# FUSION PROTEINS COMPRISING ALPHA-L-IDURONIDASE ENZYMES AND METHODS

## CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 63/291,283, filed December 17, 2021. The entire content of the application referenced above is hereby incorporated by reference herein.

## BACKGROUND

Mucopolysaccharidosis I (MPS I) (or Hurler syndrome) is a lysosomal storage disorder caused by genetic mutations in the *IDUA* gene. These mutations reduce or eliminate alpha-L-iduronidase (IDUA) protein function, which results in the accumulation of the glycosaminoglycans dermatan sulfate and heparan sulfate and to alterations in multiple organs and tissues, including the skeleton, heart, respiratory system, and brain. Treatments for MPS I remain largely supportive; while the deficient enzyme may be administered intravenously, it has little effect on the brain due to difficulties in delivering the recombinant enzyme across the blood-brain barrier (BBB). Accordingly, there is a need for more effective therapies that treat MPS I symptoms and IDUA deficiencies in both the CNS and the periphery.

## SUMMARY

Thus, provided herein is a specific enzyme replacement therapy, which has the capability of crossing the BBB and treating both the peripheral and CNS manifestations of MPS I. In particular, certain examples provide a protein comprising (a) a first Fc polypeptide linked to an alpha-L-iduronidase (IDUA) amino acid sequence, an IDUA variant amino acid sequence, or a catalytically active fragment thereof; and (b) a second Fc polypeptide; wherein the first and/or second Fc polypeptide is a modified Fc that is capable of binding (e.g., specifically binding) to a blood-brain barrier (BBB) receptor, *e.g.,* a transferrin receptor (TfR). In certain examples, the second Fc polypeptide forms an Fc dimer with the first Fc polypeptide.

In certain examples, the first Fc polypeptide is a modified Fc that is capable of binding (e.g., specifically binding) to TfR. In certain examples, the first Fc polypeptide is a modified Fc comprising a sequence having at least 90% identity to SEQ ID NO: 28 or 98 (e.g., SEQ ID NO:28) and is capable of specifically binding to a TfR. In one aspect, the present disclosure refers to a protein comprising: a. a first Fc polypeptide linked to an alpha-L-iduronidase (IDUA) amino acid sequence, an IDUA variant amino acid sequence, or a catalytically active fragment thereof; and b. a second Fc polypeptide that comprises a sequence having at least 90% identity to SEQ ID NO: 28 and that is capable of specifically binding to a transferrin receptor (TfR).

In certain examples, the first Fc polypeptide 1) comprises a sequence having at least 90% identity to SEQ ID NO: 28 or 98 (e.g., SEQ ID NO:28); 2) is capable of specifically binding to a TfR; and 3) has Ala at position 389, according to EU numbering. In certain examples, the first Fc polypeptide further comprises Glu at position 380; and Asn at position 390, according to EU numbering. In certain examples, the first Fc polypeptide further comprises at the following positions, according to EU numbering: Tyr at position 384; Thr at position 386; Glu at position 387; Trp at position 388; Thr at position 413; Glu at position 415; Glu at position 416; and Phe at position 421.

In certain examples, the second Fc polypeptide is a modified Fc that is capable of binding (e.g., specifically binding) to TfR. In certain examples, the second Fc polypeptide is a modified Fc comprising a sequence having at least 90% identity to SEQ ID NO: 28 or 98 (e.g., SEQ ID NO:28) and is capable of specifically binding to a TfR.

In certain examples, the second Fc polypeptide 1) comprises a sequence having at least 90% identity to SEQ ID NO: 28 or 98 (e.g., SEQ ID NO:28); 2) is capable of specifically binding to a TfR; and 3) has Ala at position 389, according to EU numbering. In certain examples, the second Fc polypeptide further comprises Glu at position 380; and Asn at position 390, according to EU numbering. In certain examples, the second Fc polypeptide further comprises at the following positions, according to EU numbering: Tyr at position 384; Thr at position 386; Glu at position 387; Trp at position 388; Thr at position 413; Glu at position 415; Glu at position 416; and Phe at position 421.

Certain examples provide a protein comprising:

a. a first Fc polypeptide linked to an alpha-L-iduronidase (IDUA) amino acid sequence, an IDUA variant amino acid sequence, or a catalytically active fragment thereof; and

b. a second Fc polypeptide that comprises a sequence having at least 90% identity to SEQ ID NO: 28 and that is capable of specifically binding to a transferrin receptor (TfR).

In certain examples, the second Fc polypeptide has Ala at position 389, according to EU numbering.

In certain examples, the second Fc polypeptide further comprises Glu at position 380; and Asn at position 390, according to EU numbering.

In certain examples, the second Fc polypeptide further comprises at the following positions, according to EU numbering: Tyr at position 384; Thr at position 386; Glu at position

387; Trp at position 388; Thr at position 413; Glu at position 415; Glu at position 416; and Phe at position 421.

In certain examples, the protein is capable of being transported across the blood-brain barrier of a subject.

In certain examples, the protein binds to a TfR with an affinity of from about 100 nM to about 500 nM.

In certain examples, the protein binds to a TfR with an affinity of from about 200 nM to about 400 nM.

In certain examples, the second Fc polypeptide binds to the apical domain of the TfR.

In certain examples, the binding of the protein to the TfR does not substantially inhibit binding of transferrin to the TfR.

In certain examples, the IDUA amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS: 39, 40, 45, 78, and 99.

In certain examples, the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS:39, 40, 45, 78, and 99.

In certain examples, the IDUA amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS: 41-44.

In certain examples, the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 41-44.

In certain examples, the IDUA amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS: 46-49.

In certain examples, the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 46-49.

In certain examples, the IDUA amino acid sequence comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOS: 79-82.

In certain examples, the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 79-82.

In certain examples, the first Fc polypeptide is linked to the IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In certain examples, the first Fc polypeptide is

linked to the IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof by a peptide bond. In certain examples, the first Fc polypeptide is linked to the IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof by a polypeptide linker.

In certain examples, the polypeptide linker is a flexible polypeptide linker.

In certain examples, the flexible polypeptide linker is a glycine-rich linker.

In certain examples, the polypeptide linker is GS (SEQ ID NO:71), $G_4S$ (SEQ ID NO:72) or $(G_4S)_2$ (SEQ ID NO:73).

In certain examples, the N-terminus of the first Fc polypeptide is linked to the IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof.

In certain examples, the C-terminus of the first Fc polypeptide is linked to the IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof.

In certain examples, a fusion protein as described herein comprises a single IDUA amino acid sequence, IDUA variant amino acid sequence, or a catalytically active fragment thereof.

In certain examples, the second Fc polypeptide forms an Fc dimer with the first Fc polypeptide.

In certain examples, the first Fc polypeptide and the second Fc polypeptide each contain modifications that promote heterodimerization.

In certain examples, one of the Fc polypeptides has a T366W substitution and the other Fc polypeptide has T366S, L368A, and Y407V substitutions, according to EU numbering.

In certain examples, the first Fc polypeptide contains the T366S, L368A, and Y407V substitutions and the second Fc polypeptide contains the T366W substitution.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 9-16 and 19-22; and the second Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 25-32 and 35-38.

In certain examples, the first Fc polypeptide contains the T366W substitution and the second Fc polypeptide contains the T366S, L368A, and Y407V substitutions.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 17-18 and 74-75; and the second Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 33-34 and 97-98.

In certain examples, the first Fc polypeptide and/or the second Fc polypeptide comprises a native FcRn binding site.

In certain examples, the first Fc polypeptide and the second Fc polypeptide do not have effector function.

In certain examples, the first Fc polypeptide and/or the second Fc polypeptide includes a modification that reduces effector function.

In certain examples, the modification that reduces effector function is the substitutions of Ala at position 234 and Ala at position 235; Ala at position 234, Ala at position 235 and Gly at position 329; or Ala at position 234, Ala at position 235 and Ser at position 329, according to EU numbering.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 11-16, and 19-22.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 11, 12, 19, and 20.

In certain examples, the first Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS:15, 16, 21, and 22.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 50-69 and 83-92.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 50-53.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 54-57.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 83-86.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 58-61 and 91-92.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 62-65.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 87-90.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 66-67.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 68-69.

In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 27-32 and 35-38.

In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS: 27, 28, 35 and 36.

In certain examples, the second Fc polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS:31, 32, 37 and 38.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 50-65 and 83-92; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 35-38.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 50-57 and 83-86; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 35-36.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 58-65 and 87-92; and

the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 37-38.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 66-69; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 35-38.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 66-67; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 35-36.

In certain examples, the first Fc polypeptide linked to the IDUA amino acid sequence comprises the amino acid sequence of any one of SEQ ID NOS: 68-69; and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS: 37-38.

In certain examples, uptake of the IDUA amino acid sequence into the brain is at least five-fold greater as compared to the uptake of the IDUA amino acid sequence in the absence of the first Fc polypeptide and the second Fc polypeptide or as compared to the uptake of the IDUA enzyme without the modifications to the second Fc polypeptide that result in TfR binding.

In certain examples, the first Fc polypeptide is not modified to bind to a blood-brain barrier (BBB) receptor and the second Fc polypeptide is modified to specifically bind to a TfR.

In certain examples, the protein does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

In another aspect, the present disclosure refers to a polypeptide comprising an Fc polypeptide that is linked to an alpha-L-iduronidase (IDUA) amino acid sequence, an IDUA variant amino acid sequence, or a catalytically active fragment thereof, wherein the Fc polypeptide comprises a sequence having at least 90% identity to SEQ ID NO: 12 and contains one or more modifications that promote its heterodimerization to another Fc polypeptide.

In certain examples, the Fc polypeptide is linked to the IDUA enzyme, the IDUA variant amino acid sequence, or the catalytically active fragment thereof by a peptide bond or by a polypeptide linker.

In certain examples, the polypeptide comprises from N- to C-terminus: the IDUA enzyme, the IDUA variant amino acid sequence, or the catalytically active fragment thereof; a polypeptide linker; and the Fc polypeptide.

In certain examples, the polypeptide comprises from N- to C-terminus: the Fc polypeptide; a polypeptide linker; and the IDUA enzyme, the IDUA variant amino acid sequence, or the catalytically active fragment thereof.

In certain examples, the Fc polypeptide comprises T366S, L368A, and Y407V substitutions, according to EU numbering.

In certain examples, the Fc polypeptide comprises substitutions of Ala at position 234 and Ala at position 235; Ala at position 234, Ala at position 235 and Gly at position 329; or Ala at position 234, Ala at position 235 and Ser at position 329, according to EU numbering.

In certain examples, the polypeptide comprises an amino acid sequence having at least 95% or 100% identity to any one of SEQ ID NOS:50-69 and 83-92.

Certain examples provide a protein comprising 1) a polypeptide comprising an Fc polypeptide that is linked to an alpha-L-iduronidase (IDUA) amino acid sequence, an IDUA variant amino acid sequence, or a catalytically active fragment thereof, wherein the Fc polypeptide comprises a sequence having at least 90% identity to SEQ ID NO: 12 and contains one or more modifications that promote its heterodimerization to another Fc polypeptide; and 2) the other Fc polypeptide. In another aspect, the present disclosure refers to a protein comprising the polypeptide and the other Fc polypeptide as described herein.

Certain examples provide a pharmaceutical composition comprising a fusion protein as described herein or a polypeptide as described herein and a pharmaceutically acceptable carrier and/or excipient. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the protein as described herein or the polypeptide as described herein and a pharmaceutically acceptable excipient.

Certain examples provide a polynucleotide comprising a nucleic acid sequence encoding a polypeptide as described herein (e.g., an IDUA-Fc fusion polypeptide as described herein). In another aspect, the present disclosure refers to a polynucleotide comprising a nucleic acid sequence encoding the polypeptide as described herein.

In another aspect, the present disclosure refers to a vector comprising a polynucleotide as described herein.

In another aspect, the present disclosure refers to a host cell comprising a polynucleotide as described herein or a vector as described herein. In certain examples, the host cell further comprises a polynucleotide comprising a nucleic acid sequence encoding another polypeptide described herein (e.g., the other Fc polypeptide, such as a TfR-binding modified Fc polypeptide).

In another aspect, the present disclosure refers to a method for producing a polypeptide comprising an Fc polypeptide that is linked to an IDUA amino acid sequence, IDUA variant amino acid sequence, or catalytically active fragment thereof, comprising culturing a host cell under conditions in which the polypeptide encoded by a polynucleotide as described herein is expressed.

Certain examples provide a pair of polynucleotides comprising a first nucleic acid sequence encoding a first Fc polypeptide linked to an IDUA amino acid sequence, IDUA variant amino acid sequence, or catalytically active fragment thereof; and a second nucleic acid sequence encoding a second Fc polypeptide, as described herein.

In another aspect, the present disclosure refers to one or more vectors comprising the pair of polynucleotides as described herein. For example, certain examples provide a single vector comprising the pair of polynucleotides. Other examples provide two vectors, wherein the first vector comprises the first polynucleotide from the pair and the second vector comprises the second polynucleotide from the pair.

In another aspect, the present disclosure refers to a host cell comprising the pair of polynucleotides as described herein, or the one or more vectors as described herein.

In another aspect, the present disclosure refers to a method for producing a protein comprising a first Fc polypeptide linked to an IDUA amino acid sequence, IDUA variant amino acid sequence, or catalytically active fragment thereof, and a second Fc polypeptide, comprising culturing a host cell under conditions in which a pair of polynucleotides as described herein are expressed.

Certain examples provide a method of treating MPS I, the method comprising administering a protein as described herein or a polypeptide as described herein to a patient in need thereof. In certain examples, a therapeutically effective amount of the protein or polypeptide is administered.

In another aspect, the present disclosure refers to a protein as described herein or a polypeptide as described herein for use in treating MPS I in a patient in need thereof.

In another aspect, the present disclosure refers to the use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for treating MPS I in a patient in need thereof.

Certain examples provide a method of decreasing the accumulation of a toxic metabolic product in a patient having MPS I, the method comprising administering a protein as described herein or a polypeptide as described herein to the patient. In certain examples, an effective amount (e.g., a therapeutically effective amount) of the protein or polypeptide is administered.

Certain examples provide a protein as described herein or a polypeptide as described herein for use in decreasing the accumulation of a toxic metabolic product in a patient having MPS I.

Certain examples provide the use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having MPS I.

In certain examples, the toxic metabolic product comprises heparan sulfate-derived oligosaccharides or dermatan sulfate-derived oligosaccharides. In another aspect, the present disclosure refers to a protein as described herein or a polypeptide as described herein for use in decreasing the accumulation of a toxic metabolic product in a patient having MPS I, wherein the toxic metabolic product comprises heparan sulfate-derived oligosaccharides or dermatan sulfate-derived oligosaccharides. In another aspect, the present disclosure refers to the use of a protein as described herein or a polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having MPS I, wherein the toxic metabolic product comprises heparan sulfate-derived oligosaccharides or dermatan sulfate-derived oligosaccharides.

## BRIEF DESCRIPTION OF THE FIGURES

**Figures 1A-1B.** Illustration of exemplary ETV:IDUA fusion proteins, wherein the IDUA enzyme is fused to (Fig. 1A) the N-terminus of an Fc polypeptide; or to (Fig. 1B) the C-terminus of an Fc polypeptide.

**Figure 2.** *In vitro* evaluation of enzymatic activity of IDUA-Fc fusion proteins and Aldurazyme (laronidase).

**Figure 3.** Evaluation of cellular activity of ETV:IDUA Fusion 1 as compared to laronidase in fibroblasts from Hurler (MPS I) patients and healthy controls using a LCMS quantification of heparan sulfate and dermatan sulfate.

**Figures 4A-4C.** Evaluation of (Fig. 4A) ETV:IDUA Fusion 3 serum PK, (Fig. 4B) ETV:IDUA Fusion 4 serum PK, and (Fig. 4C) ETV:IDUA Fusion 6 serum PK in TfR knock in mice ("$TfR^{mu/hu}KI$").

**Figures 5A-5C.** Evaluation of (Fig. 4A) ETV:IDUA Fusion 3 brain PK, (Fig. 4B) ETV:IDUA Fusion 4 brain PK, and (Fig. 4C) ETV:IDUA Fusion 6 brain PK in TfR knock in mice ("$TfR^{mu/hu}KI$").

**Figures 6A-6D.** Evaluation of pharmacodynamic response (total GAG levels) in CSF (Fig. 6A), brain (Fig. 6B), liver (Fig. 6C), and urine (Fig. 6D) in a comparative study carried out in healthy and disease mice models of MPS I. The healthy mouse model is represented by TfR knock in mice ("$TfR^{mu/hu}$") and the disease mouse model is represented by TfR knock in mice in which the gene for IDUA has been knocked out ("IDUA KO; $TfR^{mu/hu}$"). Graphs display mean ± SEM and p values: one-way ANOVA Dunnett's multiple comparison test; ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$.

## DETAILED DESCRIPTION

There is currently a need for new therapeutics for the treatment of MPS I, specifically therapeutics that treat severe MPS I having a neurocognitive phenotype. Described herein is a