CLAIMS

1. Use of a pharmaceutical composition comprising from 16.2 mg to 48.4 mg of a mutant Fibroblast Growth Factor-21 (FGF-21) peptide conjugate and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating nonalcoholic steatohepatitis (NASH) in a subject in need thereof,

wherein the mutant FGF-21 peptide conjugate comprises:

i) a mutant FGF-21 peptide comprising the amino acid sequence of SEQ ID NO: 2,

ii) a glycosyl moiety, and

iii) a 20 kDa polyethylene glycol (PEG), wherein the mutant FGF-21 peptide is attached to the glycosyl moiety by a covalent bond between a threonine at amino acid position 173 of SEQ ID NO: 2 and a first site of the glycosyl moiety and wherein the glycosyl moiety is attached to the 20 kDa PEG by a covalent bond between a second site of the glycosyl moiety and the 20 kDa PEG,

wherein the medicament is to be administered once every two weeks to the subject in need thereof and results in at least one of:

a reduction of liver fat,

an improvement in liver fibrosis score,

NASH resolution,

≥2 point improvement of NAFLD Activity score,

improvement of VCTE score,

improvement of FAST score,

improvement of FIB-4 score,

a reduction of liver size as assessed by Magnetic resonance imaging – Proton density fat fraction,

a reduction of levels of one or more biomarkers comprising Pro-C3, alanine transaminase (ALT), Enhanced Liver Fibrosis (ELF) panel, CK-18, inflammation marker high-sensitivity C-reactive protein (hs-CRP), Hemoglobin A1c (HbA1c), Triglycerides, LDL-c, non-HDL-c, and

an increase of the levels of HDL-c and/or adiponectin.

2. The use of claim 1, wherein the subject is a human subject.

3. The use of claim 1, wherein the medicament is to be administered sub-subcutaneously.

4. The use of claim 1, wherein the glycosyl moiety comprises at least one of an N-acetylgalactosamine (GalNAc) residue, a galactose (Gal) residue, a sialic acid (Sia) residue, a 5-amine analogue of a Sia residue, a mannose (Man) residue, mannosamine, a glucose (Glc) residue, an N-acetylglucosamine (GlcNAc) residue, a fucose residue, a xylose residue, or a combination thereof.

5. The use of claim 1, wherein the glycosyl moiety comprises at least one N-acetylgalactosamine (GalNAc) residue, at least one galactose (Gal) residue, at least one sialic acid (Sia) residue, or a combination thereof.

6. The use of claim 5, wherein the at least one Sia residue is a nine-carbon carboxylated sugar.

7. The use of claim 6, wherein the at least one Sia residue is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-1-onic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc), 2-keto-3-deoxy-nonulosonic acid (KDN), or a 9-substituted sialic acid.

8. The use of claim 7, wherein the 9-substituted sialic acid is 9-O-lactyl-Neu5Ac, 9-O-acetyl-Neu5Ac, 9-deoxy-9-fluoro-Neu5Ac, or 9-azido-9-deoxy-Neu5Ac.

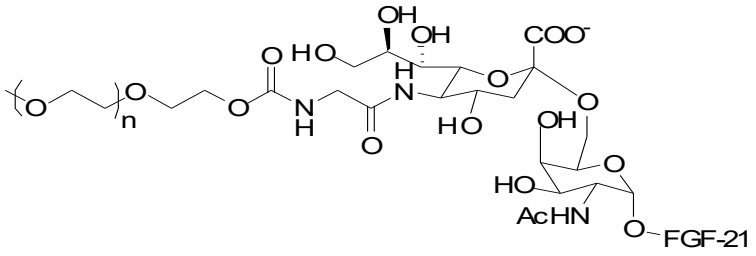
9. The use of claim 1, wherein the glycosyl moiety comprises the structure -GalNAc-Sia-.

10. The use of claim 1, wherein the 20 kDa PEG moiety is attached to the glycosyl moiety by a covalent bond to a linker, wherein the linker comprises at least one amino acid residue.

11. The use of claim 10, wherein the at least one amino acid residue is a glycine (Gly).

12. The use of claim 1, wherein the mutant FGF-21 peptide conjugate comprises the structure -GalNAc-Sia-Gly-PEG (20 kDa).

13. The use of claim 1, wherein the mutant FGF-21 peptide conjugate comprises the structure:



wherein n is an integer selected from 450 to 460.

14. The use of claim 1, wherein the 20 kDa PEG is a linear or branched PEG.

15. The use of claim 1, wherein the 20 kDa PEG is a 20 kDa methoxy-PEG.

16. The use of claim 1, wherein the mutant FGF-21 peptide conjugate displays an equal or higher potency than wild type FGF-21 when tested in vitro in KLB-FGFR1, KLB-FGFR2 and KLB-FGFR3 expressing cells.

17. The use of claim 1, wherein the medicament comprises 44 mg of the mutant FGF-21 peptide conjugate.

18. Use of a pharmaceutical composition comprising from 36 to 55 mg of a mutant Fibroblast Growth Factor-21 (FGF-21) peptide conjugate and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating nonalcoholic steatohepatitis (NASH) in a subject in need thereof,

wherein the mutant FGF-21 peptide conjugate comprises:

i) a mutant FGF-21 peptide comprising the amino acid sequence of SEQ ID NO: 2,

ii) a glycosyl moiety, and

iii) a 20 kDa polyethylene glycol (PEG), wherein the mutant FGF-21 peptide is attached to the glycosyl moiety by a covalent bond between a threonine at amino acid position 173 of SEQ ID NO: 2 and a first site of the glycosyl moiety and wherein the glycosyl moiety is attached to the 20 kDa PEG by a covalent bond between a second site of the glycosyl moiety and the 20 kDa PEG,

wherein the medicament is to be administered to the subject in need thereof and results in at least one of:

a reduction of liver fat,

an improvement in liver fibrosis score,

NASH resolution,

≥2 point improvement of NAFLD Activity score,

improvement of VCTE score,

improvement of FAST score,

improvement of FIB-4 score,

a reduction of liver size as assessed by Magnetic resonance imaging – Proton density fat fraction,

a reduction of levels of one or more biomarkers comprising Pro-C3, alanine transaminase (ALT), Enhanced Liver Fibrosis (ELF) panel, CK-18, inflammation marker high-sensitivity C-reactive protein (hs-CRP), Hemoglobin A1c (HbA1c), Triglycerides, LDL-c,

non-HDL-c, and

an increase of the levels of HDL-c and/or adiponectin.

19. Use of a pharmaceutical composition comprising from 13.5 mg to 33 mg of a mutant Fibroblast Growth Factor-21 (FGF-21) peptide conjugate and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating nonalcoholic steatohepatitis (NASH) in a subject in need thereof:

wherein the mutant FGF-21 peptide conjugate comprises:

i) a mutant FGF-21 peptide comprising the amino acid sequence of SEQ ID NO: 2,

ii) a glycosyl moiety, and

iii) a 20 kDa polyethylene glycol (PEG), wherein the mutant FGF-21 peptide is attached to the glycosyl moiety by a covalent bond between a threonine at amino acid position 173 of SEQ ID NO: 2 and a first site of the glycosyl moiety and wherein the glycosyl moiety is attached to the 20 kDa PEG by a covalent bond between a second site of the glycosyl moiety and the 20 kDa PEG,

wherein the medicament is to be administered once a week to the subject in need thereof and results in at least one of:

a reduction of liver fat,

an improvement of liver fibrosis score,

resolution of NASH,

≥2 point improvement of NAFLD Activity score,

improvement of VCTE score,

improvement of FAST score,

improvement of FIB-4 score,

a reduction of liver size as assessed by Magnetic resonance imaging – Proton density fat fraction,

a reduction of levels of one or more biomarkers comprising Pro-C3, alanine transaminase (ALT), Enhanced LiverFibrosis (ELF) panel, CK-18, inflammation marker high-sensitivity C-reactive protein (hs-CRP), Hemoglobin A1c (HbA1c), non-HDL-c, LDL-c, and Triglycerides, and

an increase of the levels of HDL-c and/or adiponectin.

20. The use of claim 19, wherein the subject is a human subject.

21. The use of claim 19, wherein the medicament is to be administered sub-subcutaneously.

22. The use of claim 19, wherein the glycosyl moiety comprises at least one of an N-acetylgalactosamine (GalNAc) residue, a galactose (Gal) residue, a sialic acid (Sia) residue, a 5-amine analogue of a Sia residue, a mannose (Man) residue, mannosamine, a glucose (Glc) residue, an N-acetylglucosamine (GlcNAc) residue, a fucose residue, a xylose residue, or a combination thereof.

23. The use of claim 19, wherein the glycosyl moiety comprises at least one N-acetylgalactosamine (GalNAc) residue, at least one galactose (Gal) residue, at least one sialic acid (Sia) residue, or a combination thereof.

24. The use of claim 23, wherein the at least one Sia residue is a nine-carbon carboxylated sugar.

25. The use of claim 24, wherein the at least one Sia residue is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-1-onic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc), 2-keto-3-deoxy-nonulosonic acid (KDN), or a 9-substituted sialic acid.

26. The use of claim 25, wherein the 9-substituted sialic acid is 9-O-lactyl-Neu5Ac, 9-O-acetyl-Neu5Ac, 9-deoxy-9-fluoro-Neu5Ac, or 9-azido-9-deoxy-Neu5Ac.

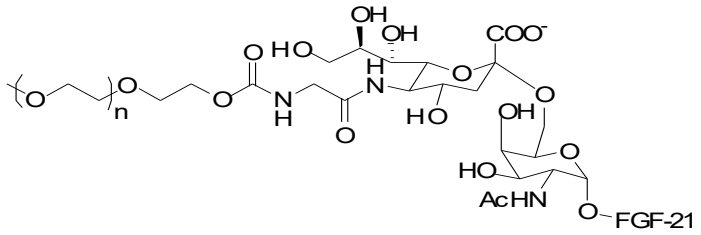
27. The use of claim 19, wherein the glycosyl moiety comprises the structure -GalNAc-Sia-.

28. The use of claim 19, wherein the 20 kDa PEG moiety is attached to the glycosyl moiety by a covalent bond to a linker, wherein the linker comprises at least one amino acid residue.

29. The use of claim 28, wherein the at least one amino acid residue is a glycine (Gly).

30. The use of claim 19, wherein the mutant FGF-21 peptide conjugate comprises the structure -GalNAc-Sia-Gly-PEG (20 kDa).

31. The use of claim 19, wherein the mutant FGF-21 peptide conjugate comprises the structure:



wherein n is an integer selected from 450 to 460.

32. The use of claim 19, wherein the 20 kDa PEG is a linear or branched PEG.

33. The use of claim 19, wherein the 20 kDa PEG is a 20 kDa methoxy-PEG.

34. The use of claim 19, wherein the mutant FGF-21 peptide conjugate displays an equal or higher potency than wild type FGF-21 when tested in vitro in KLB-FGFR1, KLB-FGFR2 and KLB-FGFR3 expressing cells.

35. The use of claim 19, wherein the medicament comprises from 22.5 to 33 mg of the mutant FGF-21 peptide conjugate.

36. The use of claim 19, wherein the medicament comprises 30 mg of the mutant FGF-21 peptide conjugate.