that produces a highly active form (therapeutic) of IDUA or IDS where the introduction of, or creation of, a population of these altered cells in a patient will supply that needed protein activity to treat (*e.g.,* reduce or eliminate one or more symptoms) MPS I or MPS II disease.

[0015a] In one aspect, the present invention refers to the use of a promoterless donor vector and an expression vector encoding a pair of zinc finger nucleases (ZFNs) targeted to an endogenous albumin gene in the manufacture of a medicament for reducing or preventing central nervous system (CNS) impairment in a subject with mucopolysaccharidosis type I (MPS I) or mucopolysaccharidosis type I (MPS II), wherein the use comprises that the promoterless donor vector and the expression vector are to be administered to the subject, wherein the promoterless donor vector comprises a transgene encoding human α-L-iduronidase (hIDUA) or human iduronate-2-sulftase (hIDS), wherein the transgene is integrated into the albumin gene in a liver cell following cleavage of the albumin gene such that the encoded hIDUA or hIDS is expressed and secreted from the liver and further wherein the hIDUA or hIDS secreted from the liver is found in the central nervous system (CNS) of the subject such that the CNS impairment is reduced or prevented.

[0015b] In another aspect, the present invention refers to a Hep2G cell comprising a promoterless donor vector encoding hIUDA or hIDS integrated into intron 1 of an albumin gene, wherein the hIUDA or hIDS is expressed and secreted from the Hep2G cell.

[0015c] In yet another aspect, the present invention refers to a cell culture comprising the Hep2G cell as disclosed herein, wherein the culture media comprises the hIUDA or hIDS.

[0015d] In yet another aspect, the present invention refers to a pharmaceutical composition comprising hIUDA and/or hIDS secreted from a cell as disclosed herein or a cell culture as disclosed herein.

[0015e] In yet another aspect, the present invention refers to the use of the composition as disclosed herein in the manufacture of a medicament for reducing or preventing central nervous system (CNS) impairment in a subject with mucopolysaccharidosis type I (MPS I) or mucopolysaccharidosis type II (MPS II).

[0016] In one aspect, provided herein is a method of reducing or preventing central nervous system (CNS) impairment in a subject with mucopolysaccharidosis

type I (MPS I) and/or mucopolysaccharidosis type I (MPS II), the method comprising administering to the subject a promoterless donor vector comprising a transgene encoding human α-L-iduronidase (hIDUA) and/or human iduronate-2-sulftase (hIDS); and administering an expression vector encoding a pair of zinc finger nucleases (ZFNs) targeted to an endogenous albumin gene, wherein the transgene is integrated into the albumin gene in a liver cell following cleavage of the albumin gene such that the encoded hIDUA and/or hIDS is expressed and secreted from the liver and further wherein the hIDUA and/or hIDS secreted from the liver is found in the central nervous system (CNS) (*e.g.,* crosses the blood-brain barrier) of the subject such that the CNS impairment is reduced or prevented. In any of the methods described herein, the CNS impairment comprises cognitive deficits (*e.g.,* loss of learning ability is decreased as compared to an untreated individual); the transgene(s) modulate(s) levels of glycosoaminoglycans in the brain of the subject; and/or neuronal or glial vacuolation in the subject is reduced or eliminated in the CNS (brain and/or spinal cord). The methods may further comprise administering an immunosuppressant to the subject prior to and after administration of the donor vector and the expression vector. In any of the methods described herein, the pair of ZFNs comprises zinc finger proteins as shown in Table 1. The ZFN expression vectors and/or donor vectors may be adeno-associated vectors (AAV), which may be administered by any method, including via intravenous injection. In certain embodiments, the ZFN:ZFN:Donor ratio administered to the subject is 1:1:8.

[0017] In another aspect, the invention describes a method of expressing a transgene encoding one or more corrective IDUA or IDS transgenes in a cell of the subject. The transgene may be inserted into the genome of a suitable target cell (*e.g.,* blood cell, liver cell, brain cell, stem cell, precursor cell, etc.) such that the IDUA or IDS product encoded by that corrective transgene is stably integrated into the genome of the cell or, alternatively, the transgene may be maintained in the cell extra-

CLAIMS

What is claimed is:

1. Use of a promoterless donor vector and an expression vector encoding a pair of zinc finger nucleases (ZFNs) targeted to an endogenous albumin gene in the manufacture of a medicament for reducing or preventing central nervous system (CNS) impairment in a subject with mucopolysaccharidosis type I (MPS I) or mucopolysaccharidosis type I (MPS II), wherein the use comprises that the promoterless donor vector and the expression vector are to be administered to the subject, wherein the promoterless donor vector comprises a transgene encoding human α-L-iduronidase (hIDUA) or human iduronate-2-sulftase (hIDS),

wherein the transgene is integrated into the albumin gene in a liver cell following cleavage of the albumin gene such that the encoded hIDUA or hIDS is expressed and secreted from the liver and further wherein the hIDUA or hIDS secreted from the liver is found in the central nervous system (CNS) of the subject such that the CNS impairment is reduced or prevented.

2. The use of claim 1, wherein the CNS impairment comprises cognitive deficits.

3. The use of claim 1 or claim 2, further comprising that an immunosuppressant is to be administered to the subject prior to and after administration of the donor vector and the expression vector.

4. The use of any of claims 1 to 3, wherein expression of the transgenes modulates levels of glycosoaminoglycans in the brain of the subject.

5. The use of any of claims 1 to 4, wherein neuronal or glial vacuolation in the subject is reduced or eliminated.

6. The use of claim 5, wherein neuronal or glial vacuolation in the subject is reduced or eliminated cellular activity in the spinal cord of the subject.

7. The use of any of claims 1 to 6, wherein loss of learning ability is decreased as compared to an untreated individual.

8. The use of any of claims 1 to 7, wherein the pair of ZFNs comprises the zinc finger proteins shown in Table 1.

9. The use of any of claims 1 to 8, wherein the ZFN expression vector and the donor vector comprise adeno-associated vectors (AAV).

10. The use of claim 9, wherein the ZFN expression vector and the donor vector AAV are to be administered via intravenous injection.

11. The use of any of claims 1 to 10, wherein the ZFN:ZFN:Donor ratio to be administered to the subject is 1:1:8.

12. A Hep2G cell comprising a promoterless donor vector encoding hIUDA or hIDS integrated into intron 1 of an albumin gene, wherein the hIUDA or hIDS is expressed and secreted from the Hep2G cell.

13. A cell culture comprising the Hep2G cell of claim 12, wherein the culture media comprises the hIUDA or hIDS.

14. A pharmaceutical composition comprising hIUDA and/or hIDS secreted from a cell according to claim 12 or a cell culture according to claim 13.

15. Use of the composition according to claim 14 in the manufacture of a medicament for reducing or preventing central nervous system (CNS) impairment in a subject with mucopolysaccharidosis type I (MPS I) or mucopolysaccharidosis type II (MPS II).