© The American Society of Gene & Cell Therapy

A Self-inactivating γ-Retroviral Vector Reduces Manifestations of Mucopolysaccharidosis I in Mice

Jason A Metcalf[1], Xiucui Ma[1], Bruce Linders[1], Susan Wu[1], Axel Schambach[2], Kevin K Ohlemiller[3], Attila Kovacs[1], Mark Bigg[1], Li He[1], Douglas M Tollefsen[1] and Katherine P Ponder[1,4]

[1]Department of Medicine, Washington University School of Medicine, St Louis, Missouri, USA; [2]Department of Experimental Hematology, Hannover Medical School, Hannover, Germany; [3]Fay and Carl Simons Center for the Biology of Hearing and Deafness, Central Institute for the Deaf, Department of Otolaryngology, Washington University Medical School, St Louis, Missouri, USA; [4]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, Missouri, USA

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease due to deficiency in α-L-iduronidase (IDUA) that results in accumulation of glycosaminoglycans (GAGs) throughout the body, causing numerous clinical defects. Intravenous administration of a γ-retroviral vector (γ-RV) with an intact long terminal repeat (LTR) reduced the clinical manifestations of MPS I, but could cause insertional mutagenesis. Although self-inactivating (SIN) γ-RVs in which the enhancer and promoter elements in the viral LTR are absent after transduction reduces this risk, such vectors could be less effective. This report demonstrates that intravenous (i.v.) injection of a SIN γ-RV expressing canine IDUA from the liver-specific human α_1-antitrypsin promoter into adult or newborn MPS I mice completely prevents biochemical abnormalities in several organs, and improved bone disease, vision, hearing, and aorta to a similar extent as was seen with administration of the LTR-intact vector to adults. Improvements were less profound than when using an LTR-intact γ-RV in newborns, which likely reflects a lower level of transduction and expression for the SIN vector-transduced mice, and might be overcome by using a higher dose of SIN vector. A SIN γ-RV vector ameliorates clinical manifestations of MPS I in mice and should be safer than an LTR-intact γ-RV.

Received 7 July 2009; accepted 9 September 2009; published online 20 October 2009. doi:10.1038/mt.2009.236

INTRODUCTION

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease that occurs in ~1 in 100,000 live births and is due to mutations in the enzyme α-L-iduronidase (IDUA; EC 3.2.1.76) (ref. 1). This results in accumulation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate, which can result in coarse facial features, joint stiffness, skeletal deformities, visual impairment, deafness, hernias, cardiac valve and aortic disease, and mental retardation.[2]

Treatment for MPS I currently consists of hematopoietic stem cell transplantation[3] or enzyme replacement therapy.[4,5] In hematopoietic stem cell transplantation, cells from a normal donor migrate to organs where they secrete IDUA modified with mannose-6-phosphate (M6P), which is taken up by adjacent cells via the M6P receptor. In enzyme replacement therapy, IDUA modified with M6P is injected intravenously, and enzyme diffuses into organs and is taken up via the M6P receptor. Both treatments have reduced clinical manifestations of MPS I, but are expensive. Furthermore, hematopoietic stem cell transplantation requires a compatible donor and enzyme replacement therapy involves weekly injections.

Gene therapy is a promising alternative to existing treatments for MPS I, and has improved clinical manifestations of the disease in a variety of animal models.[6] Neonatal administration of γ-retroviral vectors (γ-RV),[7,8] lentiviral vectors,[9] or adeno-associated virus vectors[10] expressing the IDUA gene have prevented many aspects of MPS I disease in mice, while γ-RV have been effective in dogs and cats.[11–13] However, administration of a γ-RV[7] or a lentiviral vector[9,14] to adult mice without immunosuppression resulted in a cytotoxic T lymphocyte response and subsequent loss of IDUA expression and thus had no therapeutic effect. Administration of a γ-RV expressing IDUA in conjunction with transient immunosuppression prevented an immune response and resulted in long-term expression,[15] but immunosuppression carries inherent risks, and avoiding this option would be preferred. Engineering a γ-RV to prevent expression in nonhepatic cells by inversion of the expression cassette relative to an intact long terminal repeat (LTR) resulted in stable expression, although expression was lower and the vector was less effective at preventing disease manifestations than an LTR-intact vector with the expression cassette in the same orientation as the LTR.[16] Adult administration of a lentiviral vector with a liver-specific promoter[14] or a plasmid vector to immunodeficient mice[17] reduced disease manifestations in MPS I mice.

A second problem with using integrating vectors for gene therapy is the risk of insertional mutagenesis.[18] One way to lower this risk is to create a self-inactivating (SIN) γ-RV with a deletion in the U3 region of the 3′ viral LTR that is copied to the 5′-LTR position after transduction. This results in deletion of the viral promoter/enhancer elements, leaving only an internal promoter to drive expression.[19–21] A SIN γ-RV reduced the risk of insertional

Correspondence: *Katherine P Ponder, Campus Box 8125, 660 South Euclid Avenue, St Louis, Missouri 63110, USA. E-mail: kponder@dom.wustl.edu*

© The American Society of Gene & Cell Therapy

mutagenesis affecting cellular growth to ~10% of that seen with an LTR-intact vector in hematopoietic cells.[22,23]

In this study, we produced a SIN γ-RV with the liver-specific human α_1-antitrypsin promoter (hAAT), the canine IDUA complementary DNA (cIDUA), and an optimized woodchuck hepatitis post-transcriptional regulatory element (oPRE). This vector was administered to both neonatal and adult MPS I mice, and reduced or prevented many manifestations of disease.

RESULTS

Description of RVs and injection into mice

The γ-RV designated SIN-hAAT-cIDUA-oPRE is diagrammed in Figure 1a. This vector has a deletion of the retroviral enhancer and promoter sequences in the U3 region of the 3′-LTR, resulting in a ΔLTR, which lacks promoter function at the 5′- and 3′-ends of the provirus after integration.[19] The liver-specific hAAT promoter drives expression of the canine IDUA complementary DNA, and the oPRE improves expression.[21] This vector resulted in cIDUA expression *in vitro* in human hepatoma HepG2 cells but not in murine fibroblast NIH3T3 cells (data not shown). The previously described LTR-intact γ-RV designated hAAT-cIDUA-WPRE[7] is diagrammed in Figure 1b.

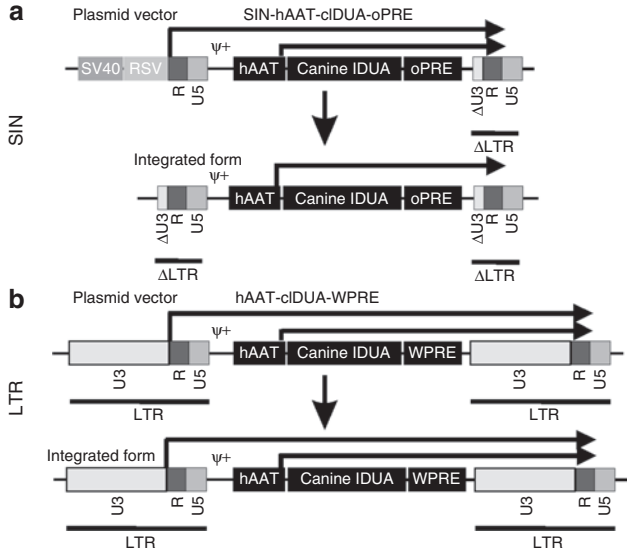

Figure 1 Vectors. (**a**). SIN-hAAT-cIDUA-oPRE. The diagram shows the SIN-hAAT-cIDUA-oPRE vector. The 5′-end contains the simian virus 40 enhancer (SV40) and the Rous sarcoma virus (RSV) long-terminal repeat (LTR) instead of the Moloney murine leukemia virus U3 (unique to the 3′-end of the RNA). Most of the U3 region of the 3′-LTR has been deleted to form a ΔU3 lacking enhancer and promoter elements, with only 22 bp from the 5′-end and 14 bp from the 3′-end of the original 449 bp U3. The U5 (unique to the 5′-end of the RNA) and the R (redundant; present at both the 5′- and the 3′-end of the RNA) are also shown. Upon integration, the U3 region of the 3′-ΔLTR is copied to the 5′-LTR position, and both ΔLTRs are transcriptionally inactive. The human α_1-antitrypsin (hAAT) promoter drives expression of the canine α-L-iduronidase gene (cIDUA) in hepatic cells, as indicated by the black arrow. The optimized post-transcriptional regulatory element (oPRE) lacks open reading frames and lacks the sequence and promoter of the potentially oncogenic X protein. (**b**) hAAT-cIDUA-WPRE. The diagram shows the LTR-intact hAAT-cIDUA-WPRE vector. The LTR is complete at both ends, and upon integration can drive expression of the cIDUA in nonhepatic cells. This vector also contains the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), which is similar to the oPRE but is longer and does not have the modifications noted above. SIN, self-inactivating.

The group designated hereafter as Neonatal-SIN are MPS I mice that received intravenous (i.v.) injection of 1×10^{10} transducing units (TU)/kg of SIN-hAAT-cIDUA-oPRE vector at 2–3 days of age. Most (81% of 16 mice) achieved stable expression of IDUA activity in serum, and animals with stable expression had an average serum IDUA activity of 127 ± 10 U/ml (Figure 2a; 63-fold the value in homozygous normal mice). The group designated as Adult-SIN were MPS I mice that received i.v. injection of 1×10^{10} TU/kg of SIN-hAAT-cIDUA-oPRE at 6 weeks of age after injection of hepatocyte growth factor to induce hepatocyte replication, as detailed in the Materials and Methods section. No immunosuppression was given to this group. Most (88% of 8 mice) achieved high and stable IDUA activity (Figure 2b), with an average of 71 ± 7 U/ml. Stable expression suggests that an immune response did not occur, which is consistent with the hypothesis that avoiding expression in antigen-presenting cells could prevent an immune response from occurring. The group designated Adult-LTR were MPS I mice that received 1.7×10^{10} TU/kg of the LTR-intact hAAT-cIDUA-WPRE vector at 6 weeks of age after administration of hepatocyte growth factor. These mice were also immunosuppressed with CTLA4-Ig and anti-CD40 ligand, as detailed in the methods. This dose was 167% of that in a previous study, and involved slightly different times of γ-RV injection relative to that of hepatocyte growth factor.[15] All mice ($N = 10$) had stable IDUA expression in serum, that averaged 235 ± 20 U/ml, as summarized in Figure 2c. Serum IDUA activity in Adult-LTR mice was threefold the value observed in Adult-SIN mice. Finally, results of the three groups analyzed in this study will be compared with historical controls from a group designated Neonatal-LTR that were analyzed with similar methods and received 1×10^{10} TU/kg of the LTR-intact vector hAAT-cIDUA-WPRE at 2–3 days after birth. These mice achieved stable serum IDUA activity at 1,240 ± 147 U/ml) (ref. 7), as summarized in Figure 2c, which was tenfold the value in the Neonatal-SIN mice.

As the efficacy of gene therapy depends on the amount of IDUA that is secreted in a usable form, the amount of serum IDUA activity with M6P modification was examined. While 49 ± 9% of serum IDUA in normal mice was M6P modified, only 11 ± 1% of serum IDUA in Neonatal-SIN mice and 14 ± 2% of serum IDUA in Adult-SIN mice were M6P modified (Figure 2d). This is similar to the percentage of M6P-modified IDUA that was seen previously with the LTR-intact hAAT-cIDUA-WPRE vector.[7,16]

Heparin cofactor II (HCII)–thrombin (IIa) complex formation is potentiated by dermatan sulfate, and the presence of such complexes in MPS I, but not normal, serum likely reflects the presence of dermatan sulfate in blood. The disappearance of these complexes has been used to evaluate the efficacy of treatment in humans.[24] An immunoblot for HCII confirms that normal mice do not form HCII–IIa complexes in their serum, whereas MPS I mice have high levels of HCII–IIa complexes. HCII–IIA complexes were absent in Neonatal-SIN and Adult-SIN mice at 8 months (Figure 2e).

Biochemical abnormalities

For the SIN-hAAT-cIDUA-oPRE vector, the liver is expected to be the organ that produces and secretes IDUA into serum, as the vector has a liver-specific hAAT promoter and the ΔLTR is

© The American Society of Gene & Cell Therapy

transcriptionally inactive. For the LTR-intact vector, we have previously shown that liver had the highest level of expression, but expression did occur in other organs.[7,15] Adult-LTR, Neonatal-SIN, and Adult-SIN mice had 20 ± 5, 33 ± 11, and 33 ± 4 U/mg of IDUA activity in liver, as shown in **Figure 3a**, which was >3.5-fold the IDUA activity in normal liver (6 ± 1 U/mg). Neonatal-SIN and Adult-SIN mice had spleen, kidney, and lung IDUA activity that was >37% of normal; these organ were not analyzed in the Adult-LTR mice evaluated in this study, as these organs were corrected biochemically in a prior study using a lower dose of the LTR-intact hAAT-cIDUA-WPRE vector.[15] IDUA activity was low in all groups including normal animals in the aorta, and could not be quantified accurately.

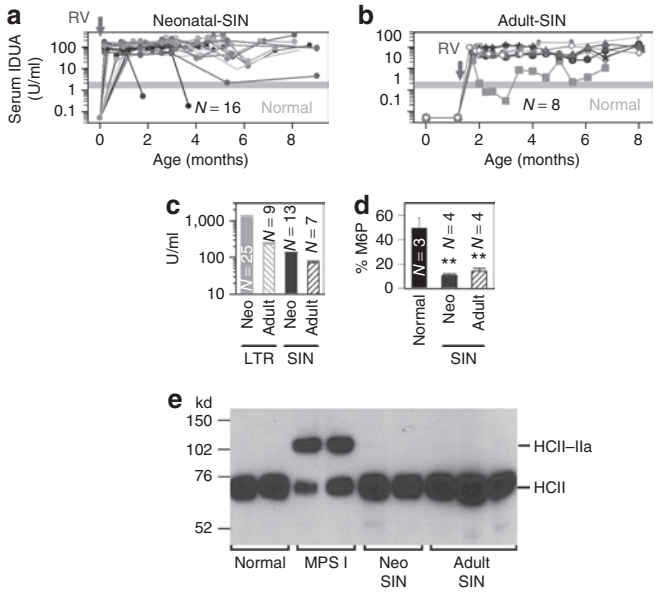

Figure 2 Serum α-L-iduronidase (IDUA) activity and heparin cofactor II (HCII) complexes after transduction. (**a**) Serum IDUA activity in Neonatal-SIN mice. Neonatal-SIN mice were mucopolysaccharidosis I (MPS I) mice that received 1 × 10^{10} transducing units (TU)/kg of SIN-hAAT-cIDUA-oPRE at 2 days of age. Serum IDUA activity was determined periodically, and each line represents an individual animal. The red arrow indicates the age of γ-retroviral vector (RV) injection. The blue bar represents the average serum IDUA activity in homozygous normal mice ±2 SD. (**b**) Serum IDUA activity in Adult-SIN mice. Adult-SIN mice were MPS I mice that received hepatocyte growth factor to induce hepatocyte replication followed by 1 × 10^{10} TU/kg of SIN-hAAT-cIDUA-oPRE at 6 weeks of age. No immunosuppression was given. (**c**) Average serum IDUA activity for different groups of treated MPS I mice. Neonatal-SIN (Neo-SIN) and Adult-SIN mice are described above; all animals with <10 U/ml of serum IDUA activity at any time of evaluation were excluded from these averages and from any subsequent analyses. The Neonatal-LTR (Neo-LTR) mice received 1 × 10^{10} TU/kg of hAAT-cIDUA-WPRE at 2 days of age and were evaluated for clinical improvement previously,[7] and will be evaluated for RNA and DNA below. The Adult-LTR mice received hepatocyte growth factor followed by 1.7 × 10^{10} TU/kg of hAAT-cIDUA-WPRE and were immunosuppressed transiently with CTLA4-Ig and αCD40 ligand as detailed in the Materials and Methods section; this vector dose was ~1.7-fold that in a previous report.[15] The average serum IDUA activity ± SEM is shown for the indicated number of mice (*N*) in each group. (**d**) Percentage of IDUA with mannose-6-phosphate (M6P) modification. The percentage of IDUA activity that was retained on an M6P receptor column and eluted with M6P was determined for samples obtained at 8 months of age. Double asterisks indicate $P < 0.01$ for the indicated group versus normal using analysis of variance with Tukey's post hoc analysis. (**e**) Immunoblot for HCII–thrombin (IIa) complexes. Sera obtained from animals of the indicated groups at 8 months of age were electrophoresed on a sodium dodecyl sulfate–polyacrylamide gel and immunoblot for HCII was performed, as dermatan sulfate can potentiate the formation of HCII–IIa complexes. The position of free HCII and the covalent HCII–IIa complexes is shown. cIDUA, canine α-L-iduronidase; hAAT, human α_1-antitrypsin promoter; LTR, long-terminal repeat; oPRE, optimized woodchuck hepatitis post-transcriptional regulatory element; SIN, self-inactivating; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element.

MPS I mice typically exhibit an increase in activity of other lysosomal enzymes and in GAG levels in organs. Therefore, we examined whether gene therapy could normalize these levels. Although reductions in β-glucuronidase (GUSB) activity in liver, spleen, kidney, and lung (**Figure 3b**) did not always achieve statistical significance for the Neonatal-SIN and Adult-SIN groups, reductions in GAG levels were significant in these organs (**Figure 3c**). The aorta has been one of the most difficult organs to correct with gene therapy. GUSB activity and GAG levels were significantly reduced in Adult-LTR, Neonatal-SIN, and Adult-SIN mice when values were compared with those in untreated MPS I mice. Although GUSB activity and GAG levels in all treated groups were not statistically different from those in normal mice, they appeared to be elevated, and the failure to detect differences was

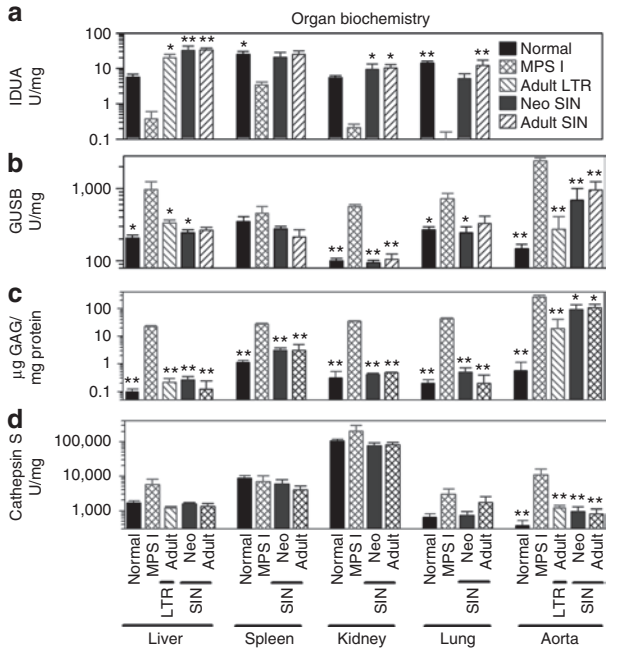

Figure 3 Enzyme activity and glycosaminoglycan (GAG) content of organs at 8 months of age. Tissues from five normal, six mucopolysaccharidosis I (MPS I), six Adult-LTR, four Neonatal-SIN, and three Adult-SIN mice were obtained at 8 months of age, and homogenates were prepared. (**a**) α-L-Iduronidase (IDUA) activity. The average IDUA activity ± SEM for each group is shown, and statistical comparisons of each group were with values in untreated MPS I mice using analysis of variance with Tukey's post hoc analysis, and asterisk indicates $P = 0.01$–0.05, while double asterisks indicate $P < 0.01$. IDUA activity is not shown for aorta as the activity was too low to be accurately determined in any samples including those from normal mice. Some organs from the Adult-LTR group were not evaluated here, as biochemical abnormalities in those sites was normalized with a lower dose of vector in a previous study.[15] (**b**) β-Glucuronidase (GUSB) activity. (**c**) GAG content. (**d**) Cathepsin S activity. LTR, long-terminal repeat; SIN, self-inactivating.

© The American Society of Gene & Cell Therapy

likely due to the small numbers of mice that were evaluated and the fact that five groups were compared statistically using analysis of variance with Tukey's post hoc analysis. Indeed, histopathology of aortas from the treated mice demonstrated that there was accumulation of lysosomal storage material to a varying extent that correlated well with the levels of GAGs determined biochemically for individual mice (data not shown).

We recently reported that cathepsin S was upregulated in the aorta of MPS I mice, and postulated that this elastin-degrading enzyme contributed to the development of elastin fragmentation in the aorta.[8] Organs were therefore evaluated for cathepsin S enzyme activity, as shown in **Figure 3d**. As is similar to our previous report, cathepsin S activity was 30-fold normal in aorta in MPS I mice ($P < 0.01$). Cathepsin S activity was significantly reduced in aorta in Adult-LTR, Neonatal-SIN, and Adult-SIN mice as compared with untreated MPS I. Values in treated mice were two- to threefold normal, which were not significantly different from values in normal mice. Cathepsin S activities in liver, kidney, and lung in MPS I mice were not statistically different from levels in normal mice, although the values were two- to fivefold normal, and the failure to detect differences with analysis of variance with Tukey's post hoc analysis may reflect the small numbers of animals and the large number of groups. These data suggest that upregulation of cathepsin S activity was much greater in the aorta than in other organs that were evaluated.

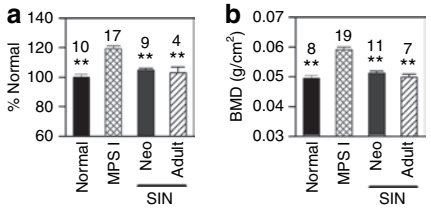

Figure 4 Bone analysis. Mice were untreated or were treated with gene therapy as described above, and bone analyses were performed at 8 months. (**a**) Femur width. The average width of the femur ± SEM was determined at the point halfway between the proximal and distal ends of the femur for the indicated number of mice. (**b**) Bone mineral density (BMD). Double asterisks indicate $P < 0.01$ for comparison of values in the indicated group with those in untreated mucopolysaccharidosis I (MPS I) mice using one-way analysis of variance with Tukey's post hoc analysis. SIN, self-inactivating.

Skeletal defects

Most MPS I mouse bones are thicker than in normal mice, with the femurs being 119 ± 2% of normal width (**Figure 4a**). Neonatal-SIN and Adult-SIN femur diameters were significantly reduced to 105 ± 2 and 103 ± 4% of normal width, respectively (for both groups, $P < 0.001$ versus MPS I, and not significant versus normal). Normal mice have a bone mineral density of 0.049 ± 0.001 g/cm^2, while MPS I bones were significantly more dense at 0.059 ± 0.001 g/cm^2 (**Figure 4b**). This defect was nearly completely corrected in Neonatal-SIN and Adult-SIN mice, who had bone mineral density of 0.051 ± 0.001 and 0.050 ± 0.001 g/cm^2, respectively (for both groups, $P < 0.001$ versus MPS I, and not significant versus normal). Because Adult-LTR mice that received a lower dose of RV in a previous study had marked improvements in bone,[15] bone was not evaluated in the Adult-LTR mice that received the higher dose in this study. These data suggest that the Neonatal-SIN and Adult-SIN mice have marked improvements in skeletal abnormalities.

Eye and ear defects

MPS I patients and mice have abnormalities in eyesight due to corneal clouding and retinal dysfunction, and in hearing due to conductive defects. To evaluate eye defects in our mice, sections of the eye were stained with hematoxylin and eosin. MPS I mice had a reduced number of photoreceptor cells per column in the outer nuclear layer of the retina (**Figure 5a,b**), as normal eyes had 9.9 ± 0.2 cells/column in the outer nuclear layer, while MPS I eyes had far fewer cells at 5.9 ± 0.3 cells/column (59% normal; $P < 0.001$ versus normal). Adult-LTR mice were almost completely corrected, with 9.1 ± 0.1 cells/column (92% normal; $P < 0.001$ versus

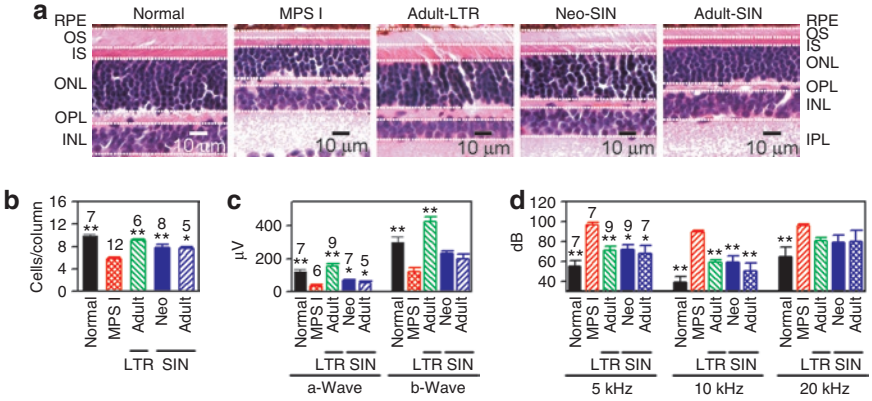

Figure 5 Analysis of eye and ear. (**a**) Histopathology of the eye. Hematoxylin and eosin stains of sections of the retina were obtained for animals that were sacrificed at 8 months. The retinal pigmented epithelium (RPE), outer segments (OS), inner segments (IS), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), and inner plexiform layer (IPL) are indicated. Bar = 10 µm. (**b**) Number of cells in the ONL. The average number of cells in one thickness of the ONL (top to bottom in the photographs) was determined for individual animals, and the average ± SEM for the group determined. Asterisk indicates $P = 0.01$–0.05, double asterisks indicate $P < 0.01$ versus mucopolysaccharidosis I (MPS I) using one-way analysis of variance with Tukey's post hoc analysis. The number of animals tested are shown above each bar. (**c**) Electroretinograms. Dark-adapted a- and b-wave measurements from electroretinograms. (**d**) Auditory-evoked brainstem responses. The decibels of sound needed to evoke a response in the brainstem that was determined at varying frequencies of sound are shown. LTR, long-terminal repeat; SIN, self-inactivating.

© The American Society of Gene & Cell Therapy

MPS I, not significant versus normal), while Neonatal-SIN and Adult-SIN mice were only partially corrected, with 7.9 ± 0.5 cells/column (80% normal; $P = 0.002$ versus MPS I and $P = 0.002$ versus normal), and 7.6 ± 0.4 cells/column (77% normal; $P = 0.02$ versus MPS I and $P = 0.005$ versus normal), respectively. In addition, the lengths of the outer segments, where light activates a signal transduction pathway that results in vision, were reduced in untreated MPS I mice and to a varying extent in the treated mice (**Figure 5a**). To evaluate eye function, electroretinograms (ERGs) were performed. Dark-adapted ERG is primarily indicative of rod function although it also measures function of the middle retinal layers. Dark-adapted ERG in MPS I mice showed a significant decrease in the a-wave (27 ± 10% of normal; $P = 0.007$ versus normal) and the b-wave (40 ± 27% of normal; $P < 0.001$ versus normal) (**Figure 5c**). This defect was completely corrected in Adult-LTR mice. For Neonatal-SIN mice, the a-wave was partially corrected at 53 ± 6% normal ($P = 0.01$ versus normal and $P = 0.02$ versus MPS I), and values for the b-wave were also intermediate at 75 ± 17% normal but were not significantly different from values in normal or MPS I mice. Similarly, for the Adult-SIN mice, the a-wave was partially corrected at 44 ± 12% normal ($P = 0.009$ versus normal and $P = 0.025$ versus MPS I) and the values for the b-wave were intermediate at 67 ± 30% normal, which was significantly different from in normal mice ($P < 0.001$) but did not differ statistically from MPS I mice. Light-adapted ERG, which is indicative of cone function, was normal in MPS I mice (data not shown).

Hearing was evaluated using auditory-evoked brainstem responses. In normal mice, 39 ± 6 decibels (dB) were required to elicit a signal in the auditory nerve at a sound frequency of 10 kHz, while MPS I mice were nearly deaf and required an average of 89 ± 2 dB (**Figure 5d**; 2.3-fold normal; $P < 0.001$ versus normal). All treated groups had significant improvements in the auditory-evoked brainstem responses at 10 kHz, as Adult-LTR mice required 59 ± 2 dB to elicit a response (1.5-fold normal, $P = 0.002$ versus MPS I), Neonatal-SIN required 61 ± 6 dB (1.6-fold normal $P = 0.004$ versus MPS I), and Adult-SIN required 50 ± 8 dB (1.3-fold normal; $P < 0.001$ versus MPS I). However, although values for the Adult-SIN mice were not statistically different from those in normal mice, those in Neonatal-SIN mice were statistically different from in normal mice ($P = 0.04$) and those in Adult-LTR mice approached significance ($P = 0.08$ versus normal). These data suggest that improvements in hearing were only partial in the treated mice.

Aorta defects

MPS I mice have dilated aortas, as shown with two-dimensional echocardiography in **Figure 6a**. At the position of the ascending aorta (see **Supplementary Figure S1** for a diagram of the regions of the aorta that were evaluated), the inner diameters of the MPS I aortas were 2.4 ± 0.2 mm (150% the normal value of 1.59 ± 0.51 mm; $P < 0.001$), as shown in **Figure 6b**. In addition, MPS I aortas were dilated at most other positions that were evaluated. The diameters of ascending aortas in Adult-LTR mice were nearly completely normal at 1.64 ± 0.11 mm (103% normal; P not significant versus normal, $P < 0.01$ versus MPS I), while values in Neonatal-SIN mice were partially corrected at 1.9 ± 0.1 mm (117% normal; P not significant versus normal; $P < 0.01$ versus MPS I). Ascending aorta diameters in Adult-SIN mice were 2.1 ± 0.2 mm (131% normal), which was not statistically better than in untreated MPS I mice, although the small numbers of animals evaluated could contribute to a failure to achieve significance. Diameters at the aortic arch and descending aorta were nearly completely corrected in all treated MPS I mice (**Figure 6b**). In addition, MPS I mice had aortic insufficiency (**Figure 6c**), with an aortic insufficiency jet that averaged 47.2 ± 8.0 grayscale units in a Doppler echocardiogram (3.1-fold the value of 15.2 ± 0.7 grayscale units of intensity in normal mice; $P = 0.01$). Aortic insufficiency was significantly reduced for Neonatal-SIN mice (21.6 ± 3.5 units, 1.4-fold normal; $P = 0.03$). Values were partially reduced for Adult-LTR mice (27 ± 5 units; 1.8-fold normal) and for Adult-SIN mice (36 ± 13 units, 2.4-fold normal), although neither of these were statistically different from values in untreated MPS I mice.

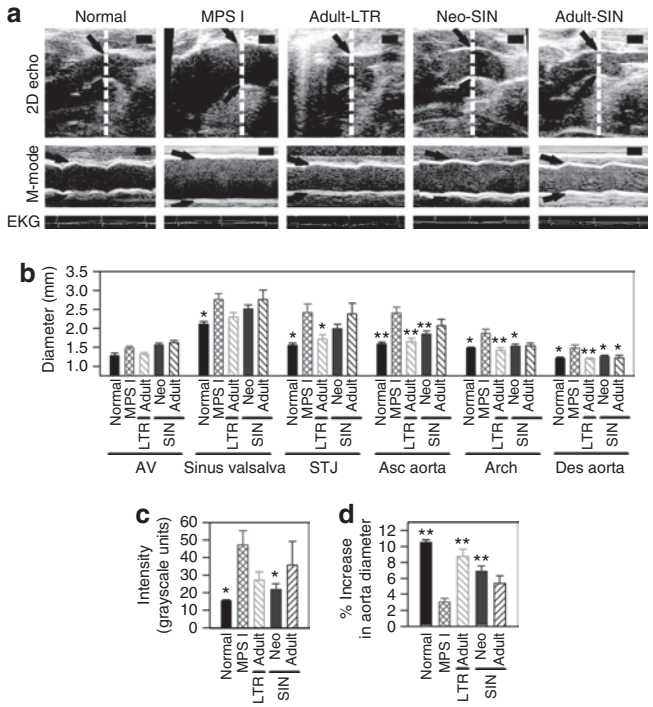

Figure 6 Aorta. (**a**) Echocardiograms at 8 months. Representative examples of 2 dimensional (2D) echos, M-mode echos, and electrocardiograms (EKGs) performed at the time of M-mode echo are shown. The dashed lines on 2D echos indicate the position at which M-mode analysis was performed; the aortic valve is on the left and the first branch of the aorta is on the right. The arrows indicate the inner edge of the aorta; for the M-mode the arrows indicate the position of the aorta during systole. (**b**) Aorta's diameter. The average inner diameter of the aorta ± SEM at the aortic valve (AV), Sinus of Valsalva, sinotubular junction (STJ), ascending aorta (Asc Aorta), aortic arch (Arch), and descending aorta (Des Aorta) were determined by 2D echo for 7 normal, 11 mucopolysaccharidosis I (MPS I), 10 Adult-LTR, 11 Neonatal-SIN, and 6 Adult-SIN mice. (**c**) Aortic insufficiency. Aortic insufficiency was determined by the intensity in grayscale units on a scale of 0–255 of the aortic insufficiency jet as measured by Doppler echocardiography. (**d**) Aortic strain. The average aortic strain ± SEM was determined using the formula: Aortic strain = (systolic diameter – diastolic diameter)/diastolic diameter and plotted as the percent increase. Asterisk indicates $P = 0.01$–0.05, double asterisks indicate $P < 0.01$ versus MPS I using one-way analysis of variance with Tukey's post hoc analysis. LTR, long-terminal repeat; SIN, self-inactivating.

© The American Society of Gene & Cell Therapy

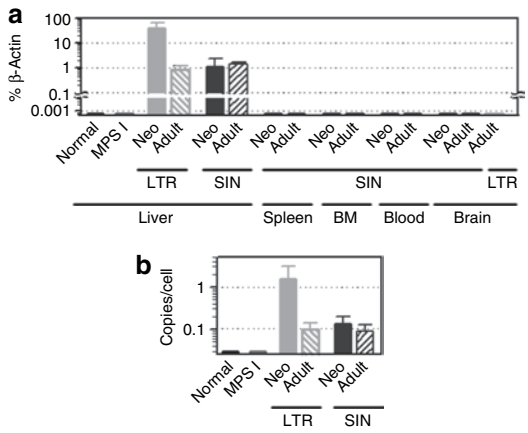

Figure 7 Nucleic acid analysis. (**a**) RNA. Real-time reverse transcriptase PCR was performed to determine the level of canine α-L-iduronidase (cIDUA) RNA ± SEM in liver, spleen, bone marrow (BM), and white blood cells (WBCs), and plotted as the ratio of the signal to that of β-actin. Note the break in the scale, and that samples that are plotted as having cIDUA RNA levels that were 0.0007% of β-actin were below the level of sensitivity, which was 0.001% of β-actin. (**b**) Liver DNA. cIDUA DNA copy numbers in mice of the indicated genotype and treatment group were determined using real-time PCR and plasmid standards. The limit of sensitivity was 0.02 copies/cell. LTR, long-terminal repeat; MPS I, mucopolysaccharidosis I; SIN, self-inactivating.

It was recently reported that human patients with MPS I have stiff aortas that have a reduced increase in diameter during systole as compared with aortas from normal controls.[25] The M-mode echocardiograms in **Figure 6a** show that the diameter of an aorta of a normal mouse increased by 10.5 ± 0.4% during contraction of the heart in systole, as quantified in **Figure 6d**. In contrast, MPS I mice have stiff aortas that only increase in diameter by 3.1 ± 0.4% during systole ($P < 0.001$ versus normal). Gene therapy partially corrects this defect, with Adult-LTR faring the best at a 8.8 ± 0.9% increase ($P < 0.001$ versus MPS I; not significant versus normal), while Neonatal-SIN were slightly lower at a 6.9 ± 0.7% increase ($P = 0.001$ versus MPS I; $P = 0.01$ versus normal). Adult-SIN mice had an increase in aortic diameter of 5.4 ± 1.0% during systole (not significant versus MPS I; $P < 0.001$ versus normal).

RNA and DNA analysis

RNA and DNA were analyzed at 8 months of age to determine the level of expression in different organs for the Neonatal-SIN and Adult-SIN groups, and to determine the expression and transduction efficiency in liver for all groups including historical controls that received the LTR-intact hAAT-cIDUA-WPRE vector. cIDUA RNA expression was undetectable (<0.001% of β-actin) in normal and MPS I livers (**Figure 7a**). Neonatal-LTR livers had the highest levels of cIDUA RNA at 39 ± 26% of β-actin, while the levels in Adult-LTR (0.8 ± 0.3% of β-actin), Neonatal-SIN (1.1 ± 1.2% of β-actin), and Adult-SIN (1.4 ± 0.2% of β-actin) mice were similar to each other and were only 2–3% of that in Neonatal-LTR mice. cIDUA RNA levels were not detected in spleen, bone marrow, white blood cells, or brain for Neonatal-SIN and Adult-SIN mice, suggesting that the SIN-hAAT-cIDUA-oPRE vector is indeed liver-specific, and that enzyme activity in other organs is due to uptake of enzyme from blood. In addition, cIDUA RNA was not detected in brain in the Adult-LTR mice, which is consistent with our prior study that used a lower dose of vector.[15]

A similar trend was seen in liver DNA levels (**Figure 7b**). cIDUA DNA was not detectable (<0.03 copies/cell) in liver in normal and MPS I mice. Neonatal-LTR mice had the highest copy number of cIDUA in liver, at 1.5 ± 1.6 copies/cell, while other treatment groups had much lower copy numbers, with 0.10 ± 0.04 copies/cell for Adult-LTR mice, 0.13 ± 0.07 copies/cell for Neonatal-SIN mice, and 0.09 ± 0.04 copies/cell for Adult-SIN mice, which were 6–8% of the values in the Neonatal-LTR mice. The relatively high amounts of RV DNA in the liver in Neonatal-LTR mice relative to that in Neonatal-SIN mice that were intended to receive the same dose of vector could reflect errors in titering of different preparations at different times with different standards, or could be due to other causes as discussed below.

DISCUSSION

Sustained IDUA activity

This study demonstrates that Neonatal-SIN and Adult-SIN mice that received 1×10^{10} TU/kg of SIN-hAAT-cIDUA-oPRE without any immunosuppression had sustained IDUA activity in serum in most mice at 127 ± 10 and 71 ± 7 U/ml, respectively, while Adult-LTR mice that received a higher dose of the LTR-intact hAAT-cIDUA-WPRE vector (1.7×10^{10} TU/kg) and transient immunosuppression achieved 235 ± 20 U/ml (**Figure 2**). All values were lower than the level of 1240 ± 147 U/ml of serum IDUA activity achieved previously after neonatal administration of the LTR-intact vector.[7] The stable expression in Adult-SIN mice without immunosuppression likely relates to the fact that the SIN vector achieved liver-specific expression (**Figure 7**), and thus avoided expression in antigen-presenting cells. This result is similar to that in our previous study in which inversion of the hAAT-cIDUA expression cassette relative to the LTR resulted in liver-specific expression and long-term expression without immunosuppression,[16] although the level of expression achieved with the SIN vector here was higher than for the reverse hAAT-cIDUA-WPRE vector, the degree of biochemical correction was greater, and the utilization of a SIN vector is an important safety advantage. This result differs from our previous result with Adult-LTR mice, where immunosuppression was necessary to achieve long-term expression,[15] which likely reflected the fact that LTR-initiated transcripts in nonhepatic cells could be translated into IDUA protein.

Correction of biochemical abnormalities

Dermatan sulfate can potentiate the formation of HCII–IIa complexes in serum. Such complexes have been used as a biomarker for patients with MPS I, and their disappearance with enzyme replacement therapy provided evidence for biochemical correction.[24] The disappearance of HCII–IIa complexes in Neonatal-SIN and Adult-SIN mice in this study (**Figure 2e**) suggests that dermatan sulfate was indeed cleared from blood.

Biochemical evaluation also involved analysis of organs for IDUA activity, secondary lysosomal enzymes, and GAG levels. Secondary lysosomal enzymes are lysosomal enzymes other than IDUA whose levels generally increase with time in untreated animals, and fall to normal levels with effective treatments. Secondary lysosomal

© The American Society of Gene & Cell Therapy

enzymes that were evaluated here include GUSB (deficient in MPS VII) and cathepsin S. Cathepsin S is an elastin-degrading proteinase that is of particular interest in the aorta, where we have postulated that it plays a role in the elastin fragmentation that contributes to aortic dilatation.[8] The liver, spleen, kidney, and lung of all groups that were evaluated had biochemical improvements in GUSB activity, GAG levels, and cathepsin S activity (**Figure 3**), which is consistent with data from several laboratories demonstrating that these organs are relatively easy to correct. The failure to achieve complete biochemical correction in the aorta is discussed below.

Reduction in manifestations of MPS I

The most important measure of the success of gene therapy for MPS I is the ability to improve manifestations that are associated with morbidity or mortality. A key question is whether or not this new SIN vector is as effective as the LTR-intact vector used in our previous studies, and to define the reasons for any differences. In our previous study with neonatal administration of the LTR-intact hAAT-cIDUA-WPRE vector, Neonatal-LTR mice had complete correction of disease manifestations in bone, eye, ear, and heart. In this study, Adult-LTR, Neonatal-SIN, and Adult-SIN mice all achieved complete correction of the femur width and bone mineral density, suggesting that expression was sufficient to correct disease in bone.

In the eye, although all treated groups evaluated in this study had improvements in the number of photoreceptors in the outer nuclear layer and in the ERG a-wave amplitude, only the Adult-LTR had statistically significant improvements in the b-wave amplitude, and some parameters in mice that were treated with the SIN vector were significantly worse than in normal mice. The greater degree of improvements in the Adult-LTR mice as compared with the groups that received the SIN vector may reflect the somewhat higher levels of serum IDUA activity (245 U/ml) in the Adult-LTR mice than in the Neonatal-SIN (127 U/ml) or the Adult-SIN (71 U/ml) mice and the fact that we have already demonstrated that improvements in the eye are only partial when serum IDUA activity is 30–100 U/ml (refs. 7,15).

In the ear, all treated groups evaluated in this study had improvements in hearing, although these were only partial. Histopathological evaluation of the ear demonstrated that all Adult-LTR, Neonatal-SIN, and Adult-LTR mice had a middle ear exudate, and that cells within the exudate had evidence of lysosomal storage (data not shown). This is consistent with the hypothesis that enzyme does not diffuse effectively from blood into the middle ear, and the fact that expression of IDUA in white blood cells was not detected for the Neonatal-SIN and the Adult-SIN groups (**Figure 7**), and was very low (0.004% of β-actin) for Adult-LTR mice in our previous study.[15] In contrast, white blood cells from Neonatal-LTR mice had 0.2% as much RV RNA as β-actin, which is 50-fold higher than for the Adult-LTR mice. We hypothesize that the loss of expression in white blood cells contributed to the failure to correct the middle ear exudate, which was likely responsible for the partial reduction in hearing.

The aorta has been one of the most difficult sites to correct with gene therapy in our experience. In this study, ascending aortas from the Adult-LTR mice with 235 U/ml of serum IDUA activity were almost completely corrected to a normal diameter, while in our previous study, aortas from Adult-LTR mice that received a lower dose of vector and achieved 84 U/ml had little or no correction in aortic diameters.[15] The partial improvement in aortic diameters seen in Neonatal-SIN mice with 127 U/ml of serum IDUA activity and the lack of improvement seen in Adult-SIN mice with 71 U/ml is not surprising, and reflects the fact that aorta is a difficult organ to treat.

A parameter of aortic function that was evaluated for the first time here was aortic strain, which is the difference in the diameter of the aorta during systole and diastole divided by the aortic diameter during diastole. MPS I mice had a marked reduction in aortic strain, which likely reflects the fragmentation of elastin in the aorta and subsequent loss of elasticity.[8] Aortic strain was partially and significantly improved for the Adult-LTR and the Neonatal-SIN mice, which reflects a reduction, but not complete prevention, in the number of fragmented elastin fibers seen with histopathology (data not shown). Aortic strain was at best minimally improved for the Adult-SIN mice, which is consistent with the increased number of fragmented elastin fibers as compared with the other treated groups, and with the degree of aortic dilatation. Loss of elasticity of the aorta likely contributes to hemodynamic abnormalities and development of heart failure in MPS I mice. All treated MPS I mice had significant reductions in GUSB activity, GAG levels, and cathepsin S levels in aorta as compared with untreated MPS I mice (**Figure 3**).

Comparison of the LTR-intact and the SIN vector

The key question for this gene therapy approach is to assess the relative efficacy of the SIN vector as compared with the LTR-intact vector, and to determine why differences exist. For MPS I mice that were treated as adults, administration of the SIN vector in this study had a similar effect as was seen in mice that received the same dose of the LTR-intact vector in our previous study,[15] and was slightly inferior to the effect of a higher dose (1.7-fold) of the LTR-intact vector in this study. For the adult-treated groups evaluated in this study, the transduction efficiency and the expression in liver were similar, which is consistent with the hypothesis that the SIN vector is almost as effective as the LTR-intact vector after transfer to adult mice.

For mice that were treated as newborns, the LTR-intact vector evaluated in a previous study[7] was clearly superior to the SIN vector in terms of its ability to correct disease in difficult-to-treat sites such as the eye, ear, and aorta in MPS I mice. To investigate why the LTR-intact vector was better, levels of DNA and RNA from these previously treated mice were compared with levels in the mice treated in this study in the same real-time PCR assays. This demonstrated that the Neonatal-LTR mice had 11-fold as many copies of the γ-RV as the Neonatal-SIN mice, and 35-fold as much RNA. This is consistent with the fact that the Neonatal-LTR mice had liver IDUA activity of 290 U/mg (ninefold that in Neonatal-SIN mice) and serum IDUA activity was 1,240 U/ml (tenfold that in Neonatal-SIN mice). This suggests that the major difference was that the Neonatal-LTR mice had a higher transduction efficiency in liver, although slightly higher expression from the LTR-intact vector may have also played a role. This difference in transduction efficiency despite administration of the same dose in TU/kg could reflect inaccuracies in titer (done at different times), or a reduced

© The American Society of Gene & Cell Therapy

efficiency of temporal vein injection in the more recent study (done by different people in the two studies). It is also possible that the lack of expression in hematopoietic cells in the Neonatal-SIN mice contributed to a reduced therapeutic effect in some sites such as the ear. Further studies will attempt to obtain the SIN-hAAT-cIDUA-oPRE vector at a higher titer, and test whether a higher dose of vector can result in more complete correction. The efficacy of the SIN-hAAT-cIDUA-oPRE vector in treating the brain is being investigated and will be reported later.

Implications for MPS I gene therapy

The availability of a safe and effective gene therapy treatment would be a major step forward for MPS I. This report demonstrates that a SIN γ-RV expressing IDUA from a liver-specific promoter results in stable expression of IDUA in serum after transfer into adult MPS I mice, and amelioration or prevention of many clinically relevant parameters to a similar extent as an LTR-intact vector after transfer to adults. Although the SIN vector was not as effective as an LTR-intact vector after transfer into newborns, this difference likely relates to a lower transduction efficiency with the SIN vector. A greater therapeutic effect should be achieved with administration of a higher dose of vector, a hypothesis that is currently being tested. The safety advantage of a SIN vector will be important if an *in vivo* gene therapy approach is to translate into a therapy for humans. No tumors were observed in animals in this study and we did not observe an increased risk in liver or other tumors in mice at 1.8 years after neonatal i.v. injection of an LTR-intact γ-RV.[26] However, one MPS VII dog that received neonatal gene therapy with an LTR-intact vector expressing GUSB developed an increase in serum GUSB activity at 7.5 years, and likely had a splenic neoplasm with a γ-RV integration that is currently being characterized (K. Ponder and M. Haskins, unpublished results), so the rationale for proceeding with development of a safe and effective SIN vector exists. It is hoped that a SIN vector will eventually be used to treat MPS I in humans, although additional studies on efficacy and safety will be required.

MATERIALS AND METHODS

All reagents were purchased from Sigma-Aldrich Chemical (St Louis, MO) unless otherwise stated.

Vector construction. The γ-RV designated hAAT-cIDUA-WPRE #835 was described previously.[7,15] SIN-hAAT-cIDUA-oPRE #925 was generated as follows: pSERS11-EFS-GFP-oPRE is a SIN γ-RV with the backbone of pSERS11 (ref. 20), the EF1α promoter upstream of GFP, and the oPRE;[21] the EFS-GFP sequence was removed with *Not*I digestion, the ends were blunted, and a *Sal*I/*Xba*I phosphorylated linker (Operon, Huntsville, AL) was ligated in to generate clone #924. The hAAT-cIDUA sequence containing the canine IDUA complementary DNA[27] was removed from hAAT-cIDUA-WPRE #835 using *Bgl*II and *Xba*I, and cloned into the *Sal*I- and *Xba*I-digested clone #924 to form SIN-hAAT-cIDUA-oPRE #925.

This plasmid was transfected into the amphotropic GP+AM12 packaging cells with PGK-neo, and G418-resistant colonies were screened for the ability to transfect the human liver hepatoma cells HepG2 and result in secretion of IDUA activity into the media. The conditioned medium from the best clone was concentrated by ultrafiltration, purified over a size exclusion column, and frozen as previously described.[7] Titer was determined by transduction of NIH 3T3 cells and assessing RV DNA copy number after 1 week (ref. 7), and the vector was shown to be replication-incompetent with a marker-rescue assay.

MPS I Mice. All animal studies were approved by the authors' institutional review board and MPS I mice on a C57BL/6 background[28] were used. Some MPS I mice were injected i.v. via the temporal vein with 1×10^{10} TU/kg of SIN-hAAT-cIDUA-oPRE in 100 µl at 2–3 days after birth (Neonatal-SIN). Other MPS I mice were injected via the tail vein with SIN-hAAT-cIDUA-oPRE at 6 weeks of age (Adult-SIN); these mice received intraperitoneal injections of 5 mg/kg hepatocyte growth factor with 15 mg/kg dextran sulfate per dose at 0, 3, 6, 9, and 12 hours after initiation of the transduction protocol to induce hepatocyte replication, and then received 0.2×10^{10} TU/kg of RV per dose via the tail vein at 30, 36, 40, 44, and 48 hours after initiation of transduction, for a cumulative dose of 1×10^{10} TU/kg. Adult-SIN mice did not receive immunosuppression. Other MPS I mice were injected via the tail vein with the LTR-intact vector hAAT-cIDUA-WPRE at 6 weeks of age (Adult-LTR) as described above except that the dose was higher at 0.33×10^{10} TU/kg per dose, for a cumulative dose of 1.7×10^{10} TU/kg. Adult-LTR mice also received transient immunosuppression with 25 mg/kg per dose of human CTLA4-Ig given 6 hours before the first dose of hAAT-cIDUA-WPRE and then again at 4, 7, and 11 days, and 25 mg/kg per dose of αCD40 ligand at day 1 and day 3 after the first dose of RV. Finally, comparisons were made between mice evaluated in this study with MPS I mice that received neonatal i.v. injection of the LTR-intact vector hAAT-cIDUA-WPRE (Neonatal-LTR); the dose stated here is higher than stated previously due to an error in the previous titer calculations. Additional MPS I animals were left untreated (MPS I), while heterozygous litter mates (Normal) were maintained as controls. ERG,[29,30] auditory-evoked brainstem responses,[31] and echocardiograms with a Vevo 770 echocardiography machine (VisualSonics, Toronto, Ontario, Canada) were performed as previously described.[16] At 8 months of age, mice were anesthetized with 80 mg/kg ketamine and 15 mg/kg xylazine given intraperitoneally and perfused transcardially with either 20 ml of phosphate-buffered saline (PBS) (when designated for biochemistry), or with 20 ml of 4% paraformaldehyde and 2% glutaraldehyde in PBS (when designated for brain pathology). Bone radiographs were taken using an MX-20 Specimen Radiography System (Faxitron, Lincolnshire, IL) at 20 kV for 15 seconds. Bone mineral density scans were performed using a PixiMus dual energy X-ray absorption machine (Lunar, Madison, WI).

Biochemical analyses and histology. Approximately 75 mg of tissue from PBS-perfused mice was homogenized in 300 µl of homogenization buffer containing 100 mmol/l sodium acetate, 150 mmol/l sodium chloride, 0.2% Triton X-100, and 0.02% sodium azide at pH 5.5, using a Kontes Pellet Pestle Motor (Kimble Chase, Vineland, NJ), and the same homogenate was used for enzyme and GAG assays. Protein concentration was determined using the Bradford assay (BioRad Laboratories, Hercules, CA), while IDUA[15] and GUSB[32] assays were performed using the fluorescent substrates 4-methylumbelliferyl-α-L-iduronide (Calbiochem, San Diego, CA) for IDUA and 4-methylumbelliferyl-β-L-glucuronide (Calbiochem) for GUSB, using a Fluoroskan Ascent microplate fluorometer (Thermo Electron, Milford, MA) as previously described. One unit of enzyme activity equals 1 nmol of product produced per hour at 37 °C. GAG levels were determined using a Sulfated Glycosaminoglycan Kit (Blyscan, Newtownabbey, Northern Ireland).[33] The amount of M6P-modified IDUA was determined using an M6P receptor column and elution with M6P as previously described.[16] Cathepsin S assays were performed with the substrate Z-Phe-Arg-AMC at 100 µmol/l in a buffer with 100 mmol/l NaAcetate at pH 7.5 as detailed previously.[8] Eyes and aortas from both PBS-perfused and fixative-perfused mice were fixed with PBS with 4% paraformaldehyde and 2% glutaraldehyde and paraffin-embedded sections were stained with hematoxylin and eosin.

HCII–IIa complexes. HCII–IIa complexes were examined with an immunoblot. The diluted equivalent of 0.5 µl of serum was run on a 7.5% polyacrylamide separating gel containing 0.375 mol/l Tris–HCl, pH 8.8, 0.1% sodium dodecyl sulfate, 0.05% ammonium persulfate, and 0.05% tetramethylethylenediamine, and a 4% polyacrylamide stacking gel containing 0.125 mol/l

© The American Society of Gene & Cell Therapy

Tris–HCl, pH 6.8, 0.1% sodium dodecyl sulfate, 0.05% ammonium persulfate, and 0.05% tetramethylethylenediamine, transferred to a nitrocellulose membrane, blocked with 5% milk, then incubated with goat antihuman HCII (Affinity Biologicals, Hamilton, Ontario, Canada) at 3 μg/ml for 1 hour at room temperature. The membrane was rinsed with water, then 5% milk, followed by incubation with horseraddish peroxidase-anti-goat IgG (Sigma-Aldrich) at 1:5,000 dilution for 1 hour at room temperature and development.

Nucleic acid analysis. RNA was isolated using an RNeasy Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions, and samples were treated with DNase I. Reverse transcription was performed using a Superscript III First Strand Reverse Transcription Kit (Invitrogen, Carlsbad, CA) with 1 μg of RNA and reverse mouse β-actin and reverse cIDUA primers in a volume of 20 μl according to the manufacturer's instructions. DNA was isolated in guanidinium as described.[7] Real-time PCR was performed using primers and Taqman probes for canine IDUA and mouse β-actin as previously described,[16] with either 2 μl of complementary DNA (10% of the reverse transcriptase reaction) or 100 ng of genomic DNA. For DNA analysis, standards were dilutions of a cIDUA-containing plasmid in mouse genomic DNA.

Statistics. All statistics were performed using Sigma Stat software (Systat Software, Point Richmond, CA). Statistical significance was analyzed using one-way analysis of variance with Tukey's post hoc analysis. Errors are reported as mean ± SEM.

SUPPLEMENTARY MATERIAL

Figure S1. Diagram of aorta.

ACKNOWLEDGMENTS

We thank Monica Bessler and Phillip Mason for the use of their real-time PCR machine, Clay Semenkovich and Trey Coleman for use of their BMD machine, and Matthew Silva for use of his radiograph machine. Funding for this work was provided by the National Institutes of Health (NIH DK066448). Histology was supported by NIH P30 DK52574. BMD equipment was supported by the Clinical Nutrition Research Unit (NIH DK56341) and the Diabetes Research Training Core (NIH DK20579). ERG and ABR equipment were supported by the NIH (NIDCD P30 DC004665; PI R. Chole and NINDS P30 NS057105; PI D. Holtzman). Echocardiography equipment was supported by the Mouse Cardiovascular Phenotyping Core Facility at the Center for Cardiovascular Research at Washington University.

REFERENCES

1. Baehner, F, Schmiedeskamp, C, Krummenauer, F, Miebach, E, Bajbouj, M, Whybra, C *et al.* (2005). Cumulative incidence rates of the mucopolysaccharidoses in Germany. *J Inherit Metab Dis* **28**: 1011–1017.
2. Neufeld, EF and Muenzer, J (2001). The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *Metabolic and Molecular Basis of Inherited Disease*. McGraw Hill: New York, pp. 3421–3452.
3. Boelens, JJ, Rocha, V, Aldenhoven, M, Wynn, R, O'Meara, A, Michel, G *et al.*; EUROCORD, Inborn error Working Party of EBMT and Duke University (2009). Risk factor analysis of outcomes after unrelated cord blood transplantation in patients with hurler syndrome. *Biol Blood Marrow Transplant* **15**: 618–625.
4. Clarke, LA, Wraith, JE, Beck, M, Kolodny, EH, Pastores, GM, Muenzer, J *et al.* (2009). Long-term efficacy and safety of laronidase in the treatment of mucopolysaccharidosis I. *Pediatrics* **123**: 229–240.
5. Sifuentes, M, Doroshow, R, Hoft, R, Mason, G, Walot, I, Diament, M *et al.* (2007). A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. *Mol Genet Metab* **90**: 171–180.
6. Ponder, KP and Haskins, ME (2007). Gene therapy for mucopolysaccharidosis. *Expert Opin Biol Ther* **7**: 1333–1345.
7. Liu, Y, Xu, L, Hennig, AK, Kovacs, A, Fu, A, Chung, S *et al.* (2005). Liver-directed neonatal gene therapy prevents cardiac, bone, ear, and eye disease in mucopolysaccharidosis I mice. *Mol Ther* **11**: 35–47.
8. Ma, X, Tittiger, M, Knutsen, RH, Kovacs, A, Schaller, L, Mecham, RP *et al.* (2008). Upregulation of elastase proteins results in aortic dilatation in mucopolysaccharidosis I mice. *Mol Genet Metab* **94**: 298–304.
9. Kobayashi, H, Carbonaro, D, Pepper, K, Petersen, D, Ge, S, Jackson, H *et al.* (2005). Neonatal gene therapy of MPS I mice by intravenous injection of a lentiviral vector. *Mol Ther* **11**: 776–789.
10. Hartung, SD, Frandsen, JL, Pan, D, Koniar, BL, Graupman, P, Gunther, R *et al.* (2004). Correction of metabolic, craniofacial, and neurologic abnormalities in MPS I mice treated at birth with adeno-associated virus vector transducing the human α-L-iduronidase gene. *Mol Ther* **9**: 866–875.
11. Ponder, KP, Wang, B, Wang, P, Ma, X, Herati, R, Wang, B *et al.* (2006). Mucopolysaccharidosis I cats mount a cytotoxic T lymphocyte response after neonatal gene therapy that can be blocked with CTLA4-Ig. *Mol Ther* **14**: 5–13.
12. Traas, AM, Wang, P, Ma, X, Tittiger, M, Schaller, L, O'donnell, P *et al.* (2007). Correction of clinical manifestations of canine mucopolysaccharidosis I with neonatal retroviral vector gene therapy. *Mol Ther* **15**: 1423–1431.
13. Herati, RS, Knox, VW, O'Donnell, P, D'Angelo, M, Haskins, ME and Ponder, KP (2008). Radiographic evaluation of bones and joints in mucopolysaccharidosis I and VII dogs after neonatal gene therapy. *Mol Genet Metab* **95**: 142–151.
14. Di Domenico, C, Di Napoli, D, Gonzalez Y Reyero, E, Lombardo, A, Naldini, L and Di Natale, P (2006). Limited transgene immune response and long-term expression of human α-L-iduronidase in young adult mice with mucopolysaccharidosis type I by liver-directed gene therapy. *Hum Gene Ther* **17**: 1112–1121.
15. Ma, X, Liu, Y, Tittiger, M, Hennig, A, Kovacs, A, Popelka, S *et al.* (2007). Improvements in mucopolysaccharidosis I mice after adult retroviral vector-mediated gene therapy with immunomodulation. *Mol Ther* **15**: 889–902.
16. Herati, RS, Ma, X, Tittiger, M, Ohlemiller, KK, Kovacs, A and Ponder, KP (2008). Improved retroviral vector design results in sustained expression after adult gene therapy in mucopolysaccharidosis I mice. *J Gene Med* **10**: 972–982.
17. Aronovich, EL, Bell, JB, Khan, SA, Belur, LR, Gunther, R, Koniar, B *et al.* (2009). Systemic correction of storage disease in MPS I NOD/SCID mice using the sleeping beauty transposon system. *Mol Ther* **17**: 1136–1144.
18. Nair, V (2008). Retrovirus-induced oncogenesis and safety of retroviral vectors. *Curr Opin Mol Ther* **10**: 431–438.
19. Kraunus, J, Schaumann, DH, Meyer, J, Modlich, U, Fehse, B, Brandenburg, G *et al.* (2004). Self-inactivating retroviral vectors with improved RNA processing. *Gene Ther* **11**: 1568–1578.
20. Schambach, A, Mueller, D, Galla, M, Verstegen, MM, Wagemaker, G, Loew, R *et al.* (2006). Overcoming promoter competition in packaging cells improves production of self-inactivating retroviral vectors. *Gene Ther* **13**: 1524–1533.
21. Schambach, A, Bohne, J, Baum, C, Hermann, FG, Egerer, L, von Laer, D *et al.* (2006). Woodchuck hepatitis virus post-transcriptional regulatory element deleted from X protein and promoter sequences enhances retroviral vector titer and expression. *Gene Ther* **13**: 641–645.
22. Zychlinski, D, Schambach, A, Modlich, U, Maetzig, T, Meyer, J, Grassman, E *et al.* (2008). Physiological promoters reduce the genotoxic risk of integrating gene vectors. *Mol Ther* **16**: 718–725.
23. Maruggi, G, Porcellini, S, Facchini, G, Perna, SK, Cattoglio, C, Sartori, D *et al.* (2009). Transcriptional enhancers induce insertional gene deregulation independently from the vector type and design. *Mol Ther* **17**: 851–856.
24. Randall, DR, Colobong, KE, Hemmelgarn, H, Sinclair, GB, Hetty, E, Thomas, A *et al.* (2008). Heparin cofactor II-thrombin complex: a biomarker of MPS disease. *Mol Genet Metab* **94**: 456–461.
25. Nemes, A, Timmermans, RG, Wilson, JH, Soliman, OI, Krenning, BJ, ten Cate, FJ *et al.* (2008). The mild form of mucopolysaccharidosis type I (Scheie syndrome) is associated with increased ascending aortic stiffness. *Heart Vessels* **23**: 108–111.
26. Tittiger, ME, Ma, X, Xu, L and Ponder K (2008). Neonatal intravenous injection of a γ-retroviral vector has a low incidence of tumor induction in mice. *Hum Gene Ther* **11**: 1217–1323.
27. Stoltzfus, LJ, Sosa-Pineda, B, Moskowitz, SM, Menon, KP, Dlott, B, Hooper, L *et al.* (1992). Cloning and characterization of cDNA encoding canine α-L-iduronidase. mRNA deficiency in mucopolysaccharidosis I dog. *J Biol Chem* **267**: 6570–6575.
28. Ohmi, K, Greenberg, DS, Rajavel, KS, Ryazantsev, S, Li, HH and Neufeld, EF (2003). Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB. *Proc Natl Acad Sci USA* **100**: 1902–1907.
29. Ohlemiller, KK, Vogler, CA, Roberts, M, Galvin, N and Sands, MS (2000). Retinal function is improved in a murine model of a lysosomal storage disease following bone marrow transplantation. *Exp Eye Res* **71**: 469–481.
30. Hennig, AK, Ogilvie, JM, Ohlemiller, KK, Timmers, AM, Hauswirth, WW and Sands, MS (2004). AAV-mediated intravitreal gene therapy reduces lysosomal storage in the retinal pigmented epithelium and improves retinal function in adult MPS VII mice. *Mol Ther* **10**: 106–116.
31. Ohlemiller, KK, Hennig, AK, Lett, JM, Heidbreder, AF and Sands, MS (2002). Inner ear pathology in the mucopolysaccharidosis VII mouse. *Hear Res* **169**: 69–84.
32. Gao, C, Sands, MS, Haskins, ME and Ponder, KP (2000). Delivery of a retroviral vector expressing human β-glucuronidase to the liver and spleen decreases lysosomal storage in mucopolysaccharidosis VII mice. *Mol Ther* **2**: 233–244.
33. Wang, B, O'Malley, TM, Xu, L, Vite, C, Wang, P, O'Donnell, PA *et al.* (2006). Expression in blood cells may contribute to biochemical and pathological improvements after neonatal intravenous gene therapy for mucopolysaccharidosis VII in dogs. *Mol Genet Metab* **87**: 8–21.