Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2901328 C 2023/08/01

(11)(21) **2 901 328**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 2014/03/13
(87) Date publication PCT/PCT Publication Date: 2014/09/25
(45) Date de délivrance/Issue Date: 2023/08/01
(85) Entrée phase nationale/National Entry: 2015/08/13
(86) N° demande PCT/PCT Application No.: US 2014/025509
(87) N° publication PCT/PCT Publication No.: 2014/151341
(30) Priorité/Priority: 2013/03/15 (US61/788,724)

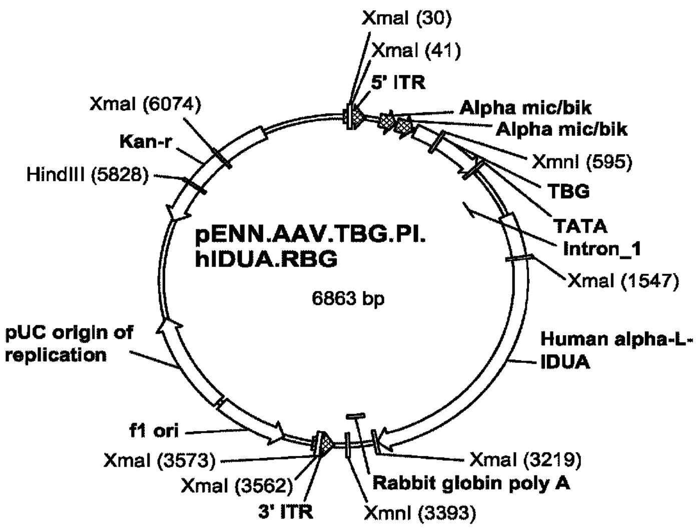
(51) Cl.Int./Int.Cl. *C12N 15/85* (2006.01), *A61K 38/47* (2006.01), *A61P 3/00* (2006.01), *C12N 15/56* (2006.01), *C12N 15/864* (2006.01), *C12N 7/01* (2006.01), *C12N 9/24* (2006.01)

(72) Inventeurs/Inventors:
WILSON, JAMES M., US;
GURDA, BRITTNEY L., US

(73) Propriétaire/Owner:
THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, US

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : COMPOSITIONS ET METHODES DE TRAITEMENT DE LA MPS1
(54) Title: COMPOSITIONS AND METHODS FOR TREATING MPS1



(57) Abrégé/Abstract:
A vector have an expression cassette containing have a hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase is provided. The vector may be a production vector or a rAAV8. Also provided are compositions containing these vectors and methods of treating MPSI and the symptoms associated with Hurler, Hurler-Scheie and Scheie syndromes.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 September 2014 (25.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/151341 A1

(51) International Patent Classification:
C12N 7/00 (2006.01) *C12N 15/00* (2006.01)
C12N 7/01 (2006.01)

(21) International Application Number: PCT/US2014/025509

(22) International Filing Date: 13 March 2014 (13.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/788,724 15 March 2013 (15.03.2013) US

(71) Applicant: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, PA 19104-6283 (US).

(72) Inventors: WILSON, James, M.; 7 Fallbrook Lane, Glen Mills, PA 19342 (US). **GURDA, Brittney, L.**; 1152 Kriebel Mill Road, Collegeville, PA 19426 (US).

(74) Agents: KODROFF, Cathy, A. et al.; Howson & Howson LLP, 350 Sentry Parkway, Building 620. Suite 210, Blue Bell, PA 19422 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: COMPOSITIONS AND METHODS FOR TREATING MPSI

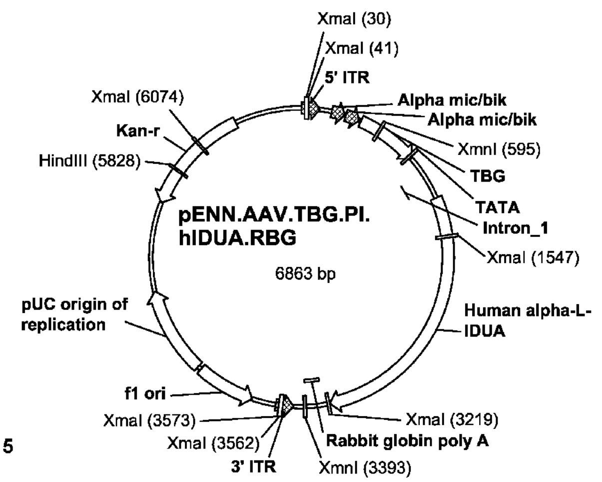


Figure 5

(57) Abstract: A vector have an expression cassette containing have a hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase is provided. The vector may be a production vector or a rAAV8. Also provided are compositions containing these vectors and methods of treating MPSI and the symptoms associated with Hurler, Hurler-Scheie and Scheie syndromes.

WO 2014/151341 A1

COMPOSITIONS AND METHODS FOR TREATING MPS1

BACKGROUND OF THE INVENTION

The mucopolysaccharidoses are a group of inherited disorders caused by a lack of specific lysosomal enzymes involved in the degradation of glycosaminoglycans (GAG), also called mucopolysaccharides. The accumulation of partially-degraded GAG causes interference with cell, tissue, and organ function. Over time, the GAG accumulates within cells, blood, and connective tissue, resulting in increasing cellular and organ damage. The most serious of the mucopolysaccharidosis (MPS) disorders, MPS I, is caused by a deficiency of the enzyme α-L-iduronidase (IDUA). This leads to three clinical syndromes which in order of severity are Hurler, Hurler-Scheie and Scheie syndromes. Each is inherited in an autosomal recessive manner with the extent of enzyme deficiency being directly related to the severity of the clinical phenotype.

The *IDUA* gene has been reported to provide instructions for producing an enzyme called alpha-L-iduronidase, which is essential for the breakdown of large sugar molecules called glycosaminoglycans (GAGs). Specifically, alpha-L-iduronidase is reported to remove sulfate from a molecule known as sulfated alpha-L-iduronic acid, which is present in two GAGs called heparan sulfate and dermatan sulfate. Alpha-L-iduronidase is located in lysosomes, compartments within cells that digest and recycle different types of molecules. More than 100 mutations in the *IDUA* gene have been found to cause mucopolysaccharidosis type I (MPS I). Mutations that change one DNA building block (nucleotide) are the most common. Mutations that cause MPS I to reduce or completely eliminate the function of alpha-L-iduronidase.

With respect to the clinical syndromes, the current standard of care for Hurler syndrome is hematopoietic stem cell transplantation (HSCT) such as bone marrow transplantation (BMT) or umbilical cord blood transplantations (UCBT). The procedure is done as early as possible, and before the age of two, to impact on both somatic and CNS aspects of the disease. However, HSCT for MPS I remains associated with a significant amount of morbidity and a 20% mortality rate. If transplantation is not an option, then enzyme replacement therapy (ERT) may be started which requires a weekly infusion of enzyme for the life of the patient. ERT does not impact on the progression of CNS disease but does partially improve the somatic manifestations. Organomegaly is significantly improved although aspects of the disease in the skeletal system, eye and heart are only partially improved. Patients may require surgery to stabilize the hip and knee and to treat carpal tunnel syndrome and finger contractions. Cardiac disease is treated medically although surgery may eventually be required.

ERT for MPS I provides exogenous enzyme for uptake into lysosomes and increased catabolism of GAG. Although the lysosomal enzymes function internally, cell-surface mannose-6-phosphate receptors are capable of binding, internalizing, and delivering these enzymes to the lysosomes. Recombinant IDUA (Aldurazyme®, BioMarin) is approved by FDA for patients with Hurler and Hurler-Scheie forms of MPS I and for patients with the Scheie form who have moderate to severe symptoms and was shown to improve pulmonary function and walking capacity. ERT has also been observed to reduce hepatomegaly in MPS I patients, as well as the levels of urinary GAG. However, because intravenous enzyme does not easily cross into the brain, ERT does not currently address the neurological symptoms experienced by some MPS I patients.

Complications of ERT revolve around immune response to the recombinant enzyme which can range from mild to full-blown anaphylaxis as well as complications of life-long peripheral access such as local and systemic infections. Up to 91% of patients receiving Aldurazyme develop antibodies to the enzyme, although it is not clear how much it affects efficacy. Furthermore, ERT requires weekly intravenous (i.v.) infusions, administered over a period of 3-8 hours in a hospital setting, which significantly impacts patient quality of life and, at a high expense, is a major strain on health care reimbursement systems.

In light of these limitations, a treatment that can more effectively correct the morbidity associated with MPS I remains an unmet medical need.

SUMMARY OF THE INVENTION

In one aspect, the invention provides an expression cassette comprising a human alpha-L-iduronidase (hIDUA) gene having the nucleotide sequence of SEQ ID NO: 1 or a sequence at least about 95% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase in human cells, wherein said expression cassette further comprises regulatory control sequences which direct expression of the human alpha-L-iduronidase in human cells, said regulatory control sequences comprising a liver-specific promoter.

In another aspect, the invention provides a vector containing the expression cassette. In one embodiment, the expression cassette is located on a cis plasmid. In another embodiment, the expression cassette is located on pENN.TBG.hIDUA.nRBG.

In yet another aspect, the invention provides a recombinant adeno-associated virus (rAAV) particle having an AAV capsid and having packaged therein a left inverted terminal repeat (ITR), a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, and an AAV right ITR, wherein said hIDUA gene has a sequence shown in SEQ ID NO: 1 (FIG 1) or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase. In one embodiment, the functional hIDUA gene is expressed under the control of a liver-specific promoter. Such a promoter may be a thyroxin binding globulin (TBG) promoter.

In a further aspect, the invention provides the recombinant adeno-associated viral particle AAV2/8.TBG.hIDUA.co.

In a yet another aspect, the invention provides a composition useful for treating mucopolysaccharidosis type I (MPS I) comprising the rAAV comprising the expression cassette described herein and a pharmaceutically acceptable carrier.

In still a further aspect, the invention provides a method for treating type I mucopolysaccharidosis comprising delivering an effective amount of a composition comprising a pharmaceutically acceptable carrier and a rAAV as described herein.

In yet another aspect, the invention provides a method for treating or ameliorating the symptoms of Hurler, Hurler-Scheie and/or Scheie syndromes.

Still other aspects and advantages of the invention will be apparent from the detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

FIGs.1A-1D provides the sequence of the pENN.AAV.TBG.PI.hIDUA.RGB plasmid [SEQ ID NO:3] described herein, which includes the nucleic acid sequence of the functional human IDUA gene. The gene of the functional IDUA located at position 1251-3213 of FIG1 and its encoded enzyme sequence are also provided in SEQ ID NO: 1 and 2. The sequence is further annotated to identify the sequences of the alpha mic/bik enhancers, the intron 1, the rabbit globin poly A, the ITRs, the origin of replication.

FIG. 2 provides the circular map of pENN.AAV.TBG.PI.hIDUA.RGB.

Date reçu/Date Received 2020-07-09

DETAILED DESCRIPTION OF THE INVENTION

The compositions described herein provide an expression cassette carrying a human IDUA gene which expresses a therapeutically effective amount of functional human alpha-L-iduronidase enzyme in a human subject.

As used herein, a "therapeutically effective amount" refers to the amount of the composition which delivers and expresses in the target cells an amount of enzyme sufficient to ameliorate or treat the symptoms of Hurler, Hurler-Scheie and/or Scheie syndromes, and or MPS I. "Treatment" may include preventing the worsening of the symptoms of one of the syndromes (or MPS I) and possibly reversal of one or more of the symptoms thereof.

As used herein a "functional human alpha-L-iduronidase" refers to a human alpha-L-iduronidase enzyme which functions normally in humans without MPS1 or an associated syndrome such as Hurler, Hurler-Scheie and/or Scheie syndromes. Conversely, a human alpha-L-iduronidase enzyme variant which causes MPS1 or one of these syndromes is considered non-functional. In one embodiment, a functional human alpha-l-iduronidase has the amino acid sequence of a wild-type human alpha-L-iduronidase described by Bremer et al, Mol. Genet. Metab. 104 (3): 289-294 (2011), NCBI Reference Sequence NP_000194.2, reproduced in SEQ ID NO:2 (653 amino acids). However, several naturally occurring functional polymorphisms (variants) of this sequence have been described and may be encompassed within the scope of this invention. These variants include, with reference to SEQ ID NO: 2, a N-linked glycosylation at position 110 [Chen *et al*, J Proteome Res. , 8:651-661 (2009)], a change from H to Q at amino acid position 33 [SEQ ID NO: 7, VAR_003350; Scott, HS, *et al*, Proc Natl Acad. Sci, 88:9695-9699 (1991); Scott, HS, *et al*, Genomics, 12:1311-1313 (1992); Scott HS, *et al*, Hum Genet, 90:327-327 (1992); Bertola F., *et al*, Hum Mutat, 32: E2189-E2210 (2011)], an H to Q reduction at amino acid position 82 [SEQ ID NO: 8, VAR_020976; Scott, HS, Hum Genet, cited above], a change from R to Q at position 105 [SEQ ID NO: 9, VAR_003356; Scott, Hum Genet, cited above; Bertola *et al*, cited above], a change from G to R at position 116 [SEQ ID NO: 12, VAR_003367], a change from V to A at position 279 [SEQ ID NO: 11, VAR_003359], a change from L to R at position 346 [SEQ ID NO: 12, VAR_017436, Teng, YN, *et al*, Clin. Genet, 57: 131-136 (2000)], a change from A to T at position 361 [SEQ ID NO: 13, VAR_003364; Scott, HS, *et al*, Hum Mol Genet, 2: 1471-1473 (1993); Yogalingam *et al*, Hum Mutat, 24: 199-207

(2004); Bertola, et al, cited above], a change from H to N at position 449 [SEQ ID NO: 14, VAR_066228, Bertola *et al*, cited above], a change from V to I at position 454 [SEQ ID NO: 15, VAR_003372; Yogalingam *et al*, cited above; Bertola, *et al*, cited above], a change from A to T at position 591 [SEQ ID NO: 16, VAR_0066231, Bertola *et al*, cited above], and a change from A to T at position 622 [SEQ ID NO: 17, Scott *et al*, Genomics, cited above]. *See, e.g.,* UniProtKB/Swiss-Prot. In another embodiment, a functional human alpha-L-iduronidase may include a synthetic amino acid sequence in which all or a portion of the first 26 amino acids of SEQ ID NO:2, which correspond to the leader (signal) peptide, are replaced with a heterologous leader peptide. This leader peptide, which is responsible for transporting the enzyme out of the cell through its secretory pathway into the circulation, may be substituted with another suitable leader peptide, *e.g.*, such as the leader peptides from interleukin-2 (IL-2) or oncostatin. Suitable leader peptides are preferably, although not necessarily of human original. Suitable leader peptides may be determined using a variety of computational programs for determining the leader (signal) peptide in a selected protein. Although not limited, such sequences may be from about 15 to about 50 amino acids in length, or about 20 to about 28 amino acids in length, or may be larger or smaller as required. In addition, at least one *in vitro* assay has been described as being useful to assess the enzymatic activity of an IDUA enzyme [*see, e.g.*, Kakkis *et al*, Mol Genet Metabol, 2001 Mar; 72(3): 199-208].

Suitably, the composition and method described herein do not require long term, repeated weekly injections of a therapeutic dose. Without wishing to be bound by theory, the method described herein is believed to be useful for correcting the central nervous system phenotype in addition to somatic symptoms associated with MPSI disorders.

Expression Cassette

The expression cassette is composed of, at a minimum, a gene and its regulatory sequences. Where the cassette is designed to be expressed from a recombinant adeno-associated virus, the expression cassette further contains 5' and 3' AAV inverted terminal repeats (ITRs). These ITR's may be full-length, or one or both of the ITRs may be truncated. For example, a truncated 5' ITR containing a deletion of the D sequence and a terminal resolution site (trs) deletion may be used, e.g., for a self-complementary AAV. In one embodiment, the rAAV is pseudotyed, *i.e.,* the AAV capsid is from a different source AAV than that the AAV which provides the ITRs. In one embodiment, the ITRs of AAV serotype 2 are used. However, ITRs from other suitable sources may be selected.

As described herein, patients suffering from one of the conditions described herein are delivered an expression cassette which carries a functional human alpha-L-iduronidase (hIDUA) gene under control of regulatory sequences which direct expression of a functional human alpha-L-iduronidase enzyme in the cells.

The expression cassette contains a hIDUA gene characterized by having the nucleotide sequence of SEQ ID NO: 1. This sequence, developed by the inventors, has an identity of about 83% with the published gene sequence of Genbank NP000194.2 encoding SEQ ID NO: 2. In another embodiment, the expression cassette contains a hIDUA gene characterized by having the nucleotide sequence at least about 80% identical to SEQ ID NO: 1 and encodes a functional human alpha-L-iduronidase. In another embodiment, the sequence is at least about 85% identity to SEQ ID NO: 1 or at least about 90% identical to SEQ ID NO:1 and encodes a functional human alpha-L-iduronidase. In one embodiment, the sequence is at least about 95% identical to SEQ ID NO:1, at least about 97% identical to SEQ ID NO:1, or at least about 99% identical to SEQ ID NO: 1 and encodes a functional human alpha-L-iduronidase. In one embodiment, this encompasses full-length hIDUA gene, including the leader peptide sequences of the human alpha-L-iduronidase (*i.e.*, encoding about amino acid 26, or about amino acid 27, to about amino acid 653 of SEQ ID NO:2), corresponding to about 1 to about 78 of SEQ ID NO:1. In another embodiment, the hIDUA gene encodes a functional synthetic human alpha-L-iduronidase enzyme which is synthetic peptide comprising a heterologous leader sequence fused to the secreted portion of a functional alpha-L-iduronidase enzyme, *i.e.*, about amino acids 27 to about 653 of SEQ ID NO: 2 or one of the functional variants thereof which are identified herein.

Identity or similarity with respect to a sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (*i.e.,* same residue) or similar (*i.e.,* amino acid residue from the same group based on common side-chain properties, see below) with the peptide and polypeptide regions provided herein, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Percent (%) identity is a measure of the relationship between two polynucleotides or two polypeptides, as determined by comparing their nucleotide or amino acid sequences, respectively. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. The alignment of the two sequences is examined and the number of positions giving an exact amino acid or nucleotide correspondence between the two sequences determined, divided by the total length of the alignment and multiplied by 100 to give a % identity figure. This % identity figure may be determined over the whole length of the sequences to be compared, which is particularly suitable for sequences of the same or very similar length and which are highly homologous, or over shorter defined lengths, which is more suitable for sequences of unequal length or which have a lower level of homology. There are a number of algorithms, and computer programs based thereon, which are available to be used the literature and/or publically or commercially available for performing alignments and percent identity. The selection of the algorithm or program is not a limitation of the present invention.

Examples of suitable alignment programs including, *e.g.,* the software CLUSTALW under Unix and then be imported into the Bioedit program (Hall, T. A. 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98); the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J. et al., Nucleic Acids Res., 12:387-395, 1984, available from Genetics Computer Group, Madison, Wis., USA). The programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity between two polypeptide sequences.

Other programs for determining identity and/or similarity between sequences include, *e.g,,* the BLAST family of programs available from the National Center for Biotechnology Information (NCB), Bethesda, Md., USA and accessible through the home page of the NCBI), the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used; and FASTA (Pearson W. R. and Lipman D. J., Proc. Natl. Acad. Sci. USA, 85:2444-2448, 1988, available as part of the Wisconsin Sequence Analysis Package). SeqWeb Software (a web-based interface to the GCG Wisconsin Package: Gap program).

As used throughout this specification and the claims, the terms “comprising” and “including” are inclusive of other components, elements, integers, steps and the like.

Date Reçue/Date Received 2022-05-24

Conversely, the term "consisting" and its variants are exclusive of other components, elements, integers, steps and the like. The term "about" encompasses a variation within and including ± 10%, unless otherwise specified.

In one embodiment, the expression cassette is designed for human liver-directed expression. Thus, a liver-specific promoter is particularly well suited for the expression cassette. In one embodiment, thyroxin binding globulin promoter is selected. In one embodiment, the TBG promoter has the sequence of nucleotides 442 to 901 of FIG. 1. Alternatively, another liver-specific promoter may be selected. Examples of promoters that are tissue-specific are well known for liver and other tissues (albumin, Miyatake *et al.*, (1997) *J. Virol.*, **71**:5124-32; hepatitis B virus core promoter, Sandig *et al.*, (1996) *Gene Ther.*, 3:1002-9; alpha-fetoprotein (AFP), Arbuthnot *et al.*, (1996) *Hum. Gene Ther.*, 7:1503-14), bone osteocalcin (Stein *et al.*, (1997) *Mol. Biol. Rep.*, **24**:185-96); bone sialoprotein (Chen *et al.*, (1996) *J. Bone Miner. Res.*, **11**:654-64), lymphocytes (CD2, Hansal *et al.*, (1998) *J. Immunol.*, **161**:1063-8; immunoglobulin heavy chain; T cell receptor chain), neuronal such as neuron-specific enolase (NSE) promoter (Andersen *et al.*, (1993) *Cell. Mol. Neurobiol.,* **13**:503-15), neurofilament light-chain gene (Piccioli *et al.*, (1991) *Proc. Natl. Acad. Sci. USA,* **88**:5611-5), and the neuron-specific vgf gene (Piccioli *et al.*, (1995) *Neuron,* **15**:373-84), among others. Other promoters (not liver-specific) may be selected, but expression cassettes containing same may not have all of the advantages of those with TBG or another liver-specific promoter. Alternatively, a regulatable promoter may be selected. See, e.g., WO 2011/126808B2 .

In one embodiment, the expression cassette comprises one or more expression enhancers. In one embodiment, the expression cassette contains two or more expression enhancers. These enhancers may be the same or may from one another different. For example, an enhancer may include an Alpha mic/bik enhancer. This enhancer may be present in two copies which are located adjacent to one another. Alternatively, the dual copies of the enhancer may be separated by one or more sequences. In still another embodiment, the expression cassette further contains an intron, e.g, the Promega intron. Other suitable introns include those known in the art, e.g., such as are described in WO 2011/126808.

Further, an expression cassette of the invention is provided with a suitable polyadenylation signal. In one embodiment, the polyA sequence is a rabbit globin poly A.

Date reçu/Date Received 2020-07-09

In one embodiment, the polyA sequence is characterized by that of nt 3261-3387 of FIG. 1. Alternatively, another polyA, *e.g.*, a human growth hormone (hGH) polyadenylation sequence, an SV40 polyA, or a synthetic polyA. Still other conventional regulatory elements may be additional or optionally included in an expression cassette.

In one embodiment, the expression cassette is engineered onto a suitable vector, e.g., a plasmid vector using techniques known to those of skill in the art. Optionally, a composition of the invention may contain a first expression cassette comprising the modified human IDUA gene and a second expression cassette comprising a different gene. In still another embodiment, the functional human IDUA may be expressed from a more than one expression cassette, which may be located on a multiple vectors, *e.g.*, as described in WO 2011/126808.

In one embodiment, the expression cassette is carried by the pENN.TBG.hIDUA.nRBG, which plasmid is used to generate a recombinant adeno-associated virus carrying the expression cassette.

Production of AAV Viral Particles

In one embodiment, the invention provides a recombinant adeno-associated virus (rAAV) particle having an AAV capsid and having packaged therein a 5' inverted terminal repeat (ITR), a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, and an AAV 3' ITR, wherein said hIDUA gene has a sequence shown in SEQ ID NO: 1 (FIG 1) or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase. One particularly desirable rAAV is AAV2/8.TBG.hIDUA.co.

Methods of preparing AAV-based vectors are known. See, e.g., US Published Patent Application No. 2007/0036760 (February 15, 2007).

The use of AAV capsids of AAV8 are particularly well suited for the compositions and methods described herein. The sequences of AAV8 and methods of generating vectors based on the AAV8 capsid are described in US Patent 7,282,199 B2, US 7,790,449, and US 8,318,480. Also well suited for use in the invention are AAV9 capsids. The sequences of AAV9 and methods of generating vectors based on the AAV9 capsid are described in US Patent 7,906,111. However, other AAV capsids may be selected or generated for use in the

Date reçu/Date Received 2020-07-09

invention. The sequences of a number of such AAV are provided in the above-cited US Patent 7,282,199 B2, US 7,790,449, US 8,318,480, and US Patent 7,906,111, and/or are available from GenBank. The sequences of any of the AAV capsids can be readily generated synthetically or using a variety of molecular biology and genetic engineering techniques. Suitable production techniques are well known to those of skill in the art. See, *e.g.*, Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY). Alternatively, oligonucleotides encoding peptides (e.g., CDRs) or the peptides themselves can generated synthetically, *e.g.*, by the well-known solid phase peptide synthesis methods (Merrifield, (1962) *J. Am. Chem. Soc.*, **85**:2149; Stewart and Young, Solid Phase Peptide Synthesis (Freeman, San Francisco, 1969) pp. 27-62). See, also,, D M McCarty et al, "Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis", Gene Therapy, (August 2001), Vol 8, Number 16, Pages 1248-1254. Self-complementary AAVs are described in, e.g., U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683.

These and other suitable production methods are within the knowledge of those of skill in the art and are not a limitation of the present invention.

The recombinant adeno-associated virus (AAV) described herein may be generated using techniques which are known. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid; a functional rep gene; a expression cassette composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the expression cassette into the AAV capsid protein.

The components required to be cultured in the host cell to package an AAV expression cassette in an AAV capsid may be provided to the host cell in *trans*. Alternatively, any one or more of the required components (*e.g.*, expression cassette, *rep* sequences, *cap* sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art. Most suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. Examples of suitable inducible and constitutive promoters are provided herein, in the discussion of regulatory elements suitable for use with the transgene. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contains the rep and/or cap proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

The expression cassette, *rep* sequences, *cap* sequences, and helper functions required for producing the rAAV of the invention may be delivered to the packaging host cell in the form of any genetic element which transfer the sequences carried thereon. The selected genetic element may be delivered by any suitable method, including those described herein. The methods used to construct any embodiment of this invention are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, *e.g.*, Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, *e.g.*, K. Fisher *et al*, (1993) *J. Virol.*, **70**:520-532 and US Patent No. 5,478,745.

Unless otherwise specified, the AAV ITRs, and other selected AAV components described herein, may be readily selected from among any AAV. These ITRs or other AAV components may be readily isolated using techniques available to those of skill in the art from an AAV sequence. Such AAV may be isolated or obtained from academic, commercial, or public sources (*e.g.*, the American Type Culture Collection, Manassas, VA). Alternatively, the AAV sequences may be obtained through synthetic or other suitable means by reference to published sequences such as are available in the literature or in databases such as, *e.g.,* GenBank®, PubMed®, or the like.

A. The Expression cassette

The expression cassette is as defined herein. In addition, the expression cassette and/or a vector as described herein may contain additional transgene or regulatory sequences. The expression cassette that is packaged into a capsid protein and delivered to a selected host cell.

1. The transgene

The invention may include the use of multiple transgenes. Suitable transgenes may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention.

2. Regulatory Elements

In addition to the major elements identified above for the expression cassette, the vector also includes conventional control elements which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, *e.g.*, Boshart *et al*, (1985) *Cell*, **41**:521-530], the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 promoter [Invitrogen]. Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied compounds, include, the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system [International Patent Publication No. WO 98/10088]; the ecdysone insect promoter [No *et al*, (1996) *Proc. Natl. Acad. Sci. USA*, **93**:3346-3351], the tetracycline-repressible system [Gossen *et al*, (1992) *Proc. Natl. Acad. Sci. USA*, **89**:5547-5551], the tetracycline-inducible system [Gossen *et al*, (1995) *Science*, **268**:1766-1769, see also Harvey *et al*, (1998) *Curr. Opin. Chem. Biol.*, **2**:512-518], the RU486-inducible system [Wang *et al*, (1997) *Nat. Biotech.*, **15**:239-243 and Wang *et al*, (1997) *Gene Ther.*, **4**:432-441] and the rapamycin-inducible system [Magari *et al*, (1997) *J. Clin. Invest.*, **100**:2865-2872], including, *e.g.*, the Argent™ system which is available from Ariad. Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, *e.g.*, temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

Another embodiment of the transgene includes a gene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle should be used. These include the promoters from genes encoding skeletal β-actin, myosin light chain 2A, dystrophin, desmin, MHC, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally-occurring promoters (see Li *et al.*, (1999) *Nat. Biotech.*, **17**:241-245). Examples of promoters that are tissue-specific are known for CNS/neuronal include, e.g., neuron-specific enolase (NSE) promoter (Andersen *et al.*, (1993) *Cell. Mol. Neurobiol.*, **13**:503-15), neurofilament light-chain gene (Piccioli *et al.*, (1991) *Proc. Natl. Acad. Sci. USA*, **88**:5611-5), and the neuron-specific vgf gene (Piccioli *et al.*, (1995) *Neuron*, **15**:373-84), among others. In another embodiment, the native promoter for the transgene will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

The combination of the transgene, promoter/enhancer, and 5' and 3' AAV ITRs is referred to as an expression cassette for ease of reference herein. Provided with the teachings of this invention, the design of such an expression cassette can be made by resort to conventional techniques.

3. Delivery of the Expression Cassette to an AAV Packaging Host Cell

The expression cassette can be carried on any suitable vector, *e.g.*, a plasmid, which is delivered to a host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and, optionally, integration in prokaryotic cells, mammalian cells, or both. These plasmids (or other vectors carrying the expression cassette) contain sequences permitting replication of the expression cassette in eukaryotes and/or prokaryotes and selection markers for these systems. Selectable markers or reporter genes may include sequences encoding kanamycin, geneticin, hygromicin or purimycin resistance, among others. The plasmids may also contain certain selectable reporters or marker genes that can be used to signal the presence of the vector in bacterial cells, such as ampicillin resistance. Other components of the plasmid may include an origin of replication and an amplicon, such as the amplicon system employing the Epstein Barr virus nuclear antigen. This amplicon system, or other similar amplicon components permit high copy episomal replication in the cells. Preferably, the molecule carrying the expression cassette is transfected into the cell, where it may exist transiently. Alternatively, the expression cassette may be stably integrated into the genome of the host cell, either chromosomally or as an episome. In certain embodiments, the expression cassette may be present in multiple copies, optionally in head-to-head, head-to-tail, or tail-to-tail concatamers. Suitable transfection techniques are known and may readily be utilized to deliver the expression cassette to the host cell.

Generally, when delivering the vector comprising the expression cassette by transfection, the vector is delivered in an amount from about 5 μg to about 100 μg DNA, about 10 μg to about 50 μg DNA to about 1×10^4 cells to about 1×10^{13} cells, or about 1×10^5 cells. However, the relative amounts of vector DNA to host cells may be adjusted, taking into consideration such factors as the selected vector, the delivery method and the host cells selected.

B. Packaging Host Cells

In addition to the expression cassette, the host cell contains the sequences which drive expression of an AAV capsid protein of the invention in the host cell and rep sequences of the same source as the source of the AAV ITRs found in the expression cassette, or a cross-complementing source. The packaging host cell also requires helper functions in order to package the rAAV of the invention. Such helper functions are well known in the art and will not be duplicated herein. Similarly, methods for producing suitable vectors having AAV capsids are known. [*See, e.g.*, US Published Patent Application No. US 2007/0036760].

The construct of a rAAV encoding an expression cassette described herein same can be suspended in a physiologically compatible carrier, may be administered to a subject. In one embodiment, the carrier is sterile saline alone or, optionally, with any of a number of buffering solutions (*e.g.*, phosphate buffered saline). Other exemplary carriers include lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, sesame oil, and water. The selection of the carrier is not a limitation of the present invention.

Optionally, the compositions of the invention may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. In one embodiment, delivery is via intravenous delivery. However, still other routes of administration may be selected. Alternatively or additionally, routes of administration may be combined, if desired.

In one embodiment, the invention includes a lyophilized composition which contains an rAAV as described herein, or a mixture of rAAV, in lyophilized form. Optionally, one or more stabilizers or preservatives is present in this composition. Suitably, for use, a lyophilized composition is reconstituted with a suitable diluent, e.g., sterile saline or a buffered saline.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective dosage of the viral vector is generally in the range of from about 0.1 mL to about 100 mL, or about 0.1 mL to about 10 mL, or about 0.1 mL to about 5 mL, or about 0.5 mL to about 1 mL, of solution containing concentrations of from about 3×10^9 to 3×10^{13} genomes viral vector (particles)/mL aqueous suspending agent. Another exemplary dosage is about 3×10^9 to 3×10^{13} AAV genomes per 1 kg. One suitable volume is about 1 mL. In another embodiment, a therapeutically effective dose of the rAAV construct is in the range of about 0.001 ng to about 1000 mg/70 kg animal, which may be delivered in a single dosage or over a series of two or more doses. Other suitable dosages may be determined. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed.

METHODS OF TREATMENT

The compositions of the present invention avoid complications of enzyme replacement therapy related to immune response to the recombinant enzyme which can range from mild to full-blown anaphylaxis as well as complications of life-long peripheral access such as local and systemic infections. Further, in contrast to ERT, the composition of the invention does not require long term, repeated weekly injections. Without wishing to be bound by theory, the liver-directed therapeutic method described herein is believed to be useful for correcting the central nervous system phenotype associated with MPSI disorders by providing efficient, long-term gene transfer afforded by vectors with high transduction efficiency could provide continuous, elevated circulating IDUA levels, which provides therapeutic leverage across the blood brain barrier. In addition, AAV liver gene transfer may provide active tolerance and prevent antibody formation against the enzyme.

A method for treating type I mucopolysaccharidosis comprising delivering a therapeutically effective amount of a modified hIDUA expression cassette as described herein is provided. Also provided is a method for treating and/or ameliorating the symptoms of Hurler, Hurler-Scheie and Scheie syndromes.

In one embodiment, the rAAV is delivered intravenously.

In another embodiment, the rAAV is delivered in an amount of about 3×10^9 to about 3×10^{12} is delivered to the subject. While a single administration of the rAAV is anticipated to be effective, since the liver is a regenerative organ, administration by be repeated (e.g., quarterly, bi-annually, annually, or as otherwise needed. Optionally, an initial dose of a therapeutically effective amount may be delivered over split infusion sessions, taking into consideration the age and ability of the subject to tolerate infusions. However, repeated weekly injections of a full therapeutic dose are not required, providing an advantage to the patient in terms of both comfort and therapeutic outcome.

The following examples are illustrative only and are not a limitation on the invention described herein.

Example 1 - Transgene and vector production

A modified nucleotide sequence encoding a functional human alpha-L-iduronidase was synthesized. The resulting sequence is particularly well suited for human expression and has less than about 90% identical to the functional human IDUA gene (hIDUA; Genbank NP000194.2). The resultant transgene was then inserted into a plasmid containing *cis* elements necessary for packaging into an AAV vector available from UPenn Vector Core using engineered *MluI* and *SalI* sites. Gene expression was driven by the human thyroid binding globulin (TBG, Hayashi Y, Mori Y, Janssen OE, et al. Human thyroxine-binding globulin gene: complete sequence and transcriptional regulation. Mol Endocrinol 1993; 7: 1049–1060]. The resulting plasmid, shown in Figure 2, pENN.AAV.TBG.PI.hIDUA.RGB contains the modified hIDUA gene under the control of expression control sequences including the liver-specific TBG promoter. The plasmid further contains the AAV2 5' ITR, tandem repeats of the alpha mic/bic enhancers, the TBG promoter, a Promega intron sequence, the modified human IDUA gene of SEQ ID NO: 1, a rabbit globin poly A, and an AAV2 – 3' ITR.

Large scale vector preparations were made essentially as described by Lock et al. [Rapid, simple, and versatile manufacturing of recombinant adeno-associated viral vectors at scale. Hum Gene Ther 21(10): 1259-1271. (2010)]. PEI-based transfections were performed in 10 layer cell stacks containing 75% confluent monolayers of HEK293 cells. 10L of feedstock culture medium from the cell stacks was clarified and then concentrated by tangential flow filtration. The concentrated clarified feedstock was purified over iodixanol (Optiprep; Sigma Chemical Co., St Louis, MO) gradients. All fractions directly below a visible contaminating protein band were collected and pooled. Pooled fractions were diafiltered against of PBS/35mM NaCl and concentrated using Amicon Ultra 15 spin concentrators (Millipore). Glycerol was added to the diafiltered, concentrated product to 5% final and the preparation was aliquoted and stored at -80 °C. The resulting vectors are termed herein AAV8.TBG.hIDUA or AAV2/8.TBG.hIDUA. In certain locations, the recombinant AAV particles are referred to as AAV8.TBG.hIDUAco or AAV2/8.TBG.hIDUAco. The plasmid further contains the AAV2 5' ITR, tandem repeats of the alpha mic/bic enhancers, the TBG promoter, a Promega intron sequence, the modified human IDUA gene of SEQ ID NO: 1, a rabbit globin poly A, and an AAV2 – 3' ITR.

Date reçu/Date Received 2020-07-09

Example 2 –

A. Cell based assays

HEK 293 cells were maintained in growth medium containing Dulbecco's Modified Eagle Medium (DMEM; Gibco®, Life Technologies™) with 5% fetal bovine serum (FBS; XXX), 1% penicillin/streptomycin (p/s; Life Technologies ™). Plasmid DNA transfections were carried out using Lipofectamine™ 2000 (Invitrogen™, Life Technologies™) according to the manufacturer's recommendations. Briefly, cells were plated at a density of 5x10^5 cells/well in 6-well tissue culture dishes in transfection medium (DMEM + 5%FBS, no p/s) and allowed to adhere overnight at 37° in 5%CO_2. On the next day, cells were checked for 90-95% confluency and the media was refreshed. Plasmid DNA and Lipofectamine™ 2000 were diluted with Opti-MEM I ® Reduced Serum Medium (without serum) for a final ratio of 1:2.5 (DNA: Lipofectamine™ 2000). The Lipofectamine™ 2000 transfection solution was incubated for five minutes at 22°C before mixing with the DNA solution. This final transfection mixture containing the DNA: Lipofectamine™ 2000 solution was incubated further for 20 minutes (22°C) before addition to the wells containing cells and media. Mock cells received no plasmid DNA and a plasmid encoding for eGFP was used as a transfection control. Three wells were transfected for each construct tested. Cells were incubated at 37° in 5%CO_2; media was replaced with growth media four hours later. Contents of each well were harvested 72 hours later and collected at 4000rpm for 15 minutes at 4°C. Cells were resuspended in 100μls/well lysis buffer (0.2% Triton™ X-100, 0.9% NaCl, pH4.0) and freeze/thawed three times with vortexing. In addition, lysates were treated with Benzonase for 30 minutes at 37°C before the final freeze/thaw. Cell debris was pelleted at 10 000rpm (4°C) for 10 minutes and final clarified cell lysates were placed on ice and immediately assayed for enzyme activity.

B. Tissue lysis and protein extraction

Frozen tissues were semi-thawed in a box on a bed of dry ice and small pieces of wet tissue were cut (~20mgs, ~10 mgs of spleen) up in a small petri dish. The pre-processed tissues were then submerged in a 2-ml eppendorph with 1ml lysis buffer (0.2% Triton X-100, 0.9% NaCl, pH4.0) and a 5mm steel bead. Samples were homogenized on a tissue-lyzer at 30Hz for 2 minutes. Homogenized samples were briefly spun at 6000 rpm for

Date Reçue/Date Received 2022-05-24

30 seconds and the 5mm steel beads were removed. Tissue lysates were further disrupted by sonication using a 1-1/16" diameter microhorn and frozen overnight at -80°C. Processed samples were thawed the next day at 22°C and clarified by centrifugation (10 000rpm/10 minutes/4°C). Floating lipid layers from brain, or other fatty tissues, were aspirated before assay. Samples were then stored on wet ice and assayed immediately for enzyme activity.

C. Protein estimation

Total protein was estimated using the Coomassie based Bradford assay (Thermo Scientific) following the manufacturer's protocol. Briefly, a standard curve was set up using bovine serum albumin (BSA) to generate a working range from 1-25μg/ml and a blank accounting for the protein dilution buffer with no BSA. Samples were diluted twofold from 1/300 -1/1200 and mixed at a 1:1 ratio of diluted protein: Bradford reagent in a 96-well flat-bottom dish. Samples were allowed to equilibrate at 22°C for 15 minutes and absorbance values were collected on a plate reader at the suggested wavelength of 595nm. Raw values were converted to μg/ml concentrations using the standard curve with blank correction. Microgram quantities were then converted and reported in milligrams.

D. Enzyme activity assays

IDUA enzyme activity was assayed using 4-Methylumbelliferyl alpha-L-Idopyranosiduronic Acid, Cyclohexylammonium Salt (4-MU-Ido; Toronto Research Chemicals, Inc.) as a substrate according to previously published methods [Kakkis et al, Mol Genet Metab, 2001 Mar; 72(3): 199-208]. Briefly, 5-15μls of lysates, or serum, were brought up to 100μls with double distilled water (ddH_2O) and 100μls of 100μM 4-MU-Ido substrate, diluted with reaction buffer (0.1M sodium acetate pH3.5, 0.15M NaCl, 0.05% Triton X-100) was combined in a methylacrylate cuvette (Thermo Scientific). Reactions were incubated for 1-3 hours in a 37°C water bath and ended with the addition of a 1x stop buffer (290mM glycine, 180mM sodium carbonate, pH 10.5). Products were read on a QuantiFluor™-ST (Promega) through the UV channel (Ex 365 nm, Em 440 - 470 nm). Raw fluorescence values were recorded and converted to nmol/ml/hr using a standard curve of known quantities of 4-Methylumbelliferone (M-5410; Biosynth®). Cell and tissue lysates were normalized to estimated protein values (nmol/mg/hr; see methods section entitled "protein estimation").

E. DNA extraction and genome copy analysis

Taqman PCR was used to determine the vector DNA load in diploid cells. For detection and quantification of vector genomes by real-time PCR, total cellular DNA was extracted from tissues using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). Primer and probe sets were designed to target the nRBG polyA region of the vector, using the following sequences; forward: GCCAAAAATTATGGGGACAT, reverse: ATTCCAACACACTATTGCAATG, probe: 6FAM-ATGAAGCCCCTTGAGCATCTGACTTCT-TAMRA. Standard curves for vector genome quantification were established with the *cis* plasmids used for the production of the corresponding vector. The PCR was performed with a TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with 200 ng total cellular DNA as template, 300 nM primers, and 200 nM probes each. Cycles were for 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C.

F. Immunoblotting

Immunoblotting was performed according to standard methods. In brief, the NuPAGE gel system (Life Technologies), 4-12% bis-tris gels was used. Transfer was following 30 minutes at 20V to a PVDF membrane. The block was 10% NFDM in T-PBS (overnight). The primary MAb was mouse anti-human IDUA 1:300 (1.5 hours); 1% NFDM in T-PBS. Secondary: HRP-linked rabbit anti-mouse 1:3000 (1hr); 1% NFDM in T-PBS Detection was by SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific), 30 second exposure on x-ray film.

Example 3 – In Vivo Studies in Mice and Dogs

IDUA deficiencies can be found in dogs, allowing for study of the disease and therapies in a large animal. MPS I dogs carry a recessive (null) mutation in the IDUA gene, in which a G>A mutation in the donor splice site of intron 1 creates a premature termination codon at the exon-intron junction [Menon, K.P., P.T. Tieu, and E.F. Neufeld, Architecture of the canine IDUA gene and mutation underlying canine mucopolysaccharidosis I. Genomics, 1992. 14(3): p. 763-8.]. The course of disease in the dog is analogous to Hurler-Scheie syndrome; disease manifestations include significant skeletal disease, including chest deformity.

Urine GAG analysis is a biochemical marker used to determine efficacy of treatments for MPS I in the clinical setting.

Accumulation of glycosaminoglycans (GAGs) were evaluated in major organs by Alcian Blue (pH1) stain of paraffin sections. The accumulation of unprocessed GAG within cells are particularly well visualized on 1 µm thin sections from plastic-embedded tissues stained with Toluidine Blue. Thin sections (1 µm) of epon-embedded tissues, stained with Toluidine Blue. This shows cells containing storage material ("foamy, sponge cells").

Immunohistochemistry was performed with antibody against ganglioside GM3 in brain. Shows abnormal storage of GM3 in neurons. The cortex was evaluated in dogs and cortex and hypothalamus in mice. Expression of human IDUA in liver was evaluated by immunofluorescence.

A. Mouse Studies

AAV8.TBG.modified hIDUA was prepared as described in Example 1. Mice (about 3 months) were injected intravenously at doses of about 1 $x10^{11}$ GC, 3 x 10^{10} GC, 3 x 10^{9} GC, 1 $x10^{9}$ GC and evaluated ~ 2 weeks post injection.

The results showed diminished or complete absence of GAG staining in the animals dosed at 1 x 10^{11}, 3 x 10^{10}, and 3 x 10^{9} . In these animals, diminished or complete absence of storage lesions was observed in thin sections.

In the animals dosed at 1 x 10^{9} GAG storage looks more or less as in untreated mice and storage lesions look more or less as in untreated mice

For GM3 storage, only the animals dosed at 1 x 10^{11} GC and 3 x 10^{10} GC have been evaluated, and a very weak improvement in GM3 storage in neurons was observed.

Strong expression (100% of hepatocytes) of IDUA was observed in animals dosed at 1 x 10^{11} GC. This drops off with lower doses, with only very few positive hepatocytes visible at 1 x 10^{9} GC.

B. Canine Studies

AAV8.TBG.modified hIDUA was prepared as described in Example 1. Dogs (about 8 months age) were injected intravenously at a dose of 1 $x10^{11}$ GC and evaluated after four months post injection (i.e. 1 year old).

This study shows reversal of storage lesions. More particularly, virtually complete clearance of GAG storage in all major organs is seen by Alcian Blue stain. No storage lesions are observed in heart, kidney or liver thin sections. A reduction or complete clearance of GM3 accumulation is observed in neurons. Expression of IDUA in liver is observed by immunofluorescence in >95% of hepatocytes.

Example 4 – Treatment of Hurler-Scheie with AAV2/8.TBG.hIDUA

AAV8-mediated gene transfer of IDUA will be evaluated in Hurler-Scheie patients. The subjects would receive a single infusion of vector into a peripheral vein which based on pre-clinical data should lead to stable production of the enzyme at levels that are close to what is obtained in normal subjects. The trial may involve different doses of vector for example, $3x10^{11}$ GC/kg; $1x10^{12}$ GC/kg; $3x10^{12}$ GC/kg and please add dose range. The most non-invasive assessment of efficacy is the level of urine GAG which are elevated in the disease and partially corrected following ERT. Transgene engraftment and its level of expression will be determined by measuring serum IDUA. Urine GAG will also be measured before and after gene therapy.

(Sequence Listing Free Text)

The following information is provided for sequences containing free text under numeric identifier <223>.

SEQ ID NO: (containing free text)	Free text under <223>

SEQ ID NO: (containing free text)	Free text under <223>
SEQ ID NO: 3	<220> <221> repeat_region <222> (1)..(130) <223> 5' ITR <220> <221> enhancer <222> (221)..(320) <223> Alpha mic/bik <220> <221> enhancer <222> (327)..(426) <223> Alpha mic/bik <220> <221> promoter <222> (442)..(901) <223> TBG promoter <220> <221> TATA_signal <222> (885)..(888) <220> <221> Intron <222> (1027)..(1159) <223> intron 1 <220> <221> CDS <222> (1251)..(3212) <223> human alpha-L-IDUA <220> <221> polyA_signal <222> (3261)..(3387) <220> <221> repeat_region <222> (3476)..(3605) <223> 3' ITR (located on complement) <220> <221> rep_origin

Date Reçue/Date Received 2021-06-14

SEQ ID NO: (containing free text)	Free text under <223>
	<222> (3782)..(4220) <223> f1\ori (located on complement) <220> <221> rep_origin <222> (4249)..(4891) <223> pUC\origin of replication\ <220> <221> misc_feature <222> (5566)..(6381) <223> kanamycin resistance located on complementary strand

A sequence listing labelled "Z6622PCT_ST25.txt" is being filed herewith in electronic form.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CA 2901328 2019-06-26

What is claimed is:

1. A recombinant vector carrying an expression cassette comprising a human alpha-L-iduronidase (hIDUA) gene having the nucleotide sequence of SEQ ID NO: 1 or a sequence at least about 80% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase, wherein said expression cassette further comprises regulatory control sequences which direct expression of the human alpha-L-iduronidase, said regulatory control sequences comprising a liver-specific promoter.

2. The recombinant vector according to claim 1, wherein the modified hIDUA gene is at least about 85% to about 90% identical to SEQ ID NO: 1.

3. The recombinant vector according to claim 2, wherein the modified hIDUA gene is at least about 95% to about 99% identical to SEQ ID NO: 1.

4. The recombinant vector according to claim 1, wherein the functional human alpha-L-iduronidase is selected from the group consisting of:

(a) about amino acid 1 to about amino acid 653 of SEQ ID NO: 2;

(b) a synthetic human enzyme comprising a heterologous leader sequence fused to about amino acid 27 to about 653 of SEQ ID NO: 2;

(c) a variant of amino acid sequence of SEQ ID NO:2 having one or more of the modifications comprising: an H to Q reduction at amino acid position 82 (SEQ ID NO: 8); a change from R to Q at position 105 (SEQ ID NO: 9); a change from G to R at position 116 (SEQ ID NO: 10); a change from V to A at position 279 (SEQ ID NO: 11); a change from L to R at position 346 (SEQ ID NO: 12); a change from A to T at position 361 (SEQ ID NO: 13); a change from H to N at position 449 (SEQ ID NO: 14); a change from V to I at position 454 (SEQ ID NO: 15); a change from A to T at position 591 (SEQ ID NO: 16); and a change from A to T at position 622 (SEQ ID NO: 17).

Date Reçue/Date Received 2022-05-24

5. The recombinant vector according to claim 1, wherein the promoter is a thyroxin binding globulin (TBG) promoter.

6. The recombinant vector according to claim 1, wherein the vector further comprises one or more enhancers.

7. The recombinant vector according to claim 6, wherein the enhancers are the same or different.

8. The recombinant vector according to claim 6, wherein the enhancers are selected from the group consisting of an intron, a CMV enhancer, and an Alpha mic/bik enhancer.

9. The recombinant vector according to claim 6, wherein more than one copy of the selected enhancer is present in the vector.

10. The recombinant vector according to claim 9, wherein the more than one copy of the selected enhancer are located in tandem.

11. The recombinant vector according to claim 1, wherein the expression cassette further comprises a poly A selected from rabbit globin poly A.

12. A recombinant vector, which is plasmid pENN.TBG.hIDUA.nRBG and has a sequence of SEQ ID NO: 3.

13. A recombinant adeno-associated virus (rAAV) particle having an AAV capsid and having packaged therein a 5' inverted terminal repeat (ITR), a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, and an AAV 3' ITR, wherein said hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase.

Date Reçue/Date Received 2022-05-24

14. The rAAV particle according to claim 13, wherein the modified hIDUA gene is expressed under the control of a liver-specific promoter.

15. The rAAV particle according to claim 14, wherein the promoter is a TBG promoter.

16. The rAAV particle according to claim 13, wherein the vector further comprises one or more enhancers.

17. The rAAV particle according to claim 16, wherein the enhancers are the same or different.

18. The rAAV particle according to claim 16, wherein the enhancers are selected from the group consisting of an intron, a CMV enhancer, and an Alpha mic/bik enhancer.

19. The rAAV particle according to claim 18, wherein more than one copy of the selected enhancer is present in the vector.

20. The rAAV particle according to claim 19, wherein the more than one copy of the selected enhancer are located in tandem.

21. The rAAV particle according to claim 13, wherein the rAAV particle comprises an AAV capsid selected from AAV8 and AAV9.

22. The rAAV particle according to claim 21, wherein the rAAV is pseudotyped.

23. The rAAV particle according to claim 22, wherein the ITRs are from an AAV2.

24. A recombinant adeno-associated (rAAV) viral particle AAV2/8.TBG.hIDUA.co wherein the rAAV viral particle comprises an AAV8 capsid and further comprises packaged in the capsid a modified human IDUA gene having SEQ ID NO: 1 under the

Date Reçue/Date Received 2022-05-24

control of expression control sequences, wherein the expression control sequences comprise an AAV2 - 5' ITR, an alpha mic/bic enhancer, a TBG promoter, an intron sequence, the modified human IDUA gene of SEQ ID NO: 1, a rabbit globin poly A, and an AAV2 – 3' ITR.

25. A composition useful for treating mucopolysaccharidosis type I (MPS I) comprising the rAAV particle according to any of claims 13 to 24 and a pharmaceutically acceptable carrier.

26. Use of a composition comprising a pharmaceutically acceptable carrier and a rAAV particle according to any of the claims 13 to 24 wherein an effective amount of the composition is for delivery for the treatment of MPS I.

27. The use according to claim 26, wherein said rAAV particle is for delivery intravenously.

28. The use according to claim 26, wherein about 3×10^9 to about 3×10^{12} genome copies of the rAAV particle is for delivery to the subject.

29. Use of a composition comprising the rAAV particle according to any of claims 13 to 24 and a pharmaceutically acceptable carrier for use in treating MPS I.

30. A vector carrying an expression cassette comprising a human alpha-L-Iduronidase (hIDUA) gene having the nucleotide sequence of SEQ ID NO: 1 or a sequence at least about 80% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase in human cells, wherein said expression cassette further comprises regulatory control sequences which direct expression of the human alpha-L-iduronidase in human cells, said regulatory control sequences comprising a liver-specific promoter.

Date Reçue/Date Received 2022-05-24

31. The vector according to claim 30, wherein the modified hIDUA gene is at least about 85% to about 90% or at least about 95% to about 99% identical to SEQ ID NO: 1.

32. The vector according to claim 30, wherein the functional human alpha-L-iduronidase is selected from the group consisting of:

(a) about amino acid 1 to about amino acid 653 of SEQ ID NO: 2;

(b) a synthetic human enzyme comprising a heterologous leader sequence fused to about amino acids 27 to about 653 of SEQ ID NO: 2;

(c) a variant of amino acid sequence of SEQ ID NO:2 having one or more of the modifications comprising: an H to Q reduction at amino acid position 82; a change from R to Q at position 105; a change from G to R at position 116; a change from V to A at position 279; a change from L to R at position 346; a change from A to T at position 361; a change from H to N at position 449; a change from V to I at position 454; a change from A to T at position 591; and a change from A to T at position 622.

33. The vector according to claim 30, wherein the expression cassette further comprises a poly A selected from rabbit globin poly A.

34. A recombinant adeno-associated virus (rAAV) particle having an AAV capsid and having packaged therein a 5' inverted terminal repeat (ITR), a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, and an AAV 3' ITR, wherein said hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical thereto which encodes a functional human alpha-L-iduronidase.

35. The rAAV according to claim 34, wherein the modified hIDUA gene is expressed under the control of said regulatory sequences, wherein the regulatory control sequences comprise a liver-specific promoter.

36. The vector according to claim 30 or the rAAV according to claim 34 or 35, wherein the promoter is a TBG promoter.

Date Reçue/Date Received 2022-05-24

37. The vector according to claim 30 or the rAAV according to claim 34, wherein the vector further comprises one or more enhancers.

38. The vector according to claim 37 or the rAAV according to claim 37, wherein the enhancers are the same or different.

39. The vector according to claim 37 or the rAAV according to claim 37, wherein the enhancers are selected from the group consisting of an intron, a CMV enhancer, and an Alpha mic/bik enhancer.

40. The vector according to claim 37 or the rAAV according to claim 37 wherein more than one copy of the selected enhancer is present in the vector.

41. The vector according to claim 40 or the rAAV according to claim 40, wherein the more than one copy of the selected enhancer are located in tandem.

42. The rAAV according to claim 34, wherein the rAAV particle comprises an AAV capsid selected from AAV8 and AAV9.

43. The rAAV according to claim 42, wherein the rAAV is pseudotyped.

44. The rAAV according to claim 43, wherein the ITRs are from an AAV2.

45. A composition for use in treating mucopolysaccharidosis type I (MPS I) comprising an effective amount of the composition comprising the rAAV according to any one of claims 34 to 44 and a pharmaceutically acceptable carrier.

46. The composition for the use according to claim 45, wherein said rAAV is for intravenous delivery or wherein rAAV is for delivery to the subject in an amount of about 3×10^9 to about 3×10^{12}.

Date Reçue/Date Received 2022-05-24

47. A recombinant adeno-associated virus (rAAV) particle having an AAV capsid and having packaged therein a 5' inverted terminal repeat (ITR), a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, and an AAV 3' ITR, wherein said hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase.

48. The rAAV particle according to claim 47, wherein said hIDUA gene is expressed under the control of a liver-specific promoter.

49. The rAAV particle according to claim 48, wherein the promoter is a thyroxin binding globulin (TBG) promoter.

50. The rAAV particle according to claim 47, wherein the regulatory sequences comprise one or more enhancers.

51. The rAAV particle according to claim 50, wherein the enhancers are the same or different.

52. The rAAV particle according to claim 50, wherein the enhancers are selected from the group consisting of an intron, a cytomegalovirus (CMV) enhancer, and an Alpha mic/bik enhancer.

53. The rAAV particle according to claim 52, wherein more than one copy of the selected enhancer are present in the vector.

54. The rAAV particle according to claim 53, wherein the more than one copy of the selected enhancer are located in tandem.

Date Reçue/Date Received 2022-05-24

55. The rAAV particle according to claim 47, wherein the rAAV particle comprises an AAV capsid selected from AAV8 and AAV9.

56. The rAAV particle according to claim 55, wherein the rAAV particle is pseudotyped.

57. The rAAV particle according to claim 47, wherein the ITRs are from an AAV2.

58. The rAAV particle according to claim 47, having an AAV8 capsid and having packaged therein an AAV2 5' ITR, a hIDUA gene of SEQ ID NO: 1, under the control of regulatory sequences which control expression thereof, and an AAV2 3' ITR, wherein the control regulatory sequences comprise a liver-specific TBG promoter, tandem repeats of alpha mic/bic enhancers, an intron sequence, and a poly A.

59. A composition useful for treating mucopolysaccharidosis type I (MPS I) comprising the rAAV particle according to claim 47 and a pharmaceutically acceptable carrier.

60. Use of a composition comprising a pharmaceutically acceptable carrier and the rAAV particle according to claim 47 wherein the composition is for delivery to a subject for treatment of MPS I.

61. The use according to claim 60, wherein said rAAV particle is for delivery intravenously.

62. The use according to claim 60, wherein about 3×10^9 to about 3×10^{12} genome copies of the rAAV is for delivery to the subject.

63. The rAAV particle according to claim 47, wherein the functional human alpha-L-iduronidase is selected from:

(a) about amino acid (aa) 1 to about aa 653 of SEQ ID NO: 2;

Date Reçue/Date Received 2022-05-24

(b) a synthetic human enzyme comprising a heterologous leader sequence fused to about aa 27 to about aa 653 of SEQ ID NO: 2; and

(c) a variant of amino acid sequence of SEQ ID NO:2 having one or more of the modifications comprising: an H to Q reduction at amino acid position 82 (SEQ ID NO:8); a change from R to Q at position 105 (SEQ ID NO: 9); a change from G to R at position 116 (SEQ ID NO: 10); a change from V to A at position 279 (SEQ ID NO: 11); a change from L to R at position 346 (SEQ ID NO: 12); a change from A to T at position 361 (SEQ ID NO: 13); a change from H to N at position 449 (SEQ ID NO: 14); a change from V to I at position 454 (SEQ ID NO: 15); a change from A to T at position 591 (SEQ ID NO: 16); and a change from A to T at position 622 (SEQ ID NO: 17).

64. The rAAV particle according to claim 47, wherein the regulatory sequences further comprise a poly A.

65. The rAAV particle according to claim 47, wherein the hIDUA has the sequence at least 95% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase.

66. The rAAV particle according to claim 47, wherein the hIDUA has the sequence at least 97% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase.

67. The rAAV particle according to claim 47, wherein the hIDUA has the sequence at least 99% identical to SEQ ID NO: 1 which encodes a functional human alpha-L-iduronidase.

68. The rAAV particle according to claim 47, wherein the hIDUA gene has the sequence of SEQ ID NO: 1.

69. A vector carrying an expression cassette comprising a modified human alpha-L-iduronidase (hIDUA) gene, wherein the modified hIDUA gene is at least 85% identical

Date Reçue/Date Received 2022-05-24

to SEQ ID NO: 1, which encodes a functional human alpha-L-iduronidase, wherein said expression cassette further comprises regulatory control sequences which direct expression of the human alpha-L-iduronidase, said regulatory control sequences comprising a liver-specific promoter.

70. The vector according to claim 69, wherein the modified hIDUA gene is at least about 95% to about 99% identical to SEQ ID NO: 1.

71. The vector according to claim 69, wherein the functional human alpha-L-iduronidase is selected from the group consisting of:

(a) about amino acid 1 to about amino acid 653 of SEQ ID NO: 2;

(b) a synthetic human enzyme comprising a heterologous leader sequence fused to about amino acids 27 to about 653 of SEQ ID NO: 2;

(c) a variant of amino acid sequence of SEQ ID NO:2 having one or more of the modifications comprising: an H to Q reduction at amino acid position 82; a change from R to Q at position 105; a change from G to R at position 116; a change from V to A at position 279; a change from L to R at position 346; a change from A to T at position 361; a change from H to N at position 449; a change from V to I at position 454; a change from A to T at position 591; and a change from A to T at position 622.

72. The vector according to claim 69, wherein the promoter is a TBG promoter.

73. The vector according to claim 69, wherein the vector further comprises one or more enhancers.

74. The vector according to claim 73, wherein the enhancers are the same or different.

75. The vector according to claim 73, wherein the enhancers are selected from the group consisting of an intron, a CMV enhancer, and an Alpha mic/bik enhancer.

Date Reçue/Date Received 2022-05-24

76. The vector according to claim 73, wherein more than one copy of the selected enhancer is present in the vector.

77. The vector according to claim 76, wherein the more than one copy of the selected enhancer are located in tandem.

78. The vector according to claim 69, wherein the expression cassette further comprises a poly A selected from rabbit globin poly A.

79. A packaging host cell comprising (a) an expression cassette comprising a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, wherein said hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical to SEQ ID NO: 1 thereto which encodes a functional human alpha-L-iduronidase, (b) rep sequences; and (c) nucleic acid sequences encoding an AAV capsid protein, and helper function sequences to package the expression cassette into the AAV capsid.

80. The packaging host cell according to claim 79, wherein the regulatory sequences comprise a liver specific promoter.

81. A method for producing recombinant adeno-associated virus (rAAV) particles in host cells comprising: culturing host cells which contain: at least (a) an expression cassette comprising a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, wherein the expression cassette is flanked by AAV inverted terminal repeat (ITR) sequences at its 5' end and its 3' ends and wherein said hIDUA gene has a sequence of SEQ ID NO: 1, or a sequence at least about 95% identical to SEQ ID NO: 1, which encodes a functional human alpha-L-iduronidase, (b) rep sequences; and (c) nucleic acid sequences encoding an AAV capsid protein , and sufficient helper sequences to package the expression cassette into the AAV capsid, wherein the host cells express the rep and capsid proteins and package the expression cassette into the assembled capsid to produce the rAAV particles.

Date Reçue/Date Received 2022-05-24

82. The method according to claim 81, wherein the host cell is selected from mammalian cell culture and prokaryotic cell culture.

83. The method according to claim 82, wherein the host cell is an HEK293 cell.

84. The method according to claim 81, wherein the functional human alpha-L-iduronidase is selected from the group consisting of:

(a) amino acids 1 to about 653 of SEQ ID NO: 2;

(b) a synthetic human enzyme comprising a heterologous leader sequence fused to amino acids 27 to 653 of SEQ ID NO: 2; and

(c) a variant of amino acid sequence of SEQ ID NO:2 having one or more of the modifications comprising: an H to Q reduction at amino acid position 82 (SEQ ID NO: 8); a change from R to Q at position 105 (SEQ ID NO: 9); a change from G to R at position 116 (SEQ ID NO: 10); a change from V to A at position 279 (SEQ ID NO: 11); a change from L to R at position 346 (SEQ ID NO: 12); a change from A to T at position 361 (SEQ ID NO: 13); a change from H to N at position 449 (SEQ ID NO: 14); a change from V to I at position 454 (SEQ ID NO: 15); a change from A to T at position 591 (SEQ ID NO: 16); and a change from A to T at position 622 (SEQ ID NO: 17).

85. The method according to claim 81, wherein the regulatory sequences comprise a liver specific promoter.

86. The method according to claim 85, wherein the promoter is a TBG promoter.

87. The method according to claim 81, wherein the AAV capsid is selected from AAV8 and AAV9.

88. The method according to claim 81, wherein the ITR sequences are full-length ITRs or wherein one or both of the ITRs are truncated.

Date Reçue/Date Received 2022-05-24

89. An rAAV produced according to the method of any of the claims 81 to 88.

90. A host cell comprising (a) an expression cassette comprising a human alpha-L-iduronidase (hIDUA) gene under the control of regulatory sequences which control expression thereof, wherein the expression cassette is flanked by AAV inverted terminal repeat (ITR) sequences at its 5' end and its 3' ends and wherein said hIDUA gene has a sequence of SEQ ID NO: 1 or a sequence at least about 95% identical to SEQ ID NO: 1 thereto which encodes a functional human alpha-L-iduronidase, (b) rep sequences; and (c) nucleic acid sequences encoding an AAV capsid protein, and sufficient helper sequences to package the expression cassette into the AAV capsid.

91. The host cell according to claim 90, wherein the regulatory sequences comprise a liver specific promoter.

92. The host cell according to claim 91, wherein the liver specific promoter is a TBG promoter.

93. A pharmaceutical composition for use in treating mucopolysaccharidosis type I (MPS I), wherein the composition comprises an effective amount of the rAAV virus particles produced according to the method of any one of claims 81 to 89 or produced from the host cells according to any one of claims 90 to 92 and a pharmaceutically acceptable carrier.

94. The pharmaceutical composition for the use according to claim 93, wherein the rAAV is formulated at a dosage comprising 3×10^9 to 3×10^{13} genome copies of the rAAV virus particle per kg.

Date Reçue/Date Received 2022-05-24

FIG. 1A

```
pENN.AAV.TBG.PI.        6863 bp    DNA     circular


FEATURES             Location/Qualifiers
     misc_structure  complement(5566..6381)
                     /vntifkey="88"
                     /label=Kan-r
     rep_origin      4249..4891
                     /vntifkey="33"
                     /label=pUC\origin\of\replication
     promoter        442..901
                     /vntifkey="29"
                     /label=TBG
     TATA_signal     885..888
                     /vntifkey="41"
                     /label=TATA
     enhancer        221..320
                     /vntifkey="9"
                     /label=Alpha\mic/bik
     enhancer        327..426
                     /vntifkey="9"
                     /label=Alpha\mic/bik
     intron          1027..1159
                     /vntifkey="15"
                     /label=Intron_1
                     /note="chimeric intron"
     polyA_signal    3261..3387
                     /vntifkey="25"
                     /label=Rabbit\globin\poly\A
     repeat_region   1..130
                     /vntifkey="34"
                     /label=5'\ITR
     repeat_region   complement(3476..3605)
                     /vntifkey="34"
                     /label=3'\ITR
     rep_origin      complement(3782..4220)
                     /vntifkey="33"
                     /label=f1\ori
     CDS             1251..3212
                     /vntifkey="4"
                     /label=Human\alpha-L-IDUA
```

FIG. 1B

BASE COUNT 1584 a 1971 c 1702 g 1606 t
ORIGIN

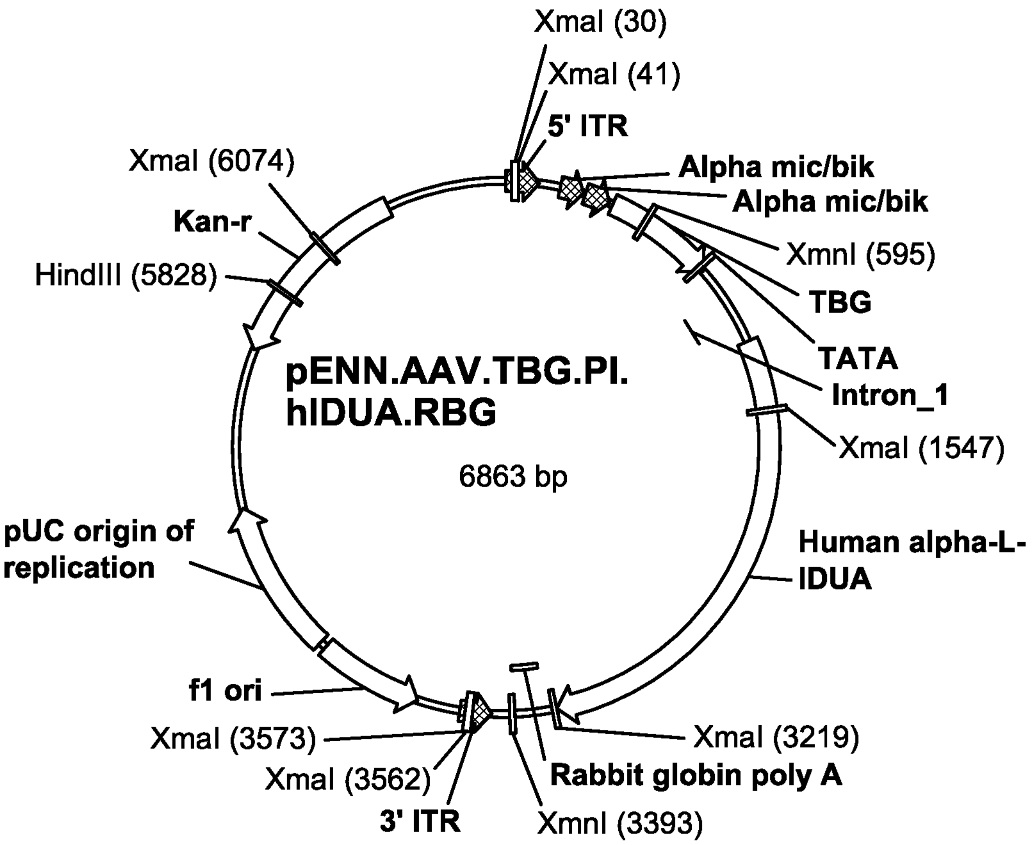
```
   1 ctgcgcgctc gctcgctcac tgaggccgcc cgggcaaagc ccgggcgtcg ggcgaccttt
  61 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact
 121 aggggttcct tgtagttaat gattaacccg ccatgctact tatctaccag ggtaatgggg
 181 atcctctaga actatagcta gaattcgccc ttaagctagc aggttaattt ttaaaaagca
 241 gtcaaaagtc caagtggccc ttggcagcat ttactctctc tgtttgctct ggttaataat
 301 ctcaggagca caaacattcc agatccaggt taatttttaa aaagcagtca aaagtccaag
 361 tggcccttgg cagcatttac tctctctgtt tgctctggtt aataatctca ggagcacaaa
 421 cattccagat ccggcgcgcc agggctggaa gctacctttg acatcatttc ctctgcgaat
 481 gcatgtataa tttctacaga acctattaga aaggatcacc cagcctctgc ttttgtacaa
 541 ctttccctta aaaaactgcc aattccactg ctgtttggcc caatagtgag aactttttcc
 601 tgctgcctct tggtgctttt gcctatggcc cctattctgc ctgctgaaga cactcttgcc
 661 agcatggact taaacccctc cagctctgac aatcctcttt ctcttttgtt ttacatgaag
 721 ggtctggcag ccaaagcaat cactcaaagt tcaaacctta tcattttttg ctttgttcct
 781 cttggccttg gttttgtaca tcagctttga aaataccatc ccagggttaa tgctggggtt
 841 aatttataac taagagtgct ctagttttgc aatacaggac atgctataaa aatggaaaga
 901 tgttgctttc tgagagacag ctttattgcg gtagtttatc acagttaaat tgctaacgca
 961 gtcagtgctt ctgacacaac agtctcgaac ttaagctgca gaagttggtc gtgaggcact
1021 gggcaggtaa gtatcaaggt tacaagacag gtttaaggag accaatagaa actgggcttg
1081 tcgagacaga gaagactctt gcgtttctga taggcaccta ttggtcttac tgacatccac
1141 tttgcctttc tctccacagg tgtccactcc cagttcaatt acagctctta aggctagagt
1201 acttaatacg actcactata ggctagcctc gagaattcac gcgtgccacc atgcggcccc
1261 tgaggcctag agctgctctg ctggcactgc tggccagtct gctggctgcc cctcctgtgg
1321 cccctgccga agcccctcac ctggtgcatg tggatgccgc cagagccctg tggcctctgc
1381 ggagattctg gcggagcacc ggcttttgcc ccccactgcc tcacagccag gccgaccagt
1441 acgtgctgag ctgggaccag cagctgaacc tggcctacgt gggcgccgtg ccccacagag
1501 gcatcaaaca ggtgagaacc cactggctgc tggaactggt gacaacccgg ggctccaccg
1561 gcagaggcct gagctacaac ttcacccacc tggacggcta cctggacctg ctgagagaga
1621 accagctgct gcccggcttc gagctgatgg gcagcgccag cggccacttc accgacttcg
1681 aggacaagca gcaagtcttt gagtggaagg acctggtgtc cagcctggcc agacggtaca
1741 tcggcagata cggactggcc cacgtgtcca agtggaactt cgagacatgg aacgagcccg
1801 accaccacga cttcgacaac gtgtcaatga ccatgcaggg ctttctgaac tactacgacg
1861 cctgctccga gggcctgaga gccgccagtc ctgccctgag actgggcgga cccggcgata
1921 gcttccacac cccccccaga agcccctga gctggggcct gctgagacac tgccacgacg
1981 gcaccaattt cttcaccggc gaggccggcg tgcggctgga ctacatcagc ctgcaccgga
2041 agggcgccag aagcagcatc agcatcctgg aacaggaaaa ggtcgtcgcc cagcagatcc
2101 ggcagctgtt ccccaagttc gccgacaccc ccatctacaa cgacgaggcc gacccccctgg
2161 tgggatggtc actgcctcag ccttggagag ccgacgtgac ctacgccgct atggtggtga
2221 aagtgatcgc ccagcatcag aacctgctgc tggccaacac caccagcgcc ttcccttacg
2281 ccctgctgag caacgacaac gccttcctga gctaccaccc ccaccccttc gcccagagaa
2341 ccctgaccgc ccggttccag gtgaacaaca ccagacccccc ccacgtgcag ctgctgagaa
2401 agcccgtgct gaccgctatg ggactgctgg ctctgctgga cgaggaacag ctgtgggccg
2461 aagtgtccca ggccggcacc gtgctggaca gcaatcatac agtgggcgtg ctggcctccg
2521 cccacagacc tcagggaccc gccgatgctt ggcgggctgc cgtgctgatc tacgccagcg
2581 acgataccag agcccacccc aacagatccg tggccgtgac cctgcggctg agaggcgtgc
2641 caccaggccc tggactggtg tacgtgacca gatacctgga caacggcctg tgcagccccg
2701 acggcgaatg gcgcagactg ggcagacctg tgttccccac cgccgagcag ttccggcgga
2761 tgagagccgc tgaggatcct gtggctgctg cccctagacc tctgcctgct ggcggcagac
```

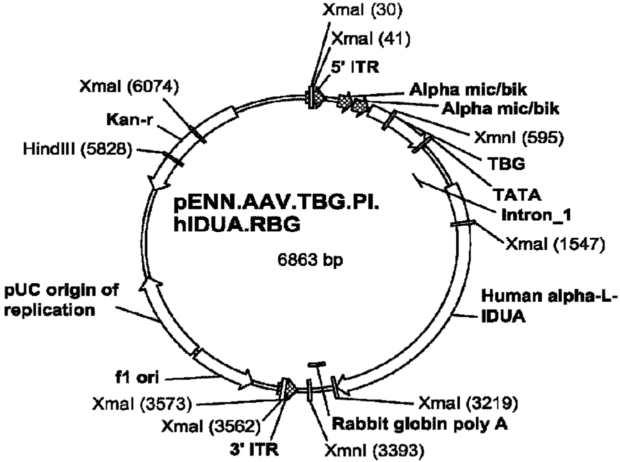
FIG. 1C

2821 tgaccctgag gcccgctctg agactgccta gtctgctgct ggtgcacgtg tgcgccaggc
2881 ccgagaagcc tcccggccag gtgacaagac tgagagccct gcccctgacc cagggccagc
2941 tggtgctggt gtggtccgat gagcacgtgg gcagcaagtg cctgtggacc tacgagatcc
3001 agttcagcca ggacggcaag gcctacaccc ccgtgtcccg gaagcccagc accttcaacc
3061 tgttcgtgtt cagccccgat acaggcgccg tgtccggctc ttatagagtg cgggccctgg
3121 actactgggc cagacccggc cctttcagcg accccgtgcc ctacctggaa gtgcccgtgc
3181 ctagaggccc ccctagcccc ggcaaccctt gagtcgaccc gggcggcctc gaggacgggg
3241 tgaactacgc ctgaggatcc gatctttttc cctctgccaa aaattatggg gacatcatga
3301 agccccttga gcatctgact tctggctaat aaaggaaatt tattttcatt gcaatagtgt
3361 gttggaattt tttgtgtctc tcactcggaa gcaattcgtt gatctgaatt tcgaccaccc
3421 ataataccca ttaccctggt agataagtag catggcgggt taatcattaa ctacaaggaa
3481 cccctagtga tggagttggc cactccctct ctgcgcgctc gctcgctcac tgaggccggg
3541 cgaccaaagg tcgcccgacg cccgggcttt gcccgggcgg cctcagtgag cgagcgagcg
3601 cgcagcctta attaacctaa ttcactggcc gtcgttttac aacgtcgtga ctgggaaaac
3661 cctggcgtta cccaacttaa tcgccttgca gcacatcccc ctttcgccag ctggcgtaat
3721 agcgaagagg cccgcaccga tcgcccttcc caacagttgc gcagcctgaa tggcgaatgg
3781 gacgcgccct gtagcggcgc attaagcgcg gcgggtgtgg tggttacgcg cagcgtgacc
3841 gctacacttg ccagcgccct agcgcccgct cctttcgctt tcttcccttc ctttctcgcc
3901 acgttcgccg gctttccccg tcaagctcta aatcgggggc tccctttagg gttccgattt
3961 agtgctttac ggcacctcga ccccaaaaaa cttgattagg gtgatggttc acgtagtggg
4021 ccatcgccct gatagacggt ttttcgccct ttgacgttgg agtccacgtt ctttaatagt
4081 ggactcttgt tccaaactgg aacaacactc aaccctatct cggtctattc ttttgattta
4141 taagggattt tgccgatttc ggcctattgg ttaaaaaatg agctgattta acaaaaattt
4201 aacgcgaatt ttaacaaaat catgtgagca aaaggccagc aaaaggccag gaaccgtaaa
4261 aaggccgcgt tgctggcgtt tttccatagg ctccgccccc ctgacgagca tcacaaaaat
4321 cgacgctcaa gtcagaggtg gcgaaacccg acaggactat aaagatacca ggcgtttccc
4381 cctggaagct ccctcgtgcg ctctcctgtt ccgaccctgc cgcttaccgg atacctgtcc
4441 gcctttctcc cttcgggaag cgtggcgctt tctcatagct cacgctgtag gtatctcagt
4501 tcggtgtagg tcgttcgctc caagctgggc tgtgtgcacg aaccccccgt tcagcccgac
4561 cgctgcgcct tatccggtaa ctatcgtctt gagtccaacc cggtaagaca cgacttatcg
4621 ccactggcag cagccactgg taacaggatt agcagagcga ggtatgtagg cggtgctaca
4681 gagttcttga agtggtggcc taactacggc tacactagaa gaacagtatt tggtatctgc
4741 gctctgctga agccagttac cttcggaaaa agagttggta gctcttgatc cggcaaacaa
4801 accaccgctg gtagcggtgg tttttttgtt tgcaagcagc agattacgcg cagaaaaaaa
4861 ggatctcaag aagatccttt gatcttttct acggggtctg acgctcagtg gaacgaaaac
4921 tcacgttaag ggattttggt catgagatta tcaaaaagga tcttcaccta gatcctttg
4981 atcctccggc gttcagcctg tgccacagcc gacaggatgg tgaccaccat ttgccccata
5041 tcaccgtcgg tactgatccc gtcgtcaata aaccgaaccg ctacaccctg agcatcaaac
5101 tcttttatca gttggatcat gtcggcggtg tcgcggccaa gacggtcgag cttcttcacc
5161 agaatgacat caccttcctc caccttcatc ctcagcaaat ccagcccttc ccgatctgtt
5221 gaactgccgg atgccttgtc ggtaaagatg cggttagctt ttacccctgc atctttgagc
5281 gctgaggtct gcctcgtgaa gaaggtgttg ctgactcata ccaggcctga atcgccccat
5341 catccagcca gaaagtgagg gagccacggt tgatgagagc tttgttgtag gtggaccagt
5401 tggtgatttt gaacttttgc tttgccacgg aacggtctgc gttgtcggga agatgcgtga
5461 tctgatcctt caactcagca aaagttcgat ttattcaaca aagccgccgt cccgtcaagt
5521 cagcgtaatg ctctgccagt gttacaacca attaaccaat tctgattaga aaaactcatc
5581 gagcatcaaa tgaaactgca atttattcat atcaggatta tcaataccat atttttgaaa
5641 aagccgtttc tgtaatgaag gagaaaactc accgaggcag ttccatagga tggcaagatc

FIG. 1D

5701 ctggtatcgg tctgcgattc cgactcgtcc aacatcaata caacctatta atttcccctc
5761 gtcaaaaata aggttatcaa gtgagaaatc accatgagtg acgactgaat ccggtgagaa
5821 tggcaaaagc ttatgcattt ctttccagac ttgttcaaca ggccagccat tacgctcgtc
5881 atcaaaatca ctcgcatcaa ccaaaccgtt attcattcgt gattgcgcct gagcgagacg
5941 aaatacgcga tcgctgttaa aaggacaatt acaaacagga atcgaatgca accggcgcag
6001 gaacactgcc agcgcatcaa caatattttc acctgaatca ggatattctt ctaatacctg
6061 gaatgctgtt ttcccgggga tcgcagtggt gagtaaccat gcatcatcag gagtacggat
6121 aaaatgcttg atggtcggaa gaggcataaa ttccgtcagc cagtttagtc tgaccatctc
6181 atctgtaaca tcattggcaa cgctaccttt gccatgtttc agaaacaact ctggcgcatc
6241 gggcttccca tacaatcgat agattgtcgc acctgattgc ccgacattat cgcgagccca
6301 tttataccca tataaatcag catccatgtt ggaatttaat cgcggcctcg agcaagacgt
6361 ttcccgttga atatggctca taacacccct tgtattactg tttatgtaag cagacagttt
6421 tattgttcat gatgatatat ttttatcttg tgcaatgtaa catcagagat tttgagacac
6481 catgttcttt cctgcgttat cccctgattc tgtggataac cgtattaccg cctttgagtg
6541 agctgatacc gctcgccgca gccgaacgac cgagcgcagc gagtcagtga gcgaggaagc
6601 ggaagagcgc ccaatacgca aaccgcctct ccccgcgcgt tggccgattc attaatgcag
6661 ctggcacgac aggtttcccg actggaaagc gggcagtgag cgcaacgcaa ttaatgtgag
6721 ttagctcact cattaggcac cccaggcttt acactttatg cttccggctc gtatgttgtg
6781 tggaattgtg agcggataac aatttcacac aggaaacagc tatgaccatg attacgccag
6841 atttaattaa ggccttaatt agg


XmaI (30)
XmaI (41)
5' ITR
Alpha mic/bik
Alpha mic/bik
XmnI (595)
TBG
TATA
Intron_1
XmaI (1547)
Human alpha-L-
IDUA
XmaI (3219)
Rabbit globin poly A
XmnI (3393)
3' ITR
XmaI (3562)
XmaI (3573)
f1 ori
pUC origin of
replication
HindIII (5828)
Kan-r
XmaI (6074)
pENN.AAV.TBG.PI.
hIDUA.RBG
6863 bp


XmaI (30)
XmaI (41)
5' ITR
XmaI (6074)
Alpha mic/bik
Alpha mic/bik
Kan-r
XmnI (595)
HindIII (5828)
TBG
TATA
pENN.AAV.TBG.PI.
hIDUA.RBG
Intron_1
XmaI (1547)
6863 bp
pUC origin of
replication
Human alpha-L-
IDUA
f1 ori
XmaI (3573)
XmaI (3219)
XmaI (3562)
Rabbit globin poly A
3' ITR
XmnI (3393)