potent and selective inhibitor of matrix metalloproteinase 8, (MMP-8) which lost its selectivity over other MMPs when its ring was opened (Cherney *et al*. (1998), J Med Chem 41 (11), 1749-51). The favorable binding properties achieved through macrocyclization are even more pronounced in multicyclic peptides having more than one peptide ring as for example in vancomycin, nisin and actinomycin.

[0005] Different research teams have previously tethered polypeptides with cysteine residues to a synthetic molecular structure (Kemp and McNamara (1985), J. Org. Chem; Timmerman *et al*. (2005), ChemBioChem). Meloen and co-workers had used tris(bromomethyl)benzene and related molecules for rapid and quantitative cyclisation of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces (Timmerman *et al*. (2005), ChemBioChem). Methods for the generation of candidate drug compounds wherein said compounds are generated by linking cysteine containing polypeptides to a molecular scaffold as for example TATA (1,1',1"-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one, Heinis *et al.* Angew Chem, Int Ed. 2014; 53:1602–1606).

[0006] Phage display-based combinatorial approaches have been developed to generate and screen large libraries of bicyclic peptides to targets of interest (Heinis *et al*. (2009), Nat Chem Biol *5* (7), 502-7 and WO 2009/098450). Briefly, combinatorial libraries of linear peptides containing three cysteine residues and two regions of six random amino acids (Cys-$(Xaa)_6$-Cys-$(Xaa)_6$-Cys) were displayed on phage and cyclised by covalently linking the cysteine side chains to a small molecule scaffold.

SUMMARY

[0006a] In an aspect of the present disclosure, there is provided a pharmaceutical composition comprising:

BT5528, or a pharmaceutically acceptable salt thereof;

about 1.31-2.62 mg histidine hydrochloride per mg of BT5528;

about 15-30 mg sucrose per mg of BT5528; and

about 0.05-0.1 mg Polysorbate 20 per mg of BT5528.

[0006b] In another aspect of the present disclosure, there is provided a solid pharmaceutical composition, which is a lyophilized powder, comprising:

about 21.2 mg BT5528, or a pharmaceutically acceptable salt thereof;

about 27.8 mg histidine hydrochloride;

about 318 mg sucrose; and

about 1.06 mg Polysorbate 20.

[0006c] In a further aspect of the present disclosure, there is provided a use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating an advanced solid tumor malignancy associated with EphA2-expression in a patient, wherein the pharmaceutical composition is to be intravenously administered to the patient.

[0006d] In another aspect of the present disclosure, there is provided a pharmaceutical composition disclosed herein for use in therapy.

[0006e] In a further aspect of the present disclosure, there is provided a pharmaceutical composition disclosed herein and Nivolumab for use in therapy.

[0007] According to one example, the disclosure provides a pharmaceutical composition comprising BT5528, or a pharmaceutically acceptable salt thereof, histidine hydrochloride, sucrose, and Polysorbate 20. In some examples, a pharmaceutical composition comprising BT5528, or a pharmaceutically acceptable salt thereof, histidine hydrochloride, sucrose, and Polysorbate 20 is a lyophilized powder.

[0008] In another example, the disclosure provides a pharmaceutical composition comprising BT5528, or a pharmaceutically acceptable salt thereof, histidine hydrochloride, sucrose, Polysorbate 20, and dextrose. In some examples, a pharmaceutical composition comprising BT5528, or a pharmaceutically acceptable salt thereof, histidine hydrochloride, sucrose, Polysorbate 20, and dextrose is a pharmaceutical formulation in water.

[0009] In another example, the disclosure provides a method for treating advanced malignancies associated with EphA2 expression in a patient comprising administering to the patient a pharmaceutical composition as described herein. In some examples, the disclosure provides a method for treating advanced malignancies associated with EphA2 expression in a patient comprising administering to the patient weekly by IV infusion a pharmaceutical formulation comprising BT5528, or a pharmaceutically acceptable salt thereof, histidine, sucrose, Polysorbate 20, and dextrose in water.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

1. General Description of Certain Embodiments of the Invention:

[0010] Stable lyophilized formulations have been developed for BT5528, which can be reconstituted for administration. During the development process, it was observed that BT5528 adsorbed to the surface of the vials and that reconstitution was challenging. The cause of adsorption of BT5528 to the surface of the vials was investigated during separate lyophilization cycles.

[0011] A number of hypotheses were presented as potential causes, high sodium chloride concentration in the final product, a more basic pH of the reconstituted product, silanised vials and over-drying of the peptide. Each of these hypotheses were assessed together with a formulation screen of alternative sugars, surfactant and a lower BT5528 concentration to investigate if this improved the reconstitution characteristics. An improvement in the reconstitution time was obtained with a lower pH pre-lyophilization, however, oily droplets remained on the surface of the vial. A reduction in the sodium chloride content, use of silanised vials and a lower secondary drying temperature (to increase the final moisture content of the product) did not improve the reconstitution characteristics. Alternative sugars or surfactant did not generate an improvement.

[0012] Surprisingly, it was found that a reduction in BT5528 concentration from 4 mg/mL to 2 mg/mL was found to improve the reconstitution characteristics. The reconstitution time was reduced and no oily droplets were observed on the surface of the vials post-reconstitution. The recovery vs theoretical was at target in the 2 mg/mL formulation compared to consistently below target for the 4 mg/mL formulations.

[0013] Accordingly, in one aspect, the invention provides a solid pharmaceutical composition comprising BT5528, or a pharmaceutically acceptable salt thereof, which is prepared by removing

CLAIMS

1. A pharmaceutical composition comprising:

BT5528, or a pharmaceutically acceptable salt thereof;

about 1.31-2.62 mg histidine hydrochloride per mg of BT5528;

about 15-30 mg sucrose per mg of BT5528; and

about 0.05-0.1 mg Polysorbate 20 per mg of BT5528.

2. The pharmaceutical composition of claim 1, which is a solid pharmaceutical composition in lyophilized powder form.

3. The pharmaceutical composition of claim 1, which is a liquid pharmaceutical composition further comprising water.

4. The pharmaceutical composition of claim 1, comprising:

about 21.2 mg BT5528, or a pharmaceutically acceptable salt thereof;

about 55.5 mg histidine hydrochloride;

about 636 mg sucrose; and

about 1.06-2.12 mg Polysorbate 20.

5. The pharmaceutical composition of claim 1, comprising:

about 21.2 mg BT5528, or a pharmaceutically acceptable salt thereof;

about 27.8 mg histidine hydrochloride;

about 318 mg sucrose; and

about 1.06 mg Polysorbate 20.

6. The pharmaceutical composition of claim 3, comprising:

about 2-4 mg/mL BT5528, or a pharmaceutically acceptable salt thereof;

about 5.25 mg/mL histidine hydrochloride;

about 60 mg/mL sucrose; and

about 0.1-0.2 mg/mL Polysorbate 20.

7. The pharmaceutical composition of claim 3, comprising:

about 2 mg/mL BT5528, or a pharmaceutically acceptable salt thereof;

about 5.24 mg/mL histidine hydrochloride;

about 60 mg/mL sucrose; and

about 0.1 mg/mL Polysorbate 20.

8. The pharmaceutical composition of claim 3, comprising:

about 2 mg/mL BT5528, or a pharmaceutically acceptable salt thereof;

about 5.24 mg/mL histidine hydrochloride;

about 60 mg/mL sucrose; and

about 0.2 mg/mL Polysorbate 20.

9. The pharmaceutical composition of claim 3, comprising:

about 4 mg/mL BT5528, or a pharmaceutically acceptable salt thereof;

about 5.24 mg/mL histidine hydrochloride;

about 60 mg/mL sucrose; and

about 0.2 mg/mL Polysorbate 20.

10. A solid pharmaceutical composition, which is a lyophilized powder, comprising:

about 21.2 mg BT5528, or a pharmaceutically acceptable salt thereof;

about 27.8 mg histidine hydrochloride;

about 318 mg sucrose; and

about 1.06 mg Polysorbate 20.

11. Use of the pharmaceutical composition of any one of claims 1-10 in the manufacture of a medicament for treating an advanced solid tumor malignancy associated with EphA2-expression in a patient, wherein the pharmaceutical composition is to be intravenously administered to the patient.

12. The use of claim 11, wherein the advanced solid tumor malignancy associated with EphA2-expression is selected from non-small-cell lung cancer (NSCLC), ovarian cancer, triple-negative breast cancer (TNBC), gastric/upper gastrointestinal (GI), pancreatic and urothelial cancers.

13. The use of claim 11 or 12, wherein the pharmaceutical composition is to be administered once every 7 days.

14. The use of any one of claims 11-13, wherein the pharmaceutical composition is to be administered at a dose of about 2.2, 4.4, 7.3, 11, 14.6, or 19.4 mg/m^2.

15. The use of any one of claims 11-14, wherein the pharmaceutical composition is to be administered via an IV infusion of about 60 minutes.

16. The use of any one of claims 11-14, wherein Nivolumab is to be further administered to the patient.

17. The pharmaceutical composition of any one of claims 1-11 for use in therapy.

18. The pharmaceutical composition of any one of claims 1-11 and Nivolumab for use in therapy.