however. For ERT, some studies of long term treatment have shown a statistically significant association between duration of ERT use and worsening quality of life (Aronovich and Hackett (2015) *Mol Gen Metabol* 114: 83-93). Additionally, ERT is used for amelioration of somatic disease (without CNS involvement), and is inefficient for treatment of neurologic disease because the exogenously given enzyme does not cross the blood brain barrier (Muenzer *ibid*). For HSCT, there is a significant rate of mortality (at least 10%) due to the HSCT process (Aronovich and Hackett, *ibid*). Thus, the most severe MPS I and MPS II patients with CNS impairment are underserved by the currently available treatments.

[0014] Thus, there remains a need for methods and compositions that can be used to treat MPS I and MPS II disease, including treatment through genome editing, for instance, to deliver a transgene encoding a gene product such that expression of the transgene will be at a therapeutically relevant level.

SUMMARY

[0015] Disclosed herein are methods and compositions for treating and/or preventing Hurler (or MPS I) disease and for treating and/or preventing Hunter (MPS II) syndrome. The disclosure describes methods for insertion of a transgene sequence into a suitable target cell wherein the transgene encodes a protein (*e.g.,* a full length or truncated IDUA protein for MPS I; a full length or truncated IDS protein for MPS II) that treats the disease, for example a protein that is lacking or deficient in MPS I or MPS II, which when supplied by the IDUA or IDS transgene treats and/or prevents MPS I or MPS II, respectively. The disclosure also describes methods for the transfection and/or transduction of a suitable target cell with an expression system such that an IDUA or IDS encoding transgene expresses a protein that treats (*e.g.,* alleviates one or more of the symptoms associated with) the disease. The IDUA or IDS protein may be excreted from the target cell such that it is able to affect or be taken up by other cells that do not harbor the transgene (bystander effect or cross correction). The disclosure also provides for methods for the production of a cell (*e.g.,* a mature or undifferentiated cell) that produces high levels of IDUA or IDS where the introduction of a population of these altered cells into a patient will supply that needed protein to treat and/or prevent MPS I or MPS II. In addition, the disclosure provides methods for the production of a cell (*e.g*. a mature or undifferentiated cell)

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 and reducing dermatan sulfate levels in the CNS 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) for a subject with mucopolysaccharidosis type I (MPS I) or human iduronate-2-sulftase (hIDS) for a subject with mucopolysaccharidosis type II (MPS II), wherein (i) 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; (ii) 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; (iii) the subject is to be treated with cyclophosphamide prior to administration of the promoterless donor vector and expression vector and (iv) heparan sulfate levels in the CNS are not reduced.

[0016] In one example, 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 examples, the ZFN:ZFN:Donor ratio administered to the subject is 1:1:8.

[0017] In another example, the disclosure 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-

chromosomally. In one example, the corrective IDUA or IDS transgene is inserted (stably or extra-chromosomally) into a cell line for the *in vitro* production of IDUA or IDS, which (optionally purified and/or isolated) protein can then be administered to a subject for treating a subject with MPS I or MPS II disease (*e.g.,* by reducing and/or eliminating one or more symptoms associated with MPS I or MPS II disease).

[0018] In another example, described herein are *ex vivo* or *in vivo* methods of treating a subject with MPS I or MPS II disease (*e.g.,* by reducing and/or eliminating one or more symptoms associates with MPS I or MPS II disease), the methods comprising providing an IDUA or IDS protein to a subject in need therefore. In certain examples, the methods comprise *in vivo* methods, for example inserting an IDUA or IDS transgene into a cell as described herein such that the protein is produced in a subject with MPS I or MPS II disease. In some examples, the active expressed IDUA and/or IDS expressed reaches 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300 or greater percent of the endogenous levels in the subject with MPSI or MPS II disease. In some examples, the active IDUA or IDS enzyme expressed reaches 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300 or greater percent of enzyme levels found in wild type individuals. In other examples, isolated cells comprising an IDUA or IDS transgene can be used to treat a patient in need thereof, for example, by administering the cells to a subject with MPS I or MPS II disease. In other examples, the corrective IDUA or IDS transgene and/or cell expressing IDUA or IDS is inserted into subject in need thereof such that the replacement protein is produced *in vivo*. In some preferred examples, the corrective transgene is inserted into the genome of cells in the subject, while in other preferred examples, the corrective transgene is inserted into the cells of the subject and is maintained in the cells extra-chromosomally. The transgene may be integrated into a variety of target tissues, including liver, brain, muscle, heart, lung, etc. In any of the methods described herein, the expressed IDUA or IDS protein may be excreted from the cell (*e.g*. via exportation into the blood) to act on or be taken up by other cell that lack the IDUA or IDS transgene (for example by receptor-mediated endocytosis via the mannose receptor or the mannose-6-phosphate receptor). Thus, in certain examples, cells expressing the IDUA or IDS transgene (*e.g.,* liver, HSPCs or cells derived from edited HSPC) secrete the IDUA or IDS protein which then acts

on one or more secondary tissues (*e.g.,* brain, muscle, bones, heart, lung, spleen, etc.). In some instances, the target tissue (which expresses the IDUA or IDS transgene) is the liver. In other instances, the target tissue is the brain. In other instances, the target is blood (*e.g.,* vasculature). In other instances, the target is skeletal muscle. In still other instances, the target tissue(s) (is)are those that display additional examples of the pathophysiology of the disease (*e.g*. cardiac valves, bones, joint tissue, airway tissue etc.).

[0019] In any of the methods described herein, the corrective IDUA or IDS gene comprises the wild type sequence of the functioning IDUA or IDS gene (including functional fragments of wild-type), while in other examples, the sequence of the corrective IDUA or IDS transgene is altered in some manner, for example to give enhanced biological activity (*e.g.,* optimized codons to increase biological activity and/or truncation of the IDUA- or IDS-encoding sequence). In preferred examples, the methods and compositions of the disclosure produce active IDUA or IDS enzyme in an MPSI or MPS II subject in need thereof. The expressed IDUA and/or IDS enzymes are able to degrade glycosoaminoglycans (GAGs). In some examples, GAGs are reduced in specific tissues of the body, including liver, spleen, kidney, lung, heart, muscle and brain. In preferred examples, the GAG levels in any tissue are reduced 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 or 100% or any value there between as compared to an untreated MPSI or MPS II subject.

[0020] In any of the methods described herein, the IDUA or IDS transgene may be inserted into the genome of a target cell using a nuclease. Non-limiting examples of suitable nucleases include zinc-finger nucleases (ZFNs), TALENs (Transcription activator like protein nucleases) and/or CRISPR/Cas nuclease systems, which include a DNA-binding molecule that binds to a target site in a region of interest (*e.g.,* a disease associated gene, a highly-expressed gene, an albumin gene or other or safe harbor gene) in the genome of the cell and one or more nuclease domains (*e.g.,* cleavage domain and/or cleavage half-domain). Cleavage domains and cleavage half domains can be obtained, for example, from various restriction endonucleases, Cas proteins (class 1 or class 2) and/or homing endonucleases. In certain examples, the zinc finger domain recognizes a target site in an albumin gene or a globin gene in red blood cells (RBCs). *See, e.g.,* U.S. Publication No. 2014001721,

incorporated by reference in its entirety herein. In other examples, the ZFN, TALEN, and/or CRISPR/Cas system binds to and/or cleaves a safe-harbor gene, for example a CCR5 gene, a PPP1R12C (also known as AAV S1) gene, albumin, HPRT or a *Rosa* gene. *See, e.g.,* U.S. Patent Nos. 7,888,121; 7,972,854; 7,914,796; 7,951,925; 8,110,379; 8,409,861; 8,586,526; U.S. Patent Publications 20030232410; 20050208489; 20050026157; 20060063231; 20080159996; 201000218264; 20120017290; 20110265198; 20130137104; 20130122591; 20130177983; 20130177960 and 20140017212. The nucleases (or components thereof) may be provided as a polynucleotide encoding one or more ZFN, TALEN, and/or CRISPR/Cas system described herein. The polynucleotide may be, for example, mRNA. In some examples, the mRNA may be chemically modified (*See e.g.* Kormann *et al*, (2011) *Nature Biotechnology* 29(2):154-157). In other examples, the mRNA may comprise an ARCA cap (see U.S. Patents 7,074,596 and 8,153,773). In further examples, the mRNA may comprise a mixture of unmodified and modified nucleotides (see U.S. Patent Publication 20120195936). In still further examples, the mRNA may comprise a WPRE element (see U.S. Patent Application 62/158,277).

[0021] In another example, the disclosure supplies an engineered nuclease protein capable of cleaving (editing) the genome of a stem or precursor cell (*e.g.,* blood cell precursor, liver stem cell, etc.) for introduction of a desired IDUA or IDS transgene. In some examples, the edited stem or precursor cells are then expanded and may be induced to differentiate into a mature edited cells *ex vivo*, and then the cells are given to the patient. In other examples, the edited precursors (*e.g.,* CD34+ stem cells) are given in a bone marrow transplant which, following successful implantation, proliferate producing edited cells that then differentiate and mature *in vivo* and contain the biologic expressed from the IDUA or IDS transgene. In other examples, the edited stem cells are muscle stem cells which are then introduced into muscle tissue. In some examples, the engineered nuclease is a Zinc Finger Nuclease (ZFN) and in others, the nuclease is a TALE nuclease (TALEN), and in other examples, a CRISPR/Cas system is used. The nucleases may be engineered to have specificity for a safe harbor locus, a gene associated with a disease, or for a gene that is highly expressed in cells. By way of non-limiting example only, the safe harbor locus may be the AAVS1 site, the CCR5 gene, albumin or the HPRT gene while the disease associated gene may be the IDUA or IDS gene.

[0022] In any of the methods described herein, the nuclease (*e.g.,* ZFN, TALEN, and/or CRISPR/Cas system) may be supplied in polynucleotide form, for example an expression vector comprising a polynucleotide, encoding one or more nucleases (or components thereof), optionally operably linked to a promoter. In one example, the expression vector is a viral vector. In a further example, described herein is an IDUA or IDS expression vector comprising a polynucleotide encoding IDUA or IDS as described herein, optionally operably linked to a promoter. In one example, the expression is a viral vector.

[0023] In another example, described herein is a host cell comprising one or more ZFN, TALEN, and/or CRISPR/Cas system expression vectors and/or an IDUA or IDS expression vector as described herein. The host cell may be stably transformed or transiently transfected or a combination thereof with one or more ZFN, TALEN, and/or CRISPR/Cas system expression vectors. In some examples, the host cell is a liver cell. Thus, provided herein is a Hep2G cell comprising a promoterless donor vector encoding hIUDA and/or hIDS integrated into intron 1 of an albumin gene, wherein the hIUDA and/or hIDS is expressed and secreted from the Hep2G cell. A cell culture comprising the Hep2G cell of claim 11, wherein the culture media comprises the hIUDA or hIDS is also provide as is a pharmaceutical composition comprising hIUDA and/or hIDS secreted from a cell and/or isolated from culture media of cells as described herein.

[0024] In other examples, methods are provided for replacing a genomic sequence in any target gene with a therapeutic IDUA or IDS transgene as described herein, for example using one or more nucleases (*e.g.,* a ZFN, TALEN, and/or CRISPR/Cas system (or vector encoding said ZFN, TALEN, and/or CRISPR/Cas system) as described herein and a "donor" sequence or IDUA or IDS transgene that is inserted into the gene following targeted cleavage with the ZFN, TALEN, and/or CRISPR/Cas system. The donor IDUA or IDS sequence may be present in the vector carrying the nuclease (or component thereof), present in a separate vector (*e.g*., Ad, AAV or LV vector or mRNA) or, alternatively, may be introduced into the cell using a different nucleic acid delivery mechanism. Such insertion of a donor nucleotide sequence into the target locus (*e.g.,* highly expressed gene, disease associated gene, other safe-harbor gene, *etc*.) results in the expression of the IDUA or IDS transgene under control of the target locus's (*e.g.,* albumin, globin, etc.) endogenous genetic

control elements. In some examples, insertion of the IDUA or IDS transgene, for example into a target gene (*e.g.,* albumin), results in expression of an intact IDUA or IDS protein sequence and lacks any amino acids encoded by the target (*e.g.,* albumin). In other examples, the expressed exogenous IDUA or IDS protein is a fusion protein and comprises amino acids encoded by the IDUA or IDS transgene and by the endogenous locus into which the IDUA or IDS transgene is inserted (*e.g.,* from the endogenous target locus or, alternatively from sequences on the transgene that encode sequences of the target locus). The target may be any gene, for example, a safe harbor gene such as an albumin gene, an AAVS1 gene, an HPRT gene; a CCR5 gene; or a highly-expressed gene such as a globin gene in an RBC (*e.g.,* beta globin or gamma globin). In some instances, the endogenous sequences will be present on the amino (N)-terminal portion of the exogenous IDUA protein, while in others, the endogenous sequences will be present on the carboxy (C)- terminal portion of the exogenous IDUA or IDS protein. In other instances, endogenous sequences will be present on both the N- and C-terminal portions of the IDUA exogenous protein. The endogenous sequences may include full-length wild-type or mutant endogenous sequences or, alternatively, may include partial endogenous amino acid sequences. In some examples, the endogenous gene-transgene fusion is located at the endogenous locus within the cell while in other examples, the endogenous sequence-transgene coding sequence is inserted into another locus within a genome (*e.g.,* an IDUA or IDS -transgene sequence inserted into an albumin, HPRT or CCR5 locus). In some examples, the IDUA or IDS transgene is expressed such that a therapeutic IDUA or IDS protein product is retained within the cell (*e.g.,* precursor or mature cell). In other examples, the IDUA or IDS transgene is fused to the extracellular domain of a membrane protein such that upon expression, a transgene IDUA or IDS fusion will result in the surface localization of the therapeutic protein. *See, e.g.,* U.S. Patent Publication No. 20140017212. In some examples, the edited cells further comprise a trans-membrane protein to traffic the cells to a particular tissue type. In one example, the trans-membrane protein is an antibody, while in others, the trans-membrane protein is a receptor. In certain examples, the cell is a precursor (*e.g.,* CD34+ or hematopoietic stem cell) or mature RBC. In some examples, the therapeutic IDUA or IDS protein product encoded on the transgene is exported out of the cell to affect or be taken up by cells lacking the transgene. In certain

examples, the cell is a liver cell which releases the therapeutic IDUA or IDS protein into the blood stream to act on distal tissues (*e.g*., brain, heart, muscle, etc.).

[0025] The disclosure also supplies methods and compositions for the production of a cell (*e.g.,* RBC) carrying an IDUA or IDS therapeutic protein for treatment of MPS I disease that can be used universally for all patients as an allogenic product. These RBC carriers may comprise trans-membrane proteins to assist in the trafficking of the cell. In one example, the trans-membrane protein is an antibody, while in others, the trans-membrane protein is a receptor.

[0026] In one example, the IDUA or IDS transgene is expressed from the albumin promoter following insertion into the albumin locus. The biologic encoded by the IDUA or IDS transgene then may be released into the blood stream if the transgene is inserted into a hepatocyte *in vivo*. In some examples, the IDUA or IDS transgene is delivered to the liver *in vivo* in a viral vector through intravenous or other injection.

[0027] In some examples, the IDUA or IDS transgene donor is transfected or transduced into a cell for episomal or extra-chromosomal maintenance of the transgene. In some examples, the IDUA or IDS transgene donor is maintained on a vector comprising regulatory domains to regulate expression of the transgene donor. In further examples, the vector comprising the transgene donor is delivered to a suitable target cell *in vivo*, such that the IDUA or IDS therapeutic protein encoded by the transgene donor is released into the blood stream if the transgene donor vector is delivered to a hepatocyte.

[0028] In another example, the disclosure describes precursor cells (hematopoietic stem cells, muscle stem cells or CD34+ hematopoietic stem cell (HSC) cells) into which the IDUA or IDS transgene has been inserted such that mature cells derived from these precursors contain high levels of the IDUA or IDS product encoded by the transgene. In some examples, these precursors are induced pluripotent stem cells (iPSC).

[0029] In some examples, the methods of the disclosure may be used *in vivo* in transgenic animal systems. In some examples, the transgenic animal may be used in model development where the transgene encodes a human IDUA or IDS protein. In some instances, the transgenic animal may be knocked out at the corresponding endogenous locus, allowing the development of an *in vivo* system

where the human protein may be studied in isolation. Such transgenic models may be used for screening purposes to identify small molecules, or large biomolecules or other entities which may interact with or modify the human protein of interest. In some examples, the IDUA or IDS transgene is integrated into the selected locus (*e.g.,* highly expressed or safe-harbor) into a stem cell (*e.g.,* an embryonic stem cell, an induced pluripotent stem cell, a hepatic stem cell, a neural stem cell etc.) or non-human animal embryo obtained by any of the methods described herein, and then the embryo is implanted such that a live animal is born. The animal is then raised to sexual maturity and allowed to produce offspring wherein at least some of the offspring comprise the integrated IDUA or IDS transgene.

[0030] In a still further example, provided herein is a method for site specific integration of a nucleic acid sequence into an endogenous locus (*e.g.,* disease-associated, highly expressed such as an albumin locus in a liver cell or globin locus in RBCs of a chromosome, for example into the chromosome of a non-human embryo. In certain examples, the method comprises: (a) injecting a non-human embryo with (i) at least one DNA vector, wherein the DNA vector comprises an upstream sequence and a downstream sequence flanking the IDUA or IDS encoding nucleic acid sequence to be integrated, and (ii) at least one RNA molecule encoding a zinc finger, TALE nuclease or CRISPR/Cas system that recognizes the site of integration in the target locus, and (b) culturing the embryo to allow expression of the ZFN, TALEN, and/or CRISPR/Cas system, wherein a double stranded break introduced into the site of integration by the ZFN, TALEN, and/or CRISPR/Cas system is repaired, via homologous recombination with the DNA vector, so as to integrate the nucleic acid sequence into the chromosome.

[0031] In any of the previous examples, the methods and compounds of the disclosure may be combined with other therapeutic agents for the treatment of subjects with MPS I or MPS II disease. In some examples, the methods and compositions are used in combination with methods and compositions to allow passage across the blood brain barrier. In other examples, the methods and compositions are used in combination with compounds known to suppress the immune response of the subject.

[0032] A kit, comprising a nuclease system and/or an IDUA or IDS donor as described herein is also provided. The kit may comprise nucleic acids encoding the

ZFN, TALEN, and/or CRISPR/Cas system, *(e.g.* RNA molecules or the ZFN, TALEN, and/or CRISPR/Cas system encoding genes contained in a suitable expression vector), donor molecules, expression vectors encoding the single-guide RNA suitable host cell lines, instructions for performing the methods of the disclosure, and the like.

[0033] These and other examples will be readily apparent to the skilled artisan in light of disclosure as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **Figures 1A and 1B** depict the results following treatment of the indicated groups of mice with the mouse albumin-specific ZFNs and the IDUA donor. In Figure 1A graphs indicate levels of ZFN activity (% indels) in the livers of mice treated with rAAV2/8 mouse surrogate ZFNs and hIDUA donor at 1 month (left panel) and 4 months (right panel) post-dosing. Genomic DNA was isolated from livers of individual mice and ZFN activity was measured by deep sequencing of the mouse albumin locus at the ZFN target site. Study group numbers are listed, and each individual symbol represents data from an individual mouse at the stated time point (at necropsy). Horizontal lines represent the mean ± standard deviation of each group. In Figure 1B, IDUA expression was measured by Western blot analysis. Surrogate mouse ZFNs and hIDUA donor were packaged as rAAV2/8 and injected IV into mice. Mice were euthanized at 1 month (panel i) or 4 months (panel ii) post-dosing and protein was extracted from the liver. Expression of hIDUA in mouse livers was determined using a human-specific IDUA antibody. GAPDH is shown as a loading control.

[0035] **Figures 2A through 2C** depict detectable IDUA enzymatic activity. Figure 2A is a graph depicting enzyme activity in the plasma. Surrogate mouse ZFNs and hIDUA donor were packaged as rAAV2/8 and injected IV into mice. Plasma was harvested on the indicated days and IDUA enzyme activity was determined by fluorimetric assay. Data show mean ± SD of 4-8 animals/group, depending on the time point. Two-way repeated-measures ANOVA followed by Dunnett's multiple comparison test revealed that the MPS I ZFN+Donor groups were significantly different from the wild type group from Day 14-120 for male animals, and from Day 28-120 for the female animals. Figure 2B shows the IDUA activity measured up to

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 and reducing dermatan sulfate levels in the CNS 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) for a subject with mucopolysaccharidosis type I (MPS I) or human iduronate-2-sulftase (hIDS) for a subject with mucopolysaccharidosis type II (MPS II),

wherein

(i) 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;

(ii) 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;

(iii) the subject is to be treated with cyclophosphamide prior to administration of the promoterless donor vector and expression vector; and

(iv) heparan sulfate levels in the CNS are not reduced.

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 reduces 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 in the spinal cord of the subject.

6. The use of any of claims 1 to 5, wherein loss of learning ability is decreased as compared to an untreated individual.

7. The use of any of claims 1 to 6, wherein the pair of ZFNs comprises the zinc finger proteins shown in Table 1.

8. The use of any of claims 1 to 7, wherein the ZFN expression vector and the donor vector comprise adeno-associated vectors (AAV).

9. The use of claim 8, wherein the ZFN expression vector and the donor vector AAV are to be administered via intravenous injection.

10. The use of any of claims 1 to 9, wherein the ZFN:ZFN:Donor ratio to be administered to the subject is 1:1:8.