. On binding the TNF receptor family members DR4 or DR5, TRAIL induces cell death via caspase-dependent apoptosis. DR5 appears to be the primary receptor on tumor cells that facilitates the observed tumor biased activity of the TRAIL pathway. DR5 is activated by the natural ligand TRAIL, which brings three DR5 receptors within close proximity thereby activating intracellular caspase-8 and initiating activation of other death-inducing caspases, such as caspases-9 and caspases-3. Thus, initiation of this cell death pathway requires clustering of DR5 receptors for efficient cell death.

SUMMARY

[0004] Provided herein are stable liquid and lyophilized pharmaceutical formulations of multivalent DR5-binding polypeptides that are capable of agonizing DR5 signaling mediating direct cell death, and the use of the pharmaceutical formulations for treating cancer.

Embodiment 1-Example 1. A pharmaceutical formulation comprising a DR5-binding polypeptide, wherein the formulation comprises 20-70 mg/mL DR5-binding polypeptide, 5-20 mM histidine, 7-10% w/v sucrose, and 0.1-0.8% poloxamer P188 at a pH of 5.3-6.7; and wherein the DR5-binding polypeptide comprises at least one VHH domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 3.

~~Embodiment 2.Example 2.~~ The pharmaceutical formulation of ~~embodimentexample~~ 1, wherein the formulation comprises 30-60 mg/mL DR5-binding polypeptide.

~~Embodiment 3.Example 3.~~ The pharmaceutical formulation of ~~embodimentexample~~ 1, wherein the formulation comprises 50 mg/mL DR5-binding polypeptide.

~~Embodiment 4.Example 4.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-3, wherein the formulation comprises 7-15 mM histidine.

~~Embodiment 5.Example 5.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-3, wherein the formulation comprises 10 mM histidine.

~~Embodiment 6.Example 6.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-5, wherein the histidine is histidine HCl.

~~Embodiment 7.Example 7.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-6, wherein the formulation comprises 8-9% sucrose.

~~Embodiment 8.Example 8.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-7, wherein the formulation comprises 8% sucrose or 9% sucrose.

~~Embodiment 9.Example 9.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-8, wherein the formulation comprises 0.2-0.4% poloxamer P188.

~~Embodiment 10.Example 10.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-9, wherein the formulation comprises 0.2% poloxamer P188.

~~Embodiment 11.Example 11.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-10, wherein the formulation comprises 1-10 mM, 2-8 mM, 3-7 mM, or 4-6 mM methionine.

~~Embodiment 12.Example 12.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-11, wherein the formulation comprises 5 mM methionine.

~~Embodiment 13.Example 13.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-12, wherein the pH of the formulation is 5.4-6.6, 5.5-6.5, 5.6-6.4, 5.7-6.3, or 5.8-6.2.

~~Embodiment 14.Example 14.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-13, wherein the pH of the formulation is about 6.

~~Embodiment 15.Example 15.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-14, wherein the formulation comprises 50 mg/mL DR5-binding polypeptide, 10 mM histidine HCl, 8% sucrose, and 0.2% poloxamer P188, and wherein the pH of the formulation is about 6.

~~Embodiment 16.Example 16.~~ The pharmaceutical formulation of ~~embodimentexample~~ 15, wherein the formulation consists essentially of 50 mg/mL DR5-binding polypeptide, 10 mM histidine HCl, 8% sucrose, 0.2% poloxamer P188, and water, and wherein the pH of the formulation is about 6.

~~Embodiment 17.Example 17.~~ The pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-16, wherein the DR5-binding polypeptide comprises a VHH domain comprising the amino acid sequence of SEQ ID NO: 4.

~~Embodiment 18~~Example 18. The pharmaceutical formulation of any one of ~~embodiment~~examples 1-17, wherein the DR5-binding polypeptide comprises an Fc region.

~~Embodiment 19~~Example 19. The pharmaceutical formulation of ~~embodiment~~example 18, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 6.

~~Embodiment 20~~Example 20. The pharmaceutical formulation of any one of ~~embodiment~~examples 1-19, wherein the DR5-binding polypeptide has the structure VHH-linker-VHH-linker-Fc.

~~Embodiment 21~~Example 21. The pharmaceutical formulation of ~~embodiment~~example 20, wherein the VHH-linker-VHH comprises the amino acid sequence of SEQ ID NO: 5.

~~Embodiment 22~~Example 22. The pharmaceutical formulation of any one of ~~embodiment~~examples 1-21, wherein the DR5-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 7.

~~Embodiment 23~~Example 23. The pharmaceutical formulation of any one of ~~embodiment~~examples 1-21, wherein the DR5-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 7.

~~Embodiment 24~~Example 24. A lyophilized formulation comprising a DR5-binding polypeptide, wherein the DR5-binding polypeptide comprises at least one VHH domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 3; and wherein upon reconstitution of the lyophilized formulation in water to form an aqueous formulation, the aqueous formulation comprises 20-70 mg/mL DR5-binding polypeptide, 5-20 mM histidine, 7-10% sucrose, and 0.1-0.5% poloxamer P188, pH 5.3-6.7.

~~Embodiment 25~~Example 25. The lyophilized formulation of ~~embodiment~~example 24, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 30-60 mg/mL DR5-binding polypeptide.

~~Embodiment 26~~Example 26. The lyophilized formulation of ~~embodiment~~example 24, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 50 mg/mL DR5-binding polypeptide.

~~Embodiment 27~~Example 27. The lyophilized formulation of any one of ~~embodiment~~examples 24-26, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 7-15 mM histidine.

~~Embodiment 28~~Example 28. The lyophilized formulation of any one of ~~embodiment~~examples 24-26, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 10 mM histidine.

~~Embodiment 29~~Example 29. The lyophilized formulation of any one of ~~embodiment~~examples 24-28, wherein the histidine is histidine HCl.

~~Embodiment 30.Example 30.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-29, wherein upon reconstitution of the lyophilized formulation in water to form an aqueous formulation, the aqueous formulation comprises 8-9% sucrose.

~~Embodiment 31.Example 31.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-30, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 8% sucrose or 9% sucrose.

~~Embodiment 32.Example 32.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-31, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 0.2-0.4% poloxamer P188.

~~Embodiment 33.Example 33.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-32, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 0.2% poloxamer P188.

~~Embodiment 34.Example 34.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-33, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 1-10 mM, 2-8 mM, 3-7 mM, or 4-6 mM methionine.

~~Embodiment 35.Example 35.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-33, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 5 mM methionine.

~~Embodiment 36.Example 36.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-35, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the pH of the aqueous formulation is 5.4-6.6, 5.5-6.5, 5.6-6.4, 5.7-6.3, or 5.8-6.2.

~~Embodiment 37.Example 37.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-35, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the pH of the aqueous formulation is about 6.

~~Embodiment 38.Example 38.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-37, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation comprises 50 mg/mL DR5-binding polypeptide, 10 mM histidine HCl, 8% sucrose, and 0.2% poloxamer P188, pH about 6.

~~Embodiment 39.Example 39.~~ The lyophilized formulation of ~~embodimentexample~~ 28, wherein upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation consists essentially of 50 mg/mL DR5-binding polypeptide, 10 mM histidine HCl, 8% sucrose, 0.2% poloxamer P188, and water, and wherein the pH of the formulation is about 6.

~~Embodiment 40.Example 40.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-39, wherein the DR5-binding polypeptide comprises a VHH domain comprising the amino acid sequence of SEQ ID NO: 4.

~~Embodiment 41.Example 41.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-40, wherein the DR5-binding polypeptide comprises an Fc region.

~~Embodiment 42.Example 42.~~ The lyophilized formulation of ~~embodimentexample~~ 41, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 6.

~~Embodiment 43.Example 43.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-42, wherein the DR5-binding polypeptide has the structure VHH-linker-VHH-linker-Fc.

~~Embodiment 44.Example 44.~~ The lyophilized formulation of ~~embodimentexample~~ 43, wherein the VHH-linker-VHH comprises the amino acid sequence of SEQ ID NO: 5.

~~Embodiment 45.Example 45.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-44, wherein the DR5-binding polypeptide comprises the amino acid sequence of SEQ ID NO: 7.

~~Embodiment 46.Example 46.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-44, wherein the DR5-binding polypeptide consists of the amino acid sequence of SEQ ID NO: 7.

~~Embodiment 47.Example 47.~~ A lyophilized formulation formed by lyophilizing the pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-23.

~~Embodiment 48.Example 48.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-47, wherein following storage at 2-8 °C for up to 3 months, up to 6 months, up to 9 months, up to 12 months, or more than 12 months, the lyophilized formulation is an off-white, uniform and elegant cake without any visible impurities.

~~Embodiment 49.Example 49.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-48, wherein following storage at 2-8 °C for up to 3 months, up to 6 months, up to 9 months, up to 12 months, or more than 12 months, upon reconstitution of the formulation in water to form an aqueous formulation, the aqueous formulation is practically free of visible particles.

~~Embodiment 50.Example 50.~~ The lyophilized formulation of any one of ~~embodimentexamples~~ 24-49, wherein following storage at 2-8 °C for up to 3 months, up to 6 months, up to 9 months, up to 12 months, or more than 12 months, upon reconstitution of the formulation in water to form an aqueous formulation, less than 3% or less than 2% of the DR5-binding polypeptide present in the aqueous formulation is aggregated, as measured by size-exclusion chromatography.

~~Embodiment 51.Example 51.~~ The lyophilized formulation of ~~embodimentexample~~ 50, wherein less than 1% or less than 0.5% of the DR5-binding polypeptide present in the aqueous formulation is degraded, as measured by size-exclusion chromatography.

~~Embodiment 52.Example 52.~~ A pharmaceutical formulation formed by reconstituting the lyophilized formulation of any one of ~~embodimentexamples~~ 24-51.

~~Embodiment 53.Example 53.~~ A method of treating cancer comprising administering to a subject with cancer the pharmaceutical formulation of any one of ~~embodimentexamples~~ 1-23 and 52.

~~Embodiment 54~~Example 54. The method of ~~embodiment~~example 53, wherein the cancer is chondrosarcoma, mesothelioma, Ewing sarcoma, colorectal cancer, or pancreatic adenocarcinoma.

[0004a] In one aspect, the disclosure provides a pharmaceutical formulation comprising a DR5-binding polypeptide, wherein the formulation comprises 20-70 mg/mL DR5-binding polypeptide, 5-20 mM histidine, 7-10% w/v sucrose, and 0.1-0.8% poloxamer P188 at a pH of 5.3-6.7; and wherein the DR5-binding polypeptide comprises at least one VHH domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 3.

[0004b] In one aspect, the disclosure provides a lyophilized formulation comprising a DR5-binding polypeptide, wherein the DR5-binding polypeptide comprises at least one VHH domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 3; and wherein upon reconstitution of the lyophilized formulation in water to form an aqueous formulation, the aqueous formulation comprises 20-70 mg/mL DR5-binding polypeptide, 5-20 mM histidine, 7-10% sucrose, and 0.1-0.5% poloxamer P188, pH 5.3-6.7.

[0004c] In one aspect, the disclosure provides a lyophilized formulation formed by lyophilizing the pharmaceutical formulation as disclosed herein.

[0004d] In one aspect, the disclosure provides a pharmaceutical formulation formed by reconstituting the lyophilized formulation as disclosed herein.

[0004e] In one aspect, the disclosure provides use of the pharmaceutical formulation as disclosed herein in the manufacture of a medicament for treating cancer in a subject with cancer.

DETAILED DESCRIPTION

[0005] Embodiments provided herein relate to formulations of DR5-binding polypeptides and their use in various methods of treating, for example, cancer.

Definitions and Various Embodiments

[0006] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0007] All references cited herein, including patent applications, patent publications, and Genbank Accession numbers are herein incorporated by reference, as if each individual reference were specifically and individually indicated to be incorporated by reference in its entirety.

[0008] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 3rd. edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, *et al. eds.*, (2003)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor *eds.* (1995)), Harlow and Lane, *eds.* (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, *ed.* (1987)); Oligonucleotide Synthesis (M. J. Gait, *ed.*, 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, *ed.*, 1998) Academic Press; Animal Cell Culture (R. I. Freshney, *ed.*, 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, *eds.*, 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, *eds.*); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, *eds.*, 1987); PCR: The Polymerase Chain Reaction, (Mullis *et al.*, *eds.*, 1994); Current Protocols in Immunology (J. E. Coligan *et al.*, *eds.*, 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty, *ed.*, IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, *eds.*, Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor