to fusion proteins of the extracellular domain of ACE2 with the Fc domain of human IgG1 or IgG4. European patent application EP 21 210 215.6 is directed to highly sialylated and/or afucosylated forms of fusion proteins of the extracellular domain of ACE2 with the Fc domain of human IgG1 or IgG4 as well as to such fusion proteins which additionally comprise IP10.

European patent application EP 21 188 832.6 discloses ACE2 fusion proteins with the Fc domain of IgM and IgG2.

WO 2021/170113, WO 2021/183404, US 2021/0284716, WO 2021/217120, WO 2021/213437, WO 2021/189772, WO 2021/231466, WO 2021/237239, WO 2021/203103, WO 2021/174107, WO 2021/236957, WO 2021/203098, WO 2021/227937, WO 2021/213421, WO 2021/205183, WO 2021/202427, WO 2021/194909, WO 2021/170131, WO 2022/012688, WO 2022/006601, WO 2021/263128 and WO 2021/257512 also disclose fusion proteins of ACE2 with the Fc part of different immunoglobulins.

There is a need for pharmaceutical compositions of ACE2 Fc fusion proteins which provide the required protein stability during storage and which are well tolerated by human patients.

In clinical trials using soluble recombinant human ACE2 a formulation comprising 5.2 mg/ml recombinant human ACE2 in 100 mM glycine, 150 mM NaCl and 50 µM zinc chloride at a pH of 7.5 was used (Haschke et al. (2013) Clin. Pharmacokinet. 52: 783-792).

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure provides stable liquid formulations of ACE2 Fc fusion proteins which are suitable for administration to human subjects.

In one aspect, t~~T~~he present ~~invention~~disclosure provides a liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising the Fc portion of a human IgG; and

(b) a buffer having a pH of 5.6 to 6.3.

In another aspect, t~~T~~he present ~~invention~~disclosure provides a liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising a variant of the Fc portion of a human IgG; and

(b) a buffer having a pH of 5.6 to 6.3.

In one ~~embodiment~~example, the buffer is selected from the group consisting of acetate buffer, histidine buffer, phosphate buffer and succinate buffer, preferably the buffer is present in a concentration of 5 mM to 60 mM.

The liquid pharmaceutical composition may further comprise a sugar or a sugar alcohol, preferably the sugar or sugar alcohol is selected from trehalose, sucrose and mannitol and/or the sugar or sugar alcohol is present in a concentration of 100 mM to 300 mM.

The liquid pharmaceutical composition may further comprise a non-ionic surfactant, preferably the non-ionic surfactant is selected from polysorbate 20 and polysorbate 80 and/or the non-ionic surfactant is present in a concentration of 0.01 % (w/v) to 0.2% (w/v).

The liquid pharmaceutical composition may further comprise an inorganic salt, preferably the inorganic salt is sodium chloride and/or the inorganic salt is present in a concentration of 30 mM to 150 mM.

The liquid pharmaceutical composition may further comprise one or more amino acids, preferably the one or more amino acids are L-arginine and/or L-methionine and/or the one or more amino acids are present in a concentration of 1 mM to 50 mM.

In one ~~embodiment~~example the fragment of human ACE2 consists of the amino acid sequence according to SEQ ID No. 2 or is the extracellular domain of ACE2 consisting of the amino acid sequence according to SEQ ID No. 3.

The IgG may be IgG1 or IgG4.

In one ~~embodiment~~example the fusion protein comprises the Fc portion of human IgG4 comprising the amino acid sequence according to SEQ ID NO: 5 or a variant of the Fc portion of human IgG4 comprising the amino acid sequence according to any one of SEQ ID NOs: 20 and 21.

In one ~~embodiment~~example the IgG is IgG4 or a variant of IgG4 and the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 18.

In one ~~embodiment~~example the fusion protein comprises the Fc portion of human IgG1 comprising the amino acid sequence according to SEQ ID NO: 4 or a variant of the Fc portion of human IgG1 comprising the amino acid sequence according to any one of SEQ ID NOs: 22 and 23.

In one ~~embodiment~~example the IgG is IgG1 or a variant of IgG1 and the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 19.

The variant of the human ACE2 fragment may be an enzymatically inactive variant of human ACE2, preferably the enzymatically inactive variant of human ACE2 comprises a H374N and a H378N mutation, the numbering referring to SEQ ID No. 1.

In one ~~embodiment~~example the fusion protein has the amino acid sequence according to any one of SEQ ID Nos. 6 to 13, 26 to 41, 44 to 55, 58 to 69, 72 to 83 and 86 to 97.

In one ~~embodiment~~example the ACE2 part of the fusion protein is N-glycosylated and 70% to 95% of the N-glycans on the ACE2 part have at least one sialic acid molecule attached thereto.

In another aspect, t~~T~~he present ~~invention~~disclosure also relates to a liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising the Fc portion of a human IgG or a variant of the Fc portion of a human IgG; and

(b) an acetate buffer having a pH of 5.6 to 5.8;

(c) polysorbate 20 or polysorbate 80;

(d) trehalose or sucrose; and

(e) optionally, one or more stabilizers selected from the group consisting of L-arginine, L-methionine and sodium chloride.

In one ~~embodiment~~example, the concentration of the ACE2 Fc fusion protein is 1-60 mg/ml.

In a further aspect, the present disclosure relates to a use of the liquid pharmaceutical composition as described herein in the manufacture of a medicament for preventing and/or treating an infection with a coronavirus binding to ACE2, preferably wherein the coronavirus binding to ACE2 is selected from the group consisting of SARS, SARS-CoV2 and NL63, preferably it is SARS-CoV2. In yet another aspect, the present disclosure relates to the liquid pharmaceutical composition as described herein for use in therapy.

The present ~~invention~~disclosure also relates to the liquid pharmaceutical composition as disclosed herein for use in preventing and/or treating an infection with a coronavirus binding to ACE2, preferably wherein the coronavirus binding to ACE2 is selected from the group consisting of SARS, SARS-CoV2 and NL63, preferably it is SARS-CoV2.

CLAIMS

1. A liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising the Fc portion of a human IgG; and

(b) a buffer having a pH of 5.4 to 6.4.

2. A liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising a variant of the Fc portion of a human IgG; and

(b) a buffer having a pH of 5.4 to 6.4.

3. The liquid pharmaceutical composition according to claim 1 or 2, wherein the buffer is selected from the group consisting of acetate buffer, histidine buffer, phosphate buffer, citrate buffer, and succinate buffer, preferably wherein the buffer is present in a concentration of 5 mM to 60 mM.

4. The liquid pharmaceutical composition according to any one of the preceding claims, further comprising a sugar or a sugar alcohol, preferably wherein the sugar or sugar alcohol is selected from trehalose, sucrose and mannitol and/or wherein the sugar or sugar alcohol is present in a concentration of 100 mM to 300 mM.

5. The liquid pharmaceutical composition according to any one of the preceding claims, further comprising a non-ionic surfactant, preferably wherein the non-ionic surfactant is selected from polysorbate 20 and polysorbate 80 and/or wherein the non-ionic surfactant is present in a concentration of 0.01 % (w/v) to 0.2% (w/v).

6. The liquid pharmaceutical composition according to any one of the preceding claims, further comprising an inorganic salt, preferably wherein the inorganic salt is sodium chloride and/or wherein the inorganic salt is present in a concentration of 30 mM to 150 mM.

7. The liquid pharmaceutical composition according to any one of the preceding claims, further comprising one or more amino acids, preferably wherein the one or more amino acids are L-arginine and/or L-methionine and/or wherein the one or more amino acids are present in a concentration of 1 mM to 50 mM.

8. The liquid pharmaceutical composition according to any one of the preceding claims, wherein the fragment of human ACE2 consists of the amino acid sequence according to SEQ ID No. 2 or wherein the fragment of human ACE2 is the extracellular domain of ACE2 consisting of the amino acid sequence according to SEQ ID No. 3.

9. The liquid pharmaceutical composition according to any one of the preceding claims, wherein the IgG is IgG1 or IgG4.

10. The liquid pharmaceutical composition according to any one of claims 1 and 3 to 9, wherein the fusion protein comprises the Fc portion of human IgG4 comprising the amino acid sequence according to SEQ ID NO: 5, preferably wherein the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 18.

11. The liquid pharmaceutical composition according to any one of claims 2 to 9, wherein the fusion protein comprises a variant of the Fc portion of human IgG4 comprising the amino acid sequence according to any one of SEQ ID NOs: 20 and 21, preferably wherein the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 18.

12. The liquid pharmaceutical composition according to any one of claims 1 and 3 to 9, wherein the fusion protein comprises the Fc portion of human IgG1 comprising the amino acid sequence according to SEQ ID NO: 4, preferably wherein the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 19.

13. The liquid pharmaceutical composition according to any one of claims 2 to 9, wherein the fusion protein comprises a variant of the Fc portion of human IgG1 comprising the amino acid sequence according to any one of SEQ ID NOs: 22 and 23, preferably wherein the first part and the second part are linked by the amino acid sequence according to SEQ ID No. 19.

14. The liquid pharmaceutical composition according to any one of the preceding claims, wherein the variant of the human ACE2 fragment is an enzymatically inactive variant of human ACE2, preferably wherein the enzymatically inactive variant of

human ACE2 comprises a H374N and a H378N mutation, the numbering referring to SEQ ID No. 1.

15. The liquid pharmaceutical composition according to any one of claims 1 and 3 to 8, wherein the fusion protein has the amino acid sequence according to any one of SEQ ID Nos. 6 to 13, 44 to 47, 58 to 61, 72 to 75, 86 to 89.

16. The liquid pharmaceutical composition according to any one of claims 2 to 8, wherein the fusion protein has the amino acid sequence according to any one of SEQ ID Nos. 26 to 41, 48 to 55, 62 to 69, 76 to 83 and 90 to 97.

17. The liquid pharmaceutical composition according to any one of the preceding claims, wherein the ACE2 part of the fusion protein is N-glycosylated and 70% to 95% of the N-glycans on the ACE2 part have at least one sialic acid molecule attached thereto.

18. A liquid pharmaceutical composition comprising:

(a) a fusion protein comprising a first part comprising a fragment of human ACE2 or a variant of said fragment, said human ACE2 having the amino acid sequence according to SEQ ID No. 1, and a second part comprising the Fc portion of a human IgG or a variant of the Fc portion of a human IgG; and

(b) an acetate buffer having a pH of 5.6 to 5.8;

(c) polysorbate 20 or polysorbate 80;

(d) trehalose or sucrose; and

(e) optionally, one or more stabilizers selected from the group consisting of L-arginine, L-methionine and sodium chloride.

19. The liquid pharmaceutical composition according to any one of the preceding claims, wherein the concentration of the ACE2 Fc fusion protein is 1-60 mg/ml.

20. Use of t~~T~~he liquid pharmaceutical composition according to any one of the preceding claims ~~for use~~ in the manufacture of a medicament for preventing and/or treating an infection with a coronavirus binding to ACE2, preferably wherein the coronavirus binding to ACE2 is selected from the group consisting of SARS, SARS-CoV2 and NL63, preferably it is SARS-CoV2.

21. The liquid pharmaceutical composition according to any one of claims 1 to 19 for use in therapy.