# GROWTH DIFFERENTIATION FACTOR 15 FUSION PROTEINS

## RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/655,108, filed on April 9, 2018, which is hereby incorporated by reference in its entirety.

## SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled A-2239-WO-PCT_SeqList_ST25.txt, created March 15, 2019, which is 109 kb in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

The instant disclosure relates to GDF15 molecules, such as GDF15 fusion proteins, compositions thereof, and methods for making and using such proteins.

## BACKGROUND

Growth differentiation factor 15 (GDF15), also referred to as macrophage inhibitory cytokine 1 (MIC1) (Bootcov MR, 1997, *Proc Natl Acad Sci* 94:11514-9), placental bone morphogenetic factor (PLAB) (Hromas R 1997, *Biochim Biophys Acta*. 1354:40-4), placental transforming growth factor beta (PTGFB) (Lawton LN 1997, *Gene*. 203:17-26), prostate derived factor (PDF) (Paralkar VM 1998, *J Biol Chem.* 273:13760-7), and nonsteroidal anti-inflammatory drug-activated gene (NAG-1) (Baek SJ 2001, *J Biol Chem.* 276: 33384-92), is a secreted protein that circulates in plasma as an ~25 kDa homodimer. GDF15 binds to GDNF family receptor α-like (GFRAL) with high affinity. GDF15-induced cell signaling is believed to require the interaction of GFRAL with the coreceptor RET.

GDF15 has been linked to multiple biological activities. Elevated GDF15 has been shown to be correlated with weight loss and administration of GDF15 has been shown to reduce food intake and body weight. Accordingly, there is a need for efficacious GDF15 molecules that can be administered as a therapeutic. The present disclosure provides GDF15 molecules that meets this need and provide related advantages.

## SUMMARY

In one aspect, the present disclosure provides a fusion protein comprising a GDF15 region joined to an Fc region via a linker, wherein the GDF15 region comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18. In another aspect, the present disclosure provides a fusion protein comprising a GDF15 region joined to an Fc region via a linker, wherein the GDF15 region comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18, and wherein the linker is a (G4Q)n linker and n is greater than 2. In yet another aspect, the present disclosure provides a fusion protein comprising the amino acid sequence of SEQ ID NO: 50.

Provided herein are GDF15 molecules, methods of making the molecules and methods of using the molecules. In some examples, the GDF15 molecule is a GDF15-Fc fusion

protein. The fusion protein can comprise a GDF15 region joined to an Fc region. In some examples, the GDF15 region is joined to the Fc via a linker.

In some examples, the GDF15 region comprises the amino acid sequence of SEQ ID NO: 6 and at least one mutation, such as a mutation of the asparagine at position 3 (N3), as a mutation of the aspartate at position 5 (D5), or mutations of the asparagine at position 3 and the aspartate at position 5. In some examples, the GDF15 region comprises a mutation of the aspartate at position 5 to glutamate (D5E). In some examples, the GDF15 region comprises the amino acid sequence of SEQ ID NO: 16. In some examples, the GDF15 region comprises a mutation of the asparagine at position 3 to glutamine (N3Q), for example, having an amino acid sequence SEQ ID NO: 14. In yet other examples, the GDF15 region comprises both N3Q and D5E mutations. In some examples, the GDF15 region comprises the amino acid sequence of SEQ ID NO: 18.

In some examples, the fusion protein has a linker that is a G4S (SEQ ID NO: 19) or G4Q (SEQ ID NO: 24) linker, such as a (G4S)n or (G4Q)n linker, wherein n is greater than 0. In some examples, the fusion protein has a linker that is a G4A (SEQ ID NO: 58) linker, such as a (G4A)n linker, wherein n is greater than 0. In some examples, n is 1 or 2. In some examples, n is greater than 2, such as 3, 4, 5, 6, 7, or 8. In some examples, the linker comprises the amino acid sequence of SEQ ID NO: 19, 20, 21, 22, 23, 24, 25, or 58.

In some examples, the fusion protein has an Fc region comprises a charged pair mutation. In some examples, the Fc region has a truncated hinge region. In some examples, the Fc region is selected from Table 3.

Also provided herein are dimers and tetramers comprising the fusion proteins disclosed herein. In one example, the dimer comprises a GDF15-Fc fusion comprising the amino acid sequence of any one of SEQ ID NOs: 39-57. In some examples, a GDF15-Fc fusion comprising the amino acid sequence of SEQ ID NO: 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57 dimerizes with an Fc domain comprising the amino acid sequence of SEQ ID NO: 32, 33, 34, 35, 36, or 37, such as shown in Table 6. In some examples, the dimers form tetramers. In one aspect, the present disclosure provides a dimer comprising a fusion protein comprising the amino acid sequence of SEQ ID NO: 50 and a protein comprising the amino acid sequence of SEQ ID NO: 36. In a further aspect, the present disclosure provides a tetramer comprising the aforementioned dimer. Methods of producing and using the GDF15 molecules disclosed herein are also provided. In one aspect, the present disclosure provides a fusion protein, dimer or tetramer as disclosed herein for use in therapy. In yet another aspect, the present disclosure provides the use of a fusion protein, dimer or tetramer as disclosed herein in the manufacture of a medicament for treating a metabolic condition or disorder wherein the metabolic condition or disorder is diabetes, obesity, dyslipidemia, elevated glucose levels, elevated insulin levels or diabetic nephropathy.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a graph showing the effect on the body weight of cynomologus monkeys dosed with vehicle, 3 mg/kg of the positive control FGF21-Fc, 1.5 mg/kg of scFc-GDF15, or 1.5 mg/kg of the dimer FcΔ10(-)-(G4S)4-GDF15:FcΔ10(+,K) weekly for six weeks, followed by a five-week washout.

## CLAIMS

What is claimed is:

1. A fusion protein comprising a GDF15 region joined to an Fc region via a linker, wherein the GDF15 region comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18.
2. The fusion protein of claim 1, wherein the linker is a (G4S)n or (G4Q)n linker, wherein n is greater than 0.
3. The fusion protein of claim 2, wherein n is 1 or 2.
4. A fusion protein comprising a GDF15 region joined to an Fc region via a linker, wherein the GDF15 region comprises the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 18, and wherein the linker is a $(G_4Q)_n$ linker and n is greater than 2.
5. The fusion protein of claim 2 or 4, wherein the n is 3 or 4.
6. The fusion protein of any one of claims 1-5, wherein the Fc region comprises a charged pair mutation.
7. The fusion protein of any one of claims 1-5, wherein the Fc region comprises a truncated hinge region.
8. The fusion protein of any one of claims 1-5, wherein the Fc region is selected from Table 3.
9. A fusion protein comprising the amino acid sequence of SEQ ID NO: 50.
10. A dimer comprising a fusion protein comprising the amino acid sequence of SEQ ID NO: 50 and a protein comprising the amino acid sequence of SEQ ID NO: 36.
11. A tetramer comprising the dimer of claim 10.
12. The fusion protein of any one of claims 1-8, a dimer according to claim 10 or a tetramer according to claim 11 for use in therapy.
13. Use of a fusion protein of any one of claims 1-8, a dimer according to claim 10 or a tetramer according to claim 11 in the manufacture of a medicament for treating a metabolic condition or disorder wherein the metabolic condition or disorder is diabetes, obesity, dyslipidemia, elevated glucose levels, elevated insulin levels or diabetic nephropathy.