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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
23 August 2018 (23.08.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/152485 A1

(72) Inventors: WILSON, James M.; 1831 Delancey Street,
Philadelphia, PA 19103 (US). RADER, Daniel J.; 333 S.
Smedley St., Philadelphia, PA 19103 (US).

(54) Title: GENE THERAPY FOR TREATING FAMILIAL HYPERCHOLESTEROLEMIA

(57) Abstract: Regimens useful in reducing the frequency of apheresis in a human patient having familial hypercholesterolemia are
described. The method involves administering to the human subject via a peripheral vein by infusion of a suspension of replication
deficient recombinant adeno-associated virus (rAAV).

WO 2018/152485 A1

GENE THERAPY FOR TREATING FAMILIAL HYPERCHOLESTEROLEMIA

5   1. INTRODUCTION

The invention relates to a gene therapy for treating Familial Hypercholesterolemia (FH), and in particular, Homozygous FH (HoFH).

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED IN
10   ELECTRONIC FORM

Applicant hereby incorporates by reference the Sequence Listing material filed in electronic form herewith. This file is labeled "16-7717C1PCT_ST25.txt".

15   2. BACKGROUND OF THE INVENTION

Familial hypercholesterolemia (FH) is a life threatening disorder caused by mutations in genes that affect LDL receptor (LDLR) function (Goldstein et al. Familial hypercholesterolemia, in The Metabolic and Molecular Bases of Inherited Disease, C.R. Scriver, et al., Editors. 2001, McGraw-Hill Information Services Company: New York. p.
20   2863-2913 (2001)). It is estimated that >90% of patients with molecularly confirmed FH carry mutations in the gene encoding for the LDLR (LDLR, MIM 606945). The remainder of the patients carry mutations on three additional genes: APOB (MIM 107730) encoding apolipoprotein (apo) B, PCSK9 (MIM 607786) encoding proprotein convertase subtilisin/kexin type 9 (PCSK9), and LDLRAP1 (MIM 695747) encoding
25   LDLR adapter protein 1. The latter is the only gene mutation that is associated with a recessive trait. Homozygosis is usually conferred by the presence of mutations in the 2 alleles of the same gene; however cases have been reported of patients with double heterozygosis (two heterozygous mutations, one each in two different genes). Based on prevalence rates of between 1 in 500 and 1 in 200 for heterozygous FH (Nordestgaard et
30   al. Eur Heart J, 2013. 34(45): p. 3478-90a (2013), Sjouke et al. Eur Heart J, (2014)), it is estimated that between 7,000 and 43,000 people worldwide have homozygous FH (HoFH).

Characterization of mutant LDLR alleles has revealed a variety of mutations including deletions, insertions, missense mutations, and nonsense mutations (Goldstein et al. 2001). More than 1700 LDLR mutations have been reported. This genotypic heterogeneity leads to variable consequences in the biochemical function of the receptor

5   which are classified in four general groups. Class 1 mutations are associated with no detectable protein and are often caused by gene deletions. Class 2 mutations lead to abnormalities in intracellular processing of the protein. Class 3 mutations specifically affect binding the ligand LDL, and Class 4 mutations encode receptor proteins that do not cluster in coated pits. Based on residual LDLR activity assessed using patients

10   cultured fibroblasts, mutations are also classified as receptor negative (<2% residual activity of the LDLR) or receptor-defective (2-25% residual activity). Patients that are receptor-defective have, on average, lower LDL-C levels and a less malignant cardiovascular course.

As a consequence of impaired LDL receptor function, untreated total plasma

15   cholesterol levels in patients with HoFH are typically greater than 500 mg/dl, resulting in premature and aggressive atherosclerosis often leading to cardiovascular disease (CVD) before age 20 and death before age 30 (Cuchel et al. Eur Heart J, 2014. 35(32): p. 2146-2157 (2014), Goldstein et al. 2001). Early initiation of aggressive treatment for these patients is therefore essential (Kolansky et al. 2008). The available options are limited.

20   Statins are considered the first line for pharmacological treatment. Even at maximal doses, only a 10-25% reduction in LDL-C plasma levels is observed in most patients (Marais et al. Atherosclerosis, 2008. 197(1): p. 400-6 (2008); Raal et al. Atherosclerosis, 2000. 150(2): p. 421-8 (2000)). The addition of the cholesterol absorption inhibitor, ezetimibe, to statin therapy may result in a further 10-20% reduction in LDL-C levels

25   (Gagne et al. Circulation, 2002. 105 (21): p. 2469-2475 (2002)). Use of other cholesterol lowering medications, including bile acid sequestrants, niacin, fibrates, and probucol have been used successfully in the pre-statin era and can be considered to achieve further LDL-C reduction in HoFH; however, their use is limited by tolerability and drug availability. This approach has been shown to reduce CVD and all-cause mortality (Raal

30   et al. Circulation, 2011. 124(20): p. 2202-7). Despite the implementation of an aggressive multi-drug therapy approach, the LDL-C levels of HoFH patients remain

elevated and their mean life expectancy remains approximately 32 years (Raal et al.
2011). Several non-pharmacological options have also been tested over the years.
Surgical interventions, such as portacaval shunting (Bilheimer Arteriosclerosis, 1989.
9(1 Suppl): p. I158-I163 (1989); Forman et al. Atherosclerosis, 1982. 41(2-3): p. 349-
5   361 (1982)) and ileal bypass (Deckelbaum et al. N. Engl. J. Med. 1977;296:465–470
1977. 296(9): p. 465-470 (1977)), have resulted only in partial and transient LDL-C
lowering and are now considered nonviable approaches. Orthotopic liver transplantation
has been demonstrated to substantially reduce LDL-C levels in HoFH patients (Ibrahim
et al. J Cardiovasc Transl Res, 2012. 5(3): p. 351-8 (2012); Kucukkartallar et al. 2
10   Pediatr Transplant, 2011. 15(3): p. 281-4 (2011)), but disadvantages and risks limit the
use of this approach, including the high risk of post-transplantation surgical
complications and mortality, the scarcity of donors, and the need for life-long treatment
with immunosuppressive therapy (Malatack Pediatr Transplant, 2011. 15(2): p. 123-5
(2011); Starzl et al. Lancet, 1984. 1(8391): p. 1382-1383 (1984)). The current standard
15   of care in HoFH includes lipoprotein apheresis, a physical method of purging the plasma
of LDL-C which can transiently reduce LDL-C by more than 50% (Thompson
Atherosclerosis, 2003. 167(1): p. 1-13 (2003); Vella et al. Mayo Clin Proc, 2001. 76(10):
p. 1039-46 (2001)). Rapid re-accumulation of LDL-C in plasma after treatment sessions
(Eder and Rader Today's Therapeutic Trends, 1996. 14: p. 165-179 (1996)) necessitates
20   weekly or biweekly apheresis. Although this procedure may delay the onset of
atherosclerosis (Thompson et al. Lancet, 1995. 345: p. 811-816; Vella et al. Mayo Clin
Proc, 2001. 76(10): p. 1039-46 (2001)), it is laborious, expensive, and not readily
available. Furthermore, although it is a procedure that is generally well tolerated, the
fact that it requires frequent repetition and intravenous access can be challenging for
25   many HoFH patients.

        Recently three new drugs have been approved by the FDA as add-on therapy
specifically for HoFH. Two of them, lomitapide and mipomersen, inhibit the assembly
and secretion of apoB-containing lipoproteins, although they do so via different
molecular mechanisms (Cuchel et al. N Engl J Med, 2007. 356(2): p. 148-156 (2007);,
30   Raal et al. Lancet, 2010. 375(9719): p. 998-1006 (2010)). This approach results in a
significant reduction of LDL-C that reaches an average of ~50% with lomitapide (Cuchel

et al. 2013) and ~25% with mipomersen (Rall et al. 2010). However their use is associated with an array of adverse events that may affect tolerance and long term adherence and that include liver fat accumulation, the long term consequences of which have not yet been fully clarified.

The third is part of a novel class of lipid-lowering drugs, monoclonal antibodies against proprotein convertase subtilisin/kexin 9 (PCSK9) that have been shown to be effective in lowering LDL-C levels with an apparently favorable safety profile in patients with heterozygous FH (Raal et al. Circulation, 2012. 126(20): p. 2408-17 (2012), Raal et al. The Lancet, 2015. 385(9965): p. 341-350 (2015); Stein et al. Circulation, 2013. 128(19): p. 2113-20 (2012)). Treatment of HoFH with the PCSK9 inhibitor evolocumab 420 mg every 4 weeks for 12 weeks has been shown to provide about a 30% reduction in LDL-C as compared with placebo (Raal et al. 2015). Efficacy of PCSK9 inhibitors is, however, dependent on the residual LDLR activity, with no effect in patients with no residual LDLR activity (Raal et al. 2015, Stein et al. Circulation, 2013. 128(19): p. 2113-20 (2013)). Although the addition of PCSK9 inhibitors may become standard of care for FH and may provide an additional further reduction to lower hypercholesterolemia in a sub-set of HoFH patients, they will not dramatically impact the clinical management of this condition.

Therefore, there remains an unmet medical need for new medical therapies for HoFH.

3. SUMMARY OF THE INVENTION

This invention relates to the use of a replication deficient adeno-associated virus (AAV) to deliver a human Low Density Lipoprotein Receptor (hLDLR) gene to liver cells of patients (human subjects) diagnosed with HoFH. The recombinant AAV vector (rAAV) used for delivering the LDLR gene ("rAAV.hLDLR") should have a tropism for the liver (*e.g.*, a rAAV bearing an AAV8 capsid), and the hLDLR transgene should be controlled by liver-specific expression control elements. Such rAAV.hLDLR vectors can be administered by intravenous (IV) infusion over a 20 to 30-minute period to achieve therapeutic levels of LDLR expression in the liver. Therapeutically effective doses of the rAAV.hLDLR range from 2.5×10^{12} to 7.5×10^{12} genome copies (GC)/kg body weight of the patient. In a preferred embodiment, the rAAV suspension has a

potency such that a dose of 5×10^{11} GC/kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%.

5   The goal of the treatment is to functionally replace the patient's defective LDLR via rAAV-based liver-directed gene therapy as a viable approach to treat this disease and improve response to current lipid-lowering treatments. The invention is based, in part, on the development of therapeutic compositions and methods that allow for the safe delivery of efficacious doses; and improved manufacturing methods to meet the purification production requirement for efficacious dosing in human subjects.

10   Efficacy of the therapy may be assessed after treatment, *e.g.*, post-dosing, using plasma LDL-C levels as a surrogate biomarker for human LDLR transgene expression in the patient. For example, a decrease in the patient's plasma LDL-C levels after the gene therapy treatment would indicate the successful transduction of functional LDLRs. Additionally, or alternatively, other parameters that can be monitored include, but are not

15   limited to measuring changes in total cholesterol (TC), non-high density lipoprotein cholesterol (non-HDL-C), HDL-C, fasting triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), lipoprotein(a) (Lp(a)), apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I) compared to baseline, as well as LDL kinetic studies (metabolic mechanism assessment) prior to vector and after vector administration, or

20   combinations thereof.

In certain embodiments, efficacy of therapy may be measured by a reduction in the frequency of apheresis required by the patient. In certain embodiments, post-AAV8.hLDLR treatment, a patient may have his or her requirement for apheresis reduced by 25%, 50%, or more. For example, a patient receiving weekly apheresis prior

25   to AAV8.hLDLR therapy may only require biweekly or monthly apheresis; in other embodiments, apheresis may be required even less frequently, or the need may be eliminated.

In certain embodiments, efficacy of therapy may be measured by a reduction in the dose of PCSK9 inhibitor required, or by an elimination of the need for such therapy

30   in a patient post-AAV8.hLDLR treatment. In certain embodiments, efficacy of therapy is measured by a reduction in the dose of a statin or bile sequestrant required.

In certain embodiments, an immunosuppressant co-therapy is used. Such immune suppressant co-therapy may be started prior to delivery of the AAV8.hLDLR, e.g., if undesirably high neutralizing antibody levels to AAV8 are detected. In certain embodiments, co-therapy may also be started prior to delivery of the AAV8.hLDLR as a precautionary measure. In certain embodiments, immunosuppressive co-therapy is started following delivery of the AAV8.hLDLR, e.g., if an undesirable immune response is observed following treatment.

Immunosuppressants for such co-therapy include, but are not limited to, a glucocorticoid, steroids, antimetabolites, T-cell inhibitors, a macrolide (e.g., a rapamycin or rapalog), and cytostatic agents including an alkylating agent, an anti-metabolite, a cytotoxic antibiotic, an antibody, or an agent active on immunophilin. The immune suppressant may include a nitrogen mustard, nitrosourea, platinum compound, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, an anthracycline, mitomycin C, bleomycin, mithramycin, IL-2 receptor- (CD25-) or CD3-directed antibodies, anti-IL-2 antibodies, ciclosporin, tacrolimus, sirolimus, IFN-β, IFN-γ, an opioid, or TNF-α (tumor necrosis factor-alpha) binding agent. In certain embodiments, the immunosuppressive therapy may be started 0, 1, 2, 7, or more days prior to the gene therapy administration, or 0, 1, 2, 3, 7, or more days post-gene therapy administration. Such therapy may involve co-administration of two or more drugs, the (e.g., prednelisone, micophenolate mofetil (MMF) and/or sirolimus (i.e., rapamycin)) on the same day. One or more of these drugs may be continued after gene therapy administration, at the same dose or an adjusted dose. Such therapy may be for about 1 week (7 days), about 60 days, or longer, as needed. In certain embodiments, a tacrolimus-free regimen is selected.

Patients who are candidates for treatment are preferably adults (male or female ≥18 years of age) diagnosed with HoFH carrying two mutations in the *LDLR* gene; *i.e.*, patients that have molecularly defined LDLR mutations at both alleles in the setting of a clinical presentation consistent with HoFH, which can include untreated LDL-C levels, *e.g.*, LDL-C levels >300 mg/dl, treated LDL-C levels, *e.g.*, LDL-C levels <300 mg/dl and/or total plasma cholesterol levels greater than 500 mg/dl and premature and aggressive atherosclerosis. Candidates for treatment include HoFH patients that are

undergoing treatment with lipid-lowering drugs, such as statins, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL and/or plasma apheresis.

Prior to treatment, the HoFH patient should be assessed for neutralizing antibodies (NAb) to the AAV serotype used to deliver the hLDLR gene. Such NAbs can interfere with transduction efficiency and reduce therapeutic efficacy. HoFH patients that have a baseline serum NAb titer ≤ 1:10, are good candidates for treatment with the rAAV.hLDLR gene therapy protocol. However, patients with other baseline levels may be selected. Treatment of HoFH patients with titers of serum NAb >1:5 may require a combination therapy, such as transient co-treatment with an immunosuppressant before and/or during treatment with rAAV.hLDLR vector delivery. Additionally, or alternatively, patients are monitored for elevated liver enzymes, which may be treated with transient immunosuppressant therapy (e.g., if at least about 2x baseline levels of aspartate transaminase (AST) or alanine transaminase (ALT) are observed). Immunosuppressants for such co-therapy include, but are not limited to, steroids, antimetabolites, T-cell inhibitors, and alkylating agents.

The invention is illustrated by way of examples that describe a protocol for the AAV8.LDLR treatment of human subjects (Section 6, Example 1); pre-clinical animal data demonstrating efficacy of the treatment in animal models of disease (Section 7, Example 2); the manufacture and formulation of therapeutic AAV.hLDLR compositions (Sections 8.1 to 8.3, Example 3); and methods for characterization of the AAV vector (Section 8.4, Example 3).

3.1. DEFINITIONS

As used herein, "AAV8 capsid" refers to the AAV8 capsid having the encoded amino acid sequence of GenBank accession:YP_077180, which is incorporated by reference herein, and reproduced in SEQ ID NO: 5. Some variation from this encoded sequence is encompassed by the present invention, which may include sequences having about 99% identity to the referenced amino acid sequence in GenBank accession:YP_077180; US Patent 7,282,199, 7,790,449; 8,319,480; 8,962,330; US 8,962,332, (*i.e.*, less than about 1% variation from the referenced sequence). In another embodiment, the AAV8 capsid may have the VP1 sequence of the AAV8 variant described in WO2014/124282 or the dj sequence described in US 2013/0059732 A1 or

US7588772 B2, which are incorporated by reference herein., which are incorporated by reference herein. Methods of generating the capsid, coding sequences therefore, and methods for production of rAAV viral vectors have been described. *See, e.g.,* Gao, et al, Proc. Natl. Acad. Sci. U.S.A. 100 (10), 6081-6086 (2003), US 2013/0045186A1, and WO 2014/124282.

As used herein, the term "NAb titer" refers to a measurement of how much neutralizing antibody (e.g., anti-AAV NAb) is produced which neutralizes the physiologic effect of its targeted epitope (e.g., an AAV). Anti-AAV NAb titers may be measured as described in, *e.g.,* Calcedo, R., et al., Worldwide Epidemiology of Neutralizing Antibodies to Adeno-Associated Viruses. Journal of Infectious Diseases, 2009. 199(3): p. 381-390, which is incorporated by reference herein.

The terms "percent (%) identity", "sequence identity", "percent sequence identity", or "percent identical" in the context of amino acid sequences refers to the residues in the two sequences which are the same when aligned for correspondence. Percent identity may be readily determined for amino acid sequences over the full-length of a protein, polypeptide, about 32 amino acids, about 330 amino acids, or a peptide fragment thereof or the corresponding nucleic acid sequence coding sequencers. A suitable amino acid fragment may be at least about 8 amino acids in length, and may be up to about 700 amino acids. Generally, when referring to "identity", "homology", or "similarity" between two different sequences, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences. "Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Sequence alignment programs are available for amino acid sequences, *e.g.,* the "Clustal X", "MAP", "PIMA", "MSA", "BLOCKMAKER", "MEME", and "Match-Box" programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, *e.g.,* J. D. Thomson et al,

Nucl. Acids. Res., "A comprehensive comparison of multiple sequence alignments", 27(13):2682-2690 (1999).

As used herein, the term "operably linked" refers to 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.

A "replication-defective virus" or "viral vector" refers to a synthetic or artificial viral particle in which an expression cassette containing a gene of interest is packaged in a viral capsid or envelope, where any viral genomic sequences also packaged within the viral capsid or envelope are replication-deficient; *i.e.,* they cannot generate progeny virions but retain the ability to infect target cells. In one embodiment, the genome of the viral vector does not include genes encoding the enzymes required to replicate (the genome can be engineered to be "gutless" - containing only the transgene of interest flanked by the signals required for amplification and packaging of the artificial genome), but these genes may be supplied during production. Therefore, it is deemed safe for use in gene therapy since replication and infection by progeny virions cannot occur except in the presence of the viral enzyme required for replication.

It is to be noted that the term "a" or "an" refers to one or more. As such, the terms "a" (or "an"), "one or more," and "at least one" are used interchangeably herein.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

As used herein, the term "about" means a variability of 10% from the reference given, unless otherwise specified.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

4. BRIEF DESCRIPTION OF THE DRAWINGS

FIGs 1A - 1H. Impact of pre-existing AAV8 NAb on EGFP expression levels in macaque livers. Macaques of different types and ages were injected via a peripheral vein with 3×10^{12} GC/kg of AAV8.TBG.EGFP and were sacrificed 7 days later and analyzed for hepatocyte transduction in several ways. FIGS 1A - 1E are micrographs which show representative sections of liver from animals with various levels of pre-existing neutralizing antibodies to AAV8 (<1:5, 1:5, 1:10 and 1:20, respectively). FIG 1F shows a quantitative morphometric analysis of the transduction efficiency based on percent transduction of hepatocytes. FIG 1G shows quantitative morphometric analysis of the transduction efficiency based on relative EGFP intensity. FIG 1H shows quantification of EGFP protein in liver lysate by ELISA. Adult cynomolgus macaques (n = 8, closed circle), adult rhesus macaques (n = 8, open triangle), juvenile rhesus macaques (n = 5, open square).

FIG 2. Long-term expression of mLDLR in DKO mice. DKO mice were dosed with 10^{11} GC/mouse (5×10^{12} GC/kg) of AAV8.TBG.mLDLR (n = 10) or AAV8.TBG.nLacZ (n = 10). Cholesterol levels in serum were monitored on a regular basis. Statistically significant differences between the two groups were realized as early as day 7 (p <0.001) and have remained throughout the duration of the experiment. Mice were sacrificed at day 180 after vector administration.

FIGs 3A - 3F. Regression of atherosclerosis in DKO mice following AAV8.TBG.mLDLR. FIG 3A is a set of three panels with En face Sudan IV staining. Mouse aortas were pinned and stained with Sudan IV, which stains neutral lipids. Representative aortas from animals treated with 10^{11} GC/mouse of AAV8.nLacZ (5×10^{12} GC/kg) (middle), 10^{11} GC/mouse of AAV8.TBG.mLDLR (5×10^{12} GC/kg) (right) at day 60 after vector administration (day 120 on high fat diet), or at baseline (day 60 on high fat diet) (left) are shown. FIG 3B is a bar chart showing the results of morphometric analyses quantified the percent of aorta stained with Oil Red O along the entire length of the aorta. FIGS 3C-3K show the aortic roots from these mice were stained with Oil Red O. FIG 3L is bar chart showing the percent Sudan IV staining of the total aortic surface in baseline (n = 10), AAV.TBG.nLacZ (n = 9), and AAV8.TBG.mLDLR (n = 10) was determined. Quantification was conducted on Oil Red O lesions. Atherosclerotic lesion

area data were subjected to a 1-way ANOVA. Experimental groups were compared with the baseline group by using the Dunnett test. Repeated-measures ANOVA was used to compare cholesterol levels among different groups of mice over time after gene transfer. Statistical significance for all comparisons was assigned at P,0.05. Graphs represent

5   mean SD values. *p <0.05, **p <0.01, ‡ p <0.001.

FIG 4. Cholesterol levels in test or control article injected DKO mice. DKO mice were injected IV with 7.5×10^{11} GC/kg, 7.5×10^{12} GC/kg or 6.0×10^{13} GC/kg of AAV8.TBG.mLDLR or 6.0×10^{13} GC/kg of AAV8.TBG.hLDLR or vehicle control (100 µl PBS). Cholesterol levels expressed as mean ± SEM. Each group demonstrated a

10 statistically significant reduction in serum cholesterol relative to PBS controls from the same necropsy time point.

FIGs 5A - 5B. Cholesterol levels in test article injected DKO mice. FIG 5A shows cholesterol levels (mg/mL) in mice treated with varying doses of vector as measured on day 0, day 7 and day 30. Values expressed as mean ± SEM. $P<0.05$.

15   FIGs 6A - 6C. Peripheral T cell responses in vector injected rhesus macaques. Data presented show the time course of T cell response and AST levels for macaques 19498 (FIG 6A), 090-0287 (FIG 6B), and 090-0263 (FIG 6C). For each Study Day, T cell responses to no stimulation, AAV8 and hLDLR measured as spot-forming unit (SFU) per million PBMCs were plotted from left to right in each figure. Macaques 19498 and

20 090-0287 developed a positive peripheral T cell response to and/or the hLDLR transgene, whereas 090-0263 did not. * denotes positive capsid responses that were significantly above background.

FIG 7. Schematic representation of AAV8.TBG.hLDLR vector.

FIGs 8A - 8B. AAV cis plasmid constructs. A) Linear representation of the

25 paternal *cis* cloning plasmid, pENN.AAV.TBG.PI, containing the liver specific TBG promoter and chimeric intron flanked by AAV2 ITR elements. B) Linear representation of the human LDLR *cis* plasmid, pENN.AAV.TBG.PI.hLDLR.RBG.KanR, in which the human LDLR cDNA was cloned into pENN.AAV.TBG.PI between the intron and the poly A signal and the ampicillin resistance gene was replaced by the kanamycin

30 resistance gene.

FIGs 9A - 9B. AAV trans plasmids. FIG 9A is a Linear representation of the AAV8 *trans* packaging plasmid, p5E18-VD2/8, with the ampicillin resistance gene. FIG 9B is a linear representation of the AAV8 trans packaging plasmid, pAAV2/8 with the kanamycin resistance gene.

FIGs 10A - 10B. Adenovirus helper plasmid. FIG 10A illustrates derivation of the ad-helper plasmid, pAdΔF6, from the parental plasmid, pBHG10, through intermediates pAdΔF1 and pAdΔF5. FIG 10B is a linear representation of the ampicillin resistance gene in pAdΔF6 was replaced by the kanamycin resistance gene to create pAdΔF6(Kan).

FIGs 11A - 11B. Flow Diagram showing AAV8.TBG.hLDLR vector manufacturing process.

5.   DETAILED DESCRIPTION OF THE INVENTION

A replication deficient rAAV is used to deliver a hLDLR gene to liver cells of patients (human subjects) diagnosed with HoFH. The rAAV.hLDLR vector should have a tropism for the liver (*e.g.,* an rAAV bearing an AAV8 capsid) and the hLDLR transgene should be controlled by liver-specific expression control elements.

Such rAAV.hLDLR vectors can be administered by intravenous (IV) infusion over about a 20 to about 30 minute period to achieve therapeutic levels of LDLR expression in the liver. In other embodiments, shorter (e.g., 10 to 20 minutes) or longer (e.g., over 30 minutes to 60 minutes, intervening times, e.g., about 45 minutes, or longer) may be selected. Therapeutically effective doses of the rAAV.hLDLR range from at least about 2.5×10^{12} to 7.5×10^{12} genome copies (GC)/kg body weight of the patient. In a preferred embodiment, the rAAV suspension has a potency such that a dose of 5×10^{11} GC/kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%. Efficacy of treatment can be assessed using Low density lipoprotein cholesterol (LDL-C) levels as a surrogate for transgene expression. Primary efficacy assessments include LDL-C levels at 1 to 3 months (e.g., week 12) post treatment, with persistence of effect followed thereafter for at least about 1 year (about 52 weeks). Long term safety and persistence of transgene expression may be measured post-treatment.

In certain embodiments, efficacy of therapy may be measured by a reduction in the frequency of apheresis required by the patient. In certain embodiments, post-AAV8.hLDLR treatment, a patient may have his or her requirement for apheresis reduced by 25%, 50%, or more. For example, a patient receiving weekly apheresis prior to AAV8.hLDLR therapy may only require biweekly or monthly apheresis; in other embodiments, apheresis may be required even less frequently or the need may be eliminated.

In certain embodiments, efficacy of therapy may be measured by a reduction in the dose of PCSK9 inhibitor required, or by an elimination of the need for such therapy in a patient post-AAV8.hLDLR treatment. In certain embodiments, efficacy of therapy is measured by a reduction in the dose of a statin or bile sequestrant required.

Patients who are candidates for treatment are preferably adults (male or female ≥18 years of age) diagnosed with HoFH carrying two mutations in the *LDLR* gene; *i.e.*, patients that have molecularly defined LDLR mutations at both alleles in the setting of a clinical presentation consistent with HoFH, which can include untreated LDL-C levels, *e.g.*, LDL-C levels >300 mg/dl, treated LDL-C levels, *e.g.*, LDL-C levels <300 mg/dl and/or total plasma cholesterol levels greater than 500 mg/dl and premature and aggressive atherosclerosis. Candidates for treatment include HoFH patients that are undergoing treatment with lipid-lowering drugs, such as statins, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL and/or plasma apheresis.

Prior to treatment, the HoFH patient should be assessed for neutralizing antibodies (NAb) to the AAV serotype used to deliver the hLDLR gene. Such NAbs can interfere with transduction efficiency and reduce therapeutic efficacy. HoFH patients that have a baseline serum NAb titer ≤ 1:10 are good candidates for treatment with the rAAV.hLDLR gene therapy protocol. Treatment of HoFH patients with titers of serum NAb >1:5 may require a combination therapy, such as transient co-treatment with an immunosuppressant before/during treatment with rAAV.hLDLR. Additionally, or alternatively, patients are monitored for elevated liver enzymes, which may be treated with transient immunosuppressant therapy (*e.g.*, if at least about 2x baseline levels of aspartate transaminase (AST) or alanine transaminase (ALT) are observed).

In certain embodiments, an immunosuppressant co-therapy is used. Such immune suppressant co-therapy may be started prior to delivery of the AAV8.hLDLR, e.g., if undesirably high neutralizing antibody levels to AAV8 are detected. In certain embodiments, co-therapy may also be started prior to delivery of the AAV8.hLDLR as a

5   precautionary measure. In certain embodiments, immunosuppressive co-therapy is started following delivery of the AAV8.hLDLR, e.g., if an undesirable immune response is observed following treatment.

Immunosuppressants for such co-therapy include, but are not limited to, a glucocorticoid, steroids, antimetabolites, T-cell inhibitors, a macrolide (e.g., a rapamycin

10   or rapalog), and cytostatic agents including an alkylating agent, an anti-metabolite, a cytotoxic antibiotic, an antibody, or an agent active on immunophilin. The immune suppressant may include a nitrogen mustard, nitrosourea, platinum compound, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, an anthracycline, mitomycin C, bleomycin, mithramycin, IL-2 receptor- (CD25-) or CD3-directed

15   antibodies, anti-IL-2 antibodies, ciclosporin, tacrolimus, sirolimus, IFN-β, IFN-γ, an opioid, or TNF-α (tumor necrosis factor-alpha) binding agent. In certain embodiments, the immunosuppressive therapy may be started 0, 1, 2, 7, or more days prior to the gene therapy administration, or 0, 1, 2, 3, 7, or more days post-gene therapy administration. Such therapy may involve co-administration of two or more drugs, the (e.g.,

20   prednelisone, micophenolate mofetil (MMF) and/or sirolimus (i.e., rapamycin)) on the same day. One or more of these drugs may be continued after gene therapy administration, at the same dose or an adjusted dose. Such therapy may be for about 1 week (7 days), about 60 days, or longer, as needed. In certain embodiments, a tacrolimus-free regimen is selected.

25

5.1 Gene Therapy Vectors

The rAAV.hLDLR vector should have a tropism for the liver (*e.g.*, an rAAV bearing an AAV8 capsid) and the hLDLR transgene should be controlled by liver-specific expression control elements. The vector is formulated in a buffer/carrier suitable

30   for infusion in human subjects. The buffer/carrier should include a component that

prevents the rAAV, from sticking to the infusion tubing but does not interfere with the rAAV binding activity *in vivo*.

5.1.1. The rAAV.hLDLR Vector

Any of a number of rAAV vectors with liver tropism can be used. Examples of

5   AAV which may be selected as sources for capsids of rAAV include, *e.g.*, rh10, AAVrh64R1, AAVrh64R2, rh8 [See, e.g., US Published Patent Application No. 2007-0036760-A1; US Published Patent Application No. 2009-0197338-A1; EP 1310571]. See also, WO 2003/042397 (AAV7 and other simian AAV), US Patent 7790449 and US Patent 7282199 (AAV8), WO 2005/033321 and US 7,906,111 (AAV9), WO

10   2006/110689 and WO 2003/042397 (rh10), AAV3B; US 2010/0047174 (AAV-DJ).

The hLDLR transgene can include, but is not limited to one or more of the sequences provided by SEQ ID NO:1, SEQ ID NO: 2, and/or SEQ ID NO: 4, which are provided in the attached Sequence Listing, which is incorporated by reference herein. With reference to SEQ ID NO:1, these sequences include a signal sequence located at

15   about base pair 188 to about base pair 250 and the mature protein for variant 1 spans about base pair 251 to about base pair 2770. SEQ ID NO: 1 also identifies exons, at least one of which is absent in the known alternative splice variants of hLDLR. Additionally, or optionally, a sequence encoding one or more of the other hLDLR isoforms may be selected. See, *e.g.*, isoforms 2, 3, 4, 5 and 6, the sequences of which are available, *e.g.*,

20   from http://www.uniprot.org/uniprot/P01130. For example, common variants lack exon 4 (bp (255)..(377) or exon 12 (bp (1546)..(1773)) of SEQ ID NO: 1). Optionally, the transgene may include the coding sequences for the mature protein with a heterologous signal sequence. SEQ ID NO: 2 provides the cDNA for human LDLR and the translated protein (SEQID NO: 3). SEQ ID NO: 4 provides an engineered cDNA for human LDLR.

25   Alternatively or additionally, web-based or commercially available computer programs, as well as service based companies may be used to back translate the amino acids sequences to nucleic acid coding sequences, including both RNA and/or cDNA. See, *e.g.*, backtranseq by EMBOSS, ebi.ac.uk/Tools/st/ ; Gene Infinity (geneinfinity.org/sms-/sms_backtranslation.html); ExPasy (expasy.org/tools/).

30   In a specific embodiment described in the Examples, *infra*, the gene therapy vector is an AAV8 vector expressing an hLDLR transgene under control of a liver-

specific promoter (thyroxine-binding globulin, TBG) referred to as rAAV8.TBG.hLDLR (see Figure 6). The external AAV vector component is a serotype 8, $T = 1$ icosahedral capsid consisting of 60 copies of three AAV viral proteins, VP1, VP2, and VP3, at a ratio of 1:1:18. The capsid contains a single-stranded DNA rAAV vector genome.

5   The rAAV8.TBG.hLDLR genome contains an hLDLR transgene flanked by two AAV inverted terminal repeats (ITRs). The hLDLR transgene includes an enhancer, promoter, intron, an hLDLR coding sequence and polyadenylation (polyA) signal. The ITRs are the genetic elements responsible for the replication and packaging of the genome during vector production and are the only viral *cis* elements required to generate

10   rAAV. Expression of the hLDLR coding sequence is driven from the hepatocyte-specific TBG promoter. Two copies of the alpha 1 microglobulin/bikunin enhancer element precede the TBG promoter to stimulate promoter activity. A chimeric intron is present to further enhance expression and a rabbit beta globin polyadenylation (polyA) signal is included to mediate termination of hLDLR mRNA transcripts.

15   An illustrative plasmid and vector described herein uses the liver-specific promoter thyroxin binding globulin (TBG). Alternatively, other liver-specific promoters may be used [*see, e.g.,* The Liver Specific Gene Promoter Database, Cold Spring Harbor, http://rulai.schl.edu/LSPD, alpha 1 anti-trypsin (A1AT); human albumin Miyatake et al., J. Virol., 71:5124 32 (1997), humAlb; and hepatitis B virus core promoter, Sandig *et al.,*

20   Gene Ther., 3:1002 9 (1996)]. TTR minimal enhancer/promoter, alpha-antitrypsin promoter, LSP (845 nt)25(requires intron-less scAAV). Although less desired, other promoters, such as viral promoters, constitutive promoters, regulatable promoters [*see, e.g.,* WO 2011/126808 and WO 2013/04943], or a promoter responsive to physiologic cues may be used may be utilized in the vectors described herein.

25   In addition to a promoter, an expression cassette and/or a vector may contain other appropriate transcription initiation, termination, 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,

30   sequences that enhance secretion of the encoded product. Examples of suitable polyA sequences include, e.g., SV40, bovine growth hormone (bGH), and TK polyA.

Examples of suitable enhancers include, e.g., the alpha fetoprotein enhancer, the TTR minimal promoter/enhancer, LSP (TH-binding globulin promoter/alpha1-microglobulin/bikunin enhancer), amongst others.

These control sequences are "operably linked" to the hLDLR gene sequences.

5   The expression cassette may be engineered onto a plasmid which is used for production of a viral vector. The minimal sequences required to package the expression cassette into an AAV viral particle are the AAV 5' and 3' ITRs, which may be of the same AAV origin as the capsid, or which of a different AAV origin (to produce an AAV pseudotype). In one embodiment, the ITR sequences from AAV2, or the deleted version

10   thereof (ΔITR), are used for convenience and to accelerate regulatory approval. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. Typically, an expression cassette for an AAV vector comprises an AAV 5' ITR, the hLDLR coding sequences and any regulatory sequences,

15   and an AAV 3' ITR. However, other configurations of these elements may be suitable. A shortened version of the 5' ITR, termed ΔITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. In other embodiments, the full-length AAV 5' and 3' ITRs are used.

The abbreviation "sc" refers to self-complementary. "Self-complementary AAV"

20   refers a plasmid or vector having an expression cassette in which a coding region carried by a recombinant AAV nucleic acid sequence has been designed to form an intra-molecular double-stranded DNA template. Upon infection, rather than waiting for cell mediated synthesis of the second strand, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate

25   replication and transcription. See, e.g., 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, each of which is incorporated herein by reference in

30   its entirety.

5.1.2. rAAV.hLDLR Formulation

The rAAV.hLDLR formulation is a suspension containing an effective amount of rAAV.hLDLR vector suspended in an aqueous solution containing buffering saline, a surfactant, and a physiologically compatible salt or mixture of salts adjusted to an ionic
5   strength equivalent to about 100 mM sodium chloride (NaCl) to about 250 mM sodium chloride, or a physiologically compatible salt adjusted to an equivalent ionic concentration. In one embodiment, the formulation may contain, *e.g.*, about 1.5×10^{11} GC/kg to about 6×10^{13} GC/kg, or about 1×10^{12} to about 1.25×10^{13} GC/kg, as measured by optimized qPCR (oqPCR) or digital droplet PCR (ddPCR) as described in,
10   *e.g.*, M. Lock et al, Hu Gene Therapy Methods, Hum Gene Ther Methods. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14, which is incorporated herein by reference. For example, a suspension as provided herein may contain both NaCl and KCl. The pH may be in the range of 6.5 to 8, or 7 to 7.5. A suitable surfactant, or combination of surfactants, may be selected from among a Poloxamers, *i.e.*, nonionic
15   triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL (Polyoxy capryllic glyceride), polyoxy 10 oleyl ether, TWEEN (polyoxyethylene sorbitan fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the
20   formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content. In one embodiment Poloxamer 188 is selected. The surfactant may be present in an amount up to about 0.0005 % to about 0.001% of the
25   suspension. In one embodiment, the rAAV.hLDLR formulation is a suspension containing at least 1×10^{13} genome copies (GC)/mL, or greater, as measured by oqPCR or digital droplet PCR (ddPCR) as described in, e.g., M. Lock et al, Hu Gene Therapy Methods, Hum Gene Ther Methods. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14, which is incorporated herein by reference. In
30   one embodiment, the vector is suspended in an aqueous solution containing 180 mM sodium chloride, 10 mM sodium phosphate, 0.001% Poloxamer 188, pH 7.3. The

formulation is suitable for use in human subjects and is administered intravenously. In one embodiment, the formulation is delivered via a peripheral vein by infusion over 20 minutes (±5 minutes). However, this time may be adjusted as needed or desired.

In order to ensure that empty capsids are removed from the dose of AAV.

5   hLDLR that is administered to patients, empty capsids are separated from vector particles during the vector purification process, *e.g.*, using cesium chloride gradient ultracentrifugation as discussed in detail herein at Section 8.3.2.5. In one embodiment, the vector particles containing packaged genomes are purified from empty capsids using the process described in International Patent Application No. PCT/US16/65976, filed

10   December 9, 2016, US Patent Appln No. 62/322,093, filed April 13, 2016 and US Patent Appln No. 62/266,341, filed on December 11, 2015, and entitled "Scalable Purification Method for AAV8", which is incorporated by reference herein. Briefly, a two-step purification scheme is described which selectively captures and isolates the genome-containing rAAV vector particles from the clarified, concentrated supernatant of a rAAV

15   production cell culture. The process utilizes an affinity capture method performed at a high salt concentration followed by an anion exchange resin method performed at high pH to provide rAAV vector particles which are substantially free of rAAV intermediates.

In certain embodiments, the method separates recombinant AAV8 viral particles containing DNA comprising pharmacologically active genomic sequences from genome-

20   deficient(empty) AAV8 capsid intermediates. The method involves (a) forming a loading suspension comprising: recombinant AAV8 viral particles and empty AAV8 capsid intermediates which have been purified to remove non-AAV materials from an AAV producer cell culture in which the particles and intermediates were generated; and a Buffer A comprising 20 mM Bis-Tris propane (BTP) and a pH of about 10.2; (b)

25   loading the suspension of (a) onto a strong anion exchange resin, said resin being in a vessel having an inlet for flow of a suspension and/or solution and an outlet permitting flow of eluate from the vessel; (c) washing the loaded anion exchange resin with Buffer 1% B which comprises 10mM NaCl and 20mM BTP with a pH of about 10.2; (d) applying an increasing salt concentration gradient to the loaded and washed anion

30   exchange resin, wherein the salt gradient ranges from 10 mM to about 190 mM NaCl,

inclusive of the endpoints, or an equivalent; and (e) collecting the rAAV particles from eluate, said rAAV particles being purified away from intermediates.

In one embodiment, the pH used is from 10 to 10.4 (about 10.2) and the rAAV particles are at least about 50% to about 90% purified from AAV8 intermediates, or a pH

5   of 10.2 and about 90% to about 99% purified from AAV8 intermediates. In one embodiment, this is determined by genome copies. A stock or preparation of rAAV8 particles (packaged genomes) is "substantially free" of AAV empty capsids (and other intermediates) when the rAAV8 particles in the stock are at least about 75% to about 100%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or

10   at least 99% of the rAAV8 in the stock and "empty capsids" are less than about 1%, less than about 5%, less than about 10%, less than about 15% of the rAAV8 in the stock or preparation.

In one embodiment, the formulation is characterized by an rAAV stock having a ratio of "empty" to "full" of 1 or less, preferably less than 0.75, more preferably, 0.5,

15   preferably less than 0.3.

In a further embodiment, the average yield of rAAV particles is at least about 70%. This may be calculated by determining titer (genome copies) in the mixture loaded onto the column and the amount presence in the final elutions. Further, these may be determined based on q-PCR analysis and/or SDS-PAGE techniques such as those

20   described herein or those which have been described in the art.

For example, to calculate empty and full particle content, VP3 band volumes for a selected sample (*e.g.*, an iodixanol gradient-purified preparation where # of GC = # of particles) are plotted against GC particles loaded. The resulting linear equation (y = mx+c) is used to calculate the number of particles in the band volumes of the test article

25   peaks. The number of particles (pt) per 20 µL loaded is then multiplied by 50 to give particles (pt) /mL. Pt/mL divided by GC/mL gives the ratio of particles to genome copies (pt/GC). Pt/mL–GC/mL gives empty pt/mL. Empty pt/mL divided by pt/mL and x 100 gives the percentage of empty particles.

Generally, methods for assaying for empty capsids and AAV vector particles

30   with packaged genomes have been known in the art. See, *e.g.*, Grimm et al., *Gene Therapy* (1999) 6:1322-1330; Sommer et al., *Molec. Ther.* (2003) 7:122-128. To test for

denatured capsid, the methods include subjecting the treated AAV stock to SDS-

polyacrylamide gel electrophoresis, consisting of any gel capable of separating the three

capsid proteins, for example, a gradient gel containing 3-8% Tris-acetate in the buffer,

then running the gel until sample material is separated, and blotting the gel onto nylon or

5   nitrocellulose membranes, preferably nylon. Anti-AAV capsid antibodies are then used

as the primary antibodies that bind to denatured capsid proteins, preferably an anti-AAV

capsid monoclonal antibody, most preferably the B1 anti-AAV-2 monoclonal antibody

(Wobus et al., *J. Virol.* (2000) 74:9281-9293). A secondary antibody is then used, one

that binds to the primary antibody and contains a means for detecting binding with the

10   primary antibody, more preferably an anti-IgG antibody containing a detection molecule

covalently bound to it, most preferably a sheep anti-mouse IgG antibody covalently

linked to horseradish peroxidase. A method for detecting binding is used to semi-

quantitatively determine binding between the primary and secondary antibodies,

preferably a detection method capable of detecting radioactive isotope emissions,

15   electromagnetic radiation, or colorimetric changes, most preferably a chemiluminescence

detection kit. For example, for SDS-PAGE, samples from column fractions can be taken

and heated in SDS-PAGE loading buffer containing reducing agent (*e.g.*, DTT), and

capsid proteins were resolved on pre-cast gradient polyacrylamide gels (*e.g.*, Novex).

Silver staining may be performed using SilverXpress (Invitrogen, CA) according to the

20   manufacturer's instructions. In one embodiment, the concentration of AAV vector

genomes (vg) in column fractions can be measured by quantitative real time PCR (Q-

PCR). Samples are diluted and digested with DNase I (or another suitable nuclease) to

remove exogenous DNA. After inactivation of the nuclease, the samples are further

diluted and amplified using primers and a TaqMan™ fluorogenic probe specific for the

25   DNA sequence between the primers. The number of cycles required to reach a defined

level of fluorescence (threshold cycle, Ct) is measured for each sample on an Applied

Biosystems Prism 7700 Sequence Detection System. Plasmid DNA containing identical

sequences to that contained in the AAV vector is employed to generate a standard curve

in the Q-PCR reaction. The cycle threshold (Ct) values obtained from the samples are

30   used to determine vector genome titer by normalizing it to the Ct value of the plasmid

standard curve. End-point assays based on the digital PCR can also be used.

In one aspect, an optimized q-PCR method is provided herein which utilizes a broad spectrum serine protease, e.g., proteinase K (such as is commercially available from Qiagen). More particularly, the optimized qPCR genome titer assay is similar to a standard assay, except that after the DNase I digestion, samples are diluted with

5 proteinase K buffer and treated with proteinase K followed by heat inactivation. Suitably samples are diluted with proteinase K buffer in an amount equal to the sample size. The proteinase K buffer may be concentrated to 2-fold or higher. Typically, proteinase K treatment is about 0.2 mg/mL, but may be varied from 0.1 mg/mL to about 1 mg/mL. The treatment step is generally conducted at about 55 °C

10 for about 15 minutes, but may be performed at a lower temperature (e.g., about 37 °C to about 50 °C) over a longer time period (e.g., about 20 minutes to about 30 minutes), or a higher temperature (e.g., up to about 60 °C) for a shorter time period (e.g., about 5 to 10 minutes). Similarly, heat inactivation is generally at about 95 °C for about 15 minutes, but the temperature may be lowered (e.g., about 70 to about 90 °C) and the time

15 extended (e.g., about 20 minutes to about 30 minutes). Samples are then diluted (e.g., 1000 fold) and subjected to TaqMan analysis as described in the standard assay.

Additionally, or alternatively, droplet digital PCR (ddPCR) may be used. For example, methods for determining single-stranded and self-complementary AAV vector genome titers by ddPCR have been described. See, e.g., M. Lock et al, Hu Gene

20 Therapy Methods, Hum Gene Ther Methods. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14.

5.1.3 Manufacturing

The rAAV.hLDLR vector can be manufactured as shown in the flow diagram shown in Fig. 11. Briefly, cells (e.g. HEK 293 cells) are propagated in a suitable cell

25 culture system and transfected for vector generation. The rAAV.hLDLR vector can then be harvested, concentrated and purified to prepare bulk vector which is then filled and finished in a downstream process.

Methods for manufacturing the gene therapy vectors described herein include methods well known in the art such as generation of plasmid DNA used for production

30 of the gene therapy vectors, generation of the vectors, and purification of the vectors. In some embodiments, the gene therapy vector is an AAV vector and the plasmids

generated are an AAV cis-plasmid encoding the AAV genome and the gene of interest, an AAV trans-plasmid containing AAV rep and cap genes, and an adenovirus helper plasmid. The vector generation process can include method steps such as initiation of cell culture, passage of cells, seeding of cells, transfection of cells with the plasmid DNA,

5   post-transfection medium exchange to serum free medium, and the harvest of vector-containing cells and culture media. The harvested vector-containing cells and culture media are referred to herein as crude cell harvest.

The crude cell harvest may thereafter be subject method steps such as concentration of the vector harvest, diafiltration of the vector harvest, microfluidization

10   of the vector harvest, nuclease digestion of the vector harvest, filtration of microfluidized intermediate, purification by chromatography, purification by ultracentrifugation, buffer exchange by tangential flow filtration, and formulation and filtration to prepare bulk vector.

In certain embodiments, methods similar to those of FIG 11 may be used in

15   conjunction with other AAV producer cells. Numerous methods are known in the art for production of rAAV vectors, including transfection, stable cell line production, and infectious hybrid virus production systems which include Adenovirus-AAV hybrids, herpesvirus-AAV hybrids and baculovirus-AAV hybrids. See, e.g., G Ye, et al, Hu Gene Ther Clin Dev, 25: 212-217 (Dec 2014); RM Kotin, Hu Mol Genet, 2011, Vol. 20, Rev

20   Issue 1, R2-R6; M. Mietzsch, et al, Hum Gene Therapy, 25: 212-222 (Mar 2014); T Virag et al, Hu Gene Therapy, 20: 807-817 (August 2009); N. Clement et al, Hum Gene Therapy, 20: 796-806 (Aug 2009); DL Thomas et al, Hum Gene Ther, 20: 861-870 (Aug 2009). rAAV production cultures for the production of rAAV virus particles all require; 1) suitable host cells, including, for example, human-derived cell lines such as HeLa,

25   A549, or 293 cells, or insect-derived cell lines such as SF-9, in the case of baculovirus production systems; 2) suitable helper virus function, provided by wild type or mutant adenovirus (such as temperature sensitive adenovirus), herpes virus, baculovirus, or a nucleic acid construct providing helper functions in trans or in cis; 3) functional AAV rep genes, functional cap genes and gene products; 4) a transgene (such as a therapeutic

30   transgene) flanked by AAV ITR sequences; and 5) suitable media and media components to support rAAV production.

A variety of suitable cells and cell lines have been described for use in production of AAV. The cell itself may be selected from any biological organism, including prokaryotic (e.g., bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. Particularly desirable host cells are selected from

5   among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, a HEK 293 cell (which express functional adenoviral E1), Saos, C2C12, L cells, HT1080, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. In certain

10   embodiments, the cells are suspension-adapted cells. The selection of the mammalian species providing the cells is not a limitation of this invention; nor is the type of mammalian cell, i.e., fibroblast, hepatocyte, tumor cell, etc.

In a specific embodiment, the methods used for manufacturing the gene therapy vectors are described in Example 3 at Section 8, *infra*.

15   ## 5.2 Patient Population

Patients who are candidates for treatment are preferably adults (male or female ≥18 years of age) diagnosed with HoFH carrying two mutations in the *LDLR* gene; *i.e.*, patients that have molecularly defined LDLR mutations at both alleles in the setting of a clinical presentation consistent with HoFH, which can include untreated LDL-C levels,

20   *e.g.*, LDL-C levels >300 mg/dl, treated LDL-C levels, *e.g.*, LDL-C levels <300 mg/dl and/or total plasma cholesterol levels greater than 500 mg/dl and premature and aggressive atherosclerosis. In some embodiments, a patient <18 years of age can be treated. In some embodiments, the patient that is treated is a male ≥18 years of age. In some embodiments, the patient that is treated is a female ≥18 years of age. Candidates

25   for treatment include HoFH patients that are undergoing treatment with lipid-lowering drugs, such as statins, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL and/or plasma apheresis.

Prior to treatment, the HoFH patient should be assessed for NAb to the AAV serotype used to deliver the hLDLR gene. Such NAbs can interfere with transduction

30   efficiency and reduce therapeutic efficacy. HoFH patients that have a baseline serum NAb titer ≤ 1:10 are good candidates for treatment with the rAAV.hLDLR gene therapy

protocol. However, patients with higher ratios may be selected under certain circumstances. Treatment of HoFH patients with titers of serum NAb >1:5 may require a combination therapy, such as transient co-treatment with an immunosuppressant, although such therapy may be selected for patients with lower ratios.

5   Immunosuppressants for such co-therapy include, but are not limited to, steroids, antimetabolites, T-cell inhibitors, and alkylating agents. For example, such transient treatment may include a steroid (e.g., prednisole) dosed once daily for 7 days at a decreasing dose, in an amount starting at about 60 mg, and decreasing by 10 mg/day (day 7 no dose). Other doses and medications may be selected.

10   Subjects may be permitted to continue their standard of care treatment(s) (e.g., LDL apheresis and/or plasma exchange, and other lipid lowering treatments) prior to and concurrently with the gene therapy treatment at the discretion of their caring physician. In the alternative, the physician may prefer to stop standard of care therapies prior to administering the gene therapy treatment and, optionally, resume standard of care

15   treatments as a co-therapy after administration of the gene therapy. Desirable endpoints of the gene therapy regimen are low density lipoprotein cholesterol (LDL-C) reduction and change in fractional catabolic rate (FCR) of LDL apolipoprotein B (apoB) from baseline up to 12 weeks after administration of the gene therapy treatment. Other desired endpoints include, e.g., reduction in one or more of: total cholesterol (TC), non-high

20   density lipoprotein cholesterol (non-HDL-C), decrease in fasting triglycerides (TG), and changes in HDL-C (e.g., increased levels are desirable), very low density lipoprotein cholesterol (VLDL-C), lipoprotein(a) (Lp(a)), apolipoprotein B (apoB), and/or apolipoprotein A-I (apoA-I).

                In one embodiment, patients achieve desired LDL-C thresholds (e.g., LDL-C

25   <200, <130, or <100, mg/dl) after treatment with AAV8.hLDLR, alone and/or combined with the use of adjunctive treatments over the duration of the study.

                In certain embodiments, patients will have a reduced need for lipid lowering therapy, including frequency of LDL and/or plasma apheresis.

                In still other embodiments, there will be a reduction in number, size or extent of

30   assessable xanthomas compared to baseline.

                Nevertheless, patients having one or more of the following characteristics may be

excluded from treatment at the discretion of their caring physician:

- Heart failure defined by the NYHA classification as functional Class III with history of hospitalization(s) within 12 weeks of the baseline visit or functional Class IV.

- History within 12 weeks of the baseline visit of a myocardial infarction (MI), unstable angina leading to hospitalization, coronary artery bypass graft surgery (CABG), percutaneous coronary intervention (PCI), uncontrolled cardiac arrhythmia, carotid surgery or stenting, stroke, transient ischemic attack, carotid revascularization, endovascular procedure or surgical intervention.

- Uncontrolled hypertension defined as: systolic blood pressure >180 mmHg, diastolic blood pressure > 95 mmHg.

- History of cirrhosis or chronic liver disease based on documented histological evaluation or non-invasive imaging or testing.

- Documented diagnosis of any of the following liver diseases: Nonalcoholic steatohepatitis (biopsy-proven); Alcoholic liver disease; Autoimmune hepatitis; Liver cancer; Primary biliary cirrhosis; Primary sclerosing cholangitis; Wilson's disease; Hemochromatosis; α_1 anti-trypsin deficiency.

- Abnormal LFTs at screening (AST or ALT >2x upper limit of normal (ULN) and/or Total Bilirubin of >1.5x ULN unless patient has unconjugated hyperbilirubinemia due to Gilbert's syndrome).

- Hepatitis B as defined by positive for HepB SAg, or Hep B Core Ab, and/or viral DNA, or Chronic active Hepatitis C as defined by positive for HCV Ab and viral RNA.

- History of alcohol abuse within 52 weeks.

- Certain prohibited medications known to be potentially hepatotoxic, especially those that can induce microvesicular or macrovesicular steatosis. These include but are not limited to: acutane, amiodarone, HAART medications, heavy acetaminophen use (2g/day > 3 x q week), isoniazid, methotrexate, tetracyclines, tamoxifen, valproate.

- Current use of systemic corticosteroids or active tuberculosis, systemic fungal disease, or other chronic infection.

- History of immunodeficiency diseases, including a positive HIV test result.

- Chronic renal insufficiency defined as estimated GRF < 30 mL/min.

- History of cancer within the past 5 years, except for adequately treated basal cell skin cancer, squamous cell skin cancer, or in situ cervical cancer.

- Previous organ transplantation.

- Any major surgical procedure occurring less than 3 months prior to determination of baselines and/or treatment.

A baseline serum AAV8 NAb titer > 1:5, > 1:10. In other embodiments, a caring physician may determine that the presence of one or more of these physical characteristics (medical history) should not preclude treatment as provided herein.

5.3.   Dosing & Route of Administration

Patients receive a single dose of rAAV.hLDLR administered via a peripheral vein by infusion; *e.g.*, over about 20 to about 30 minutes. The dose of rAAV.hLDLR administered to a patient is at least 2.5×10^{12} GC/kg or 7.5×10^{12} GC/kg, or at least 5×10^{11} GC/kg to about 7.5×10^{12} GC/kg (as measured by oqPCR or ddPCR). However, other doses may be selected. In a preferred embodiment, the rAAV suspension used has a potency such that a dose of 5×10^{11} GC/kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%.

In some embodiments, the dose of rAAV.hLDLR administered to a patient is in the range of 2.5×10^{12} GC/kg to 7.5×10^{12} GC/kg. Preferably, the rAAV suspension used has a potency such that a dose of 5×10^{11} GC/kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%. In specific embodiments, the dose of rAAV.hLDLR administered to a patient is at least 5×10^{11} GC/kg 2.5×10^{12} GC/kg, 3.0×10^{12} GC/kg, 3.5×10^{12} GC/kg, 4.0×10^{12} GC/kg, 4.5×10^{12} GC/kg, 5.0×10^{12} GC/kg, 5.5×10^{12} GC/kg, 6.0×10^{12} GC/kg, 6.5×10^{12} GC/kg, 7.0×10^{12} GC/kg, or 7.5×10^{12} GC/kg.

In some embodiments, rAAV.hLDLR is administered in combination with one or more therapies for the treatment of HoFH. In some embodiments, rAAV.hLDLR is administered in combination with standard lipid-lowering therapy that is used to treat HoFH, including but not limited to statin, ezetimibe, ezedia, bile acid sequestrants, LDL apheresis, plasma apheresis, plasma exchange, lomitapide, mipomersen, and/or PCSK9 inhibitors. In some embodiments, rAAV.hLDLR is administered in combination with niacin. In some embodiments, rAAV.hLDLR is administered in combination with fibrates.

5.4. Measuring Clinical Objectives

Safety of the gene therapy vector after administration can be assessed by the number of adverse events, changes noted on physical examination, and/or clinical laboratory parameters assessed at multiple time points up to about 52 weeks post vector administration. Although physiological effect may be observed earlier, e.g., in about 1 day to one week, in one embodiment, steady state levels expression levels are reached by about 12 weeks.

LDL-C reduction achieved with rAAV.hLDLR administration can be assessed as a defined percent change in LDL-C at about 12 weeks, or at other desired time points, compared to baseline.

Other lipid parameters can also be assessed at about 12 weeks, or at other desired time points, compared to baseline values, specifically percent change in total cholesterol (TC), non-high density lipoprotein cholesterol (non-HDL-C), HDL-C, fasting triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), lipoprotein(a) (Lp(a)), apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I). The metabolic mechanism by which LDL-C is reduced can be assessed by performing LDL kinetic studies prior to rAAV.hLDLR administration and again 12 weeks after administration. The primary parameter to be evaluated is the fractional catabolic rate (FCR) of LDL apoB.

As used herein, the rAAV.hLDLR vector herein "functionally replaces" or "functionally supplements" the patients defective LDLR with active LDLR when the patient expresses a sufficient level of LDLR to achieve at least one of these clinical endpoints. Expression levels of hLDLR which achieve as low as about 10% to less than

100% of normal wild-type clinical endpoint levels in a non-FH patient may provide functional replacement.

In one embodiment, expression may be observed as early as about 8 hours to about 24 hours post-dosing. One or more of the desired clinical effects described above may be observed within several days to several weeks post-dosing.

Long term (up to 260 weeks) safety and efficacy can be assessed after rAAV.hLDLR administration.

Standard clinical laboratory assessments and other clinical assays described in Sections 6.4.1 through 6.7 *infra*, can be used to monitor adverse events, efficacy endpoints that assess percent change in lipid parameters, pharmacodynamic assessments, lipoprotein kinetics, ApoB-100 concentrations, as well as immune responses to the rAAV.hLDLR vector.

The following examples are illustrative only and are not intended to limit the present invention.

EXAMPLES

6. Example 1: Protocol for Treating Human Subjects

This Example relates to a gene therapy treatment for patients with genetically confirmed homozygous familial hypercholesterolemia (HoFH) due to mutations in the low density lipoprotein receptor (LDLR) gene. In this example, the gene therapy vector, AAV8.TBG.hLDLR, a replication deficient adeno-associated viral vector 8 (AAV8) expressing hLDLR is administered to patients with HoFH. Efficacy of treatment can be assessed using Low density lipoprotein cholesterol (LDL-C) levels as a surrogate for transgene expression. Primary efficacy assessments include LDL-C levels at about 12 weeks post treatment, with persistence of effect followed thereafter for at least 52 weeks. Long term safety and persistence of transgene expression may be measured post-treatment in liver biopsy samples.

6.1. Gene Therapy Vector

The gene therapy vector is an AAV8 vector expressing the transgene human low density lipoprotein receptor (hLDLR) under control of a liver-specific promoter (thyroxine-binding globulin, TBG) and is referred to in this Example as AAV8.TBG.hLDLR (see Figure 7). The AAV8.TBG.hLDLR vector consists of the AAV

5 vector active ingredient and a formulation buffer. The external AAV vector component is a serotype 8, $T = 1$ icosahedral capsid consisting of 60 copies of three AAV viral proteins, VP1, VP2, and VP3, at a ratio of 1:1:18. The capsid contains a single-stranded DNA recombinant AAV (rAAV) vector genome. The genome contains an hLDLR transgene flanked by two AAV inverted terminal repeats (ITRs). An enhancer, promoter, intron,

10 hLDLR coding sequence and polyadenylation (polyA) signal comprise the hLDLR transgene. The ITRs are the genetic elements responsible for the replication and packaging of the genome during vector production and are the only viral *cis* elements required to generate rAAV. Expression of the hLDLR coding sequence is driven from the hepatocyte-specific TBG promoter. Two copies of the alpha 1 microglobulin/bikunin

15 enhancer element precede the TBG promoter to stimulate promoter activity. A chimeric intron is present to further enhance expression and a rabbit beta globin polyadenylation (polyA) signal is included to mediate termination of hLDLR mRNA transcripts. The sequence of pAAV.TBG.PI.hLDLRco.RGB which was used to produce this vector is provided in SEQ ID NO: 6.

20   The formulation of the investigational agent is at least 1×10^{13} genome copies (GC)/mL in aqueous solution containing 180 mM sodium chloride, 10 mM sodium phosphate, 0.001% Poloxamer 188, pH 7.3 and is administered via a peripheral vein by infusion over 20 minutes (±5 minutes).

6.2. Patient Population

25   Patients treated are adults with homozygous familial hypercholesterolemia (HoFH) carrying two mutations in the LDLR gene. The patients can be males or females that are 18 years old or older. The patients have molecularly defined LDLR mutations at both alleles in the setting of a clinical presentation consistent with HoFH, which can include untreated LDL-C levels, e.g., LDL-C levels >300 mg/dl, treated LDL-C levels,

30 e.g., LDL-C levels <300 mg/dl and/or total plasma cholesterol levels greater than 500 mg/dl and premature and aggressive atherosclerosis. The treated patients can be

concurrently undergoing treatment with lipid-lowering drugs, such as statins, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL apheresis and/or plasma apheresis.

Patients that are treated can have a baseline serum AAV8 neutralizing antibody (NAb) titer $\leq 1:10$. If a patient does not have a baseline serum AAV8 neutralizing

5   antibody (NAb) titer $\leq 1:10$, the patient can be transiently co-treated with an immunosuppressant during the transduction period. In certain embodiments, a patient with an AAV8 neutralizing antibody titer may be higher (e.g., $\leq 1:5$ to $\leq 1:15$, or $\leq 1:20$) or lower (e.g., $\leq 1:2$ to $\leq 1:5$). Immunosuppressants for co-therapy include, but are not limited to, steroids, antimetabolites, T-cell inhibitors, and alkylating agents.

10   Subjects may be permitted to continue their standard of care treatment(s) (e.g., LDL apheresis and/or plasma exchange, and other lipid lowering treatments) prior to and concurrently with the gene therapy treatment at the discretion of their caring physician. In the alternative, the physician may prefer to stop standard of care therapies prior to administering the gene therapy treatment and, optionally, resume standard of care

15   treatments as a co-therapy after administration of the gene therapy. Desirable endpoints of the gene therapy regimen are low density lipoprotein cholesterol (LDL-C) reduction and change in fractional catabolic rate (FCR) of LDL apolipoprotein B (apoB) from baseline up to about 12 weeks after administration of the gene therapy treatment.

In still other embodiments, desirable endpoints include reduction in the need for

20   LDL apheresis and/or plasma apheresis is a desirable endpoint. The term "LDL apheresis" is used to refer to low-density lipoprotein (LDL) apheresis which is a process in which LDL is eliminated from the bloodstream using a process similar to dialysis. LDL apheresis is a procedure that removes LDL cholesterol from the blood of patients. During the LDL-apheresis procedure, the blood cells are separated from the plasma.

25   Specialized filters are used to remove the LDL cholesterol from the plasma, and the filtered blood is returned to the patient. A single LDL apheresis treatment can remove 60-70% of harmful LDL cholesterol from the blood. There are currently two machines that are approved in the U.S by the Food and Drug Administration. The Liposorber uses a filter covered with dextran, which attaches to the LDL and removes it from the

30   circulation. The other machine is called HELP and uses heparin to remove the LDL. Neither of these machines causes significant changes in the amount of HDL (good)

cholesterol. These are currently approved for patients with LDL cholesterol of 2000

ng/mdl or higher with a history of coronary artery disease and patients with LDL

cholesterol levels of 300 mg/dl or higher without coronary artery disease. *See, e.g.,*

American Society for Apheresis, www.apheresis.com, and

5   http://c.ymcdn.com/sites/www.apheresis.org/resource/resmgr/-

fact_sheets_file/ldl_apheresis.pdf. See, also, World Apheresis Association

[http://worldapheresis.org/] and The National Lipid Association (USA)

[https://www.lipid.org/]. In certain embodiments, plasma apheresis (plasmapheresis)

which is unselective for LDL may have been used prior to gene therapy treatment and

10   the need for such treatment may be reduced as described herein for LDL apheresis.   As

used herein, "reduction" in apheresis refers to a decrease in the number of times a month

and/or a year which a patient is required to undergo apheresis. Such a reduction may be

10%, 25%, 50%, 75%, or 100% (e.g., eliminating the need) less apheresis treatments

post- therapy as compared to the level of apheresis used prior to the rAAV8-hLDLR

15   therapy. For example, a selected patient who had been undergoing apheresis weekly pre-

treatment with rAAV8.hLDLR may only require apheresis every two weeks, monthly, or

less frequently post-treatment. In another example, a selected patient who had been

undergoing apheresis twice a month pre-treatment with rAAV8.hLDLR may only require

apheresis every monthly, bi-monthly, quarterly or less frequently post-treatment. Still

20   other

In certain embodiments, a desirable endpoint includes reduction in the dose of a

PCSK9 inhibitor used to treat the patient is a desirable endpoint. As used herein,

"reduction" in apheresis refers to a decrease in the number of times a month and/or a

year which a patient is required to undergo apheresis. Such a reduction may be 10%,

25   25%, 50%, 75%, or 100% (e.g., eliminating the need) less PCSK9 inhibitor required

post- therapy as compared to the level of PCSK9 inhibitor used prior to the rAAV8-

hLDLR therapy. For example, treating a HoFH patient on a PCSK9 inhibitor pre-

rAAV8.hLDLR therapy (e.g., receiving 300 mg - 500 mg dose) once a month by

infusion, may result in the ability to reduce treatment with the PCSK9 inhibitor to a

30   treatment level consistent with a HeFH patient. This may result in the patient being able

to receive less intrusive therapy (*e.g.,* eliminating the need for infusion of high doses).

For example, rather than a monthly infusion of 420 mg/by infusion, the patient may be electable for administration of a lower dose with a syringe or autoinjector (*e.g.,* 100 - 140 ng/mL) once a month or every two weeks (HeFH dose), or less frequently.

6.3. Dosing & Route of Administration

5   Patients receive a single dose of AAV8.TBG.hLDLR administered via a peripheral vein by infusion. The dose of AAV8.TBG.hLDLR administered to a patient is about 2.5×10^{12} GC/kg or 7.5×10^{12} GC/kg. In order to ensure that empty capsids are removed from the dose of AAV8.TBG.hLDLR that is administered to patients, empty capsids are separated from vector particles by cesium chloride gradient

10   ultracentrifugation or by ion exchange chromatography during the vector purification process, as discussed in Section 8.3.2.5.

6.4. Measuring Clinical Objectives

• LDL-C reduction achieved with AAV8.TBG.hLDLR administration can be assessed as a defined percent change in LDL-C at about 12 weeks compared to baseline.

15   • Other lipid parameters can be assessed at about 12 weeks compared to baseline values, specifically percent change in total cholesterol (TC), non-high density lipoprotein cholesterol (non-HDL-C), HDL-C, fasting triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), lipoprotein(a) (Lp(a)), apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I).

20   • The metabolic mechanism by which LDL-C is reduced can be assessed by performing LDL kinetic studies prior to vector administration and again at about 12 weeks after administration. The primary parameter to be evaluated is the fractional catabolic rate (FCR) of LDL apoB.

• Long term (up to 52 weeks or up to 260 weeks) safety and efficacy can be

25   assessed after AAV8.TBG.hLDLR administration

6.4.1. Standard Clinical Laboratory Assessments that can be performed:

The following clinical profiles can be tested before and after treatment:

• Biochemical Profile: sodium, potassium, chloride, carbon dioxide, glucose,

30   blood urea nitrogen, lactate dehydrogenase (LDH) creatinine, creatinine phosphokinase,

calcium, total protein, albumin, aspartate aminotransferase (AST), alanine

aminotransferase (ALT), alkaline phosphatase, total bilirubin, GGT.

 • CBC: white blood cell (WBC) count, hemoglobin, hematocrit, platelet count,

red cell distribution width, mean corpuscular volume, mean corpuscular hemoglobin, and

mean corpuscular hemoglobin concentration.

 • Coagulation: PT, INR, PTT (at screening and baseline, and as needed.

 • Urinalysis: urinary color, turbidity, pH, glucose, bilirubin, ketones, blood,

protein, WBC's.

6.4.2. Adverse Events of Interest

The following clinical assays can be used to monitor toxicity:

 • Liver injury

     o   CTCAE v4.0 grade 3 or higher lab result for bilirubin or liver

                enzymes (AST, ALT, AlkPhos).

     o   Bilirubin and AlkPhos CTCAE v4.0 grade 2 (bilirubin >1.5xULN;

                AlkPhos >2.5xULN).

 • Hepatotoxicity (i.e., meet criteria for "Hy's law")

     o $\geq 3 \times$ ULN (Upper limit of normal) for AST or ALT, and

     o $> 2 \times$ ULN serum total bilirubin without elevated alkaline

                phosphatase, and

     o   No other reason can be found to explain the increased

                transaminase levels combined with increased total bilirubin.

Additionally, ALT or AST elevations that may trigger corticosteroid therapy for presumed

T-cell mediated immune transaminitis (>2x baseline AND 1xULN) will be flagged and

reported.

6.5. Efficacy Endpoints

Assessment of the percent change in lipid parameters at about 12 weeks

following administration of AAV8.TBG.hLDLR can be assessed and compared to

baseline. This includes:

 • Percent changes in LDL-C directly measured (primary efficacy endpoint).

 • Percent changes in Total Cholesterol, VLDL-C, HDL-C, calculated non-

       HDL-cholesterol, Changes in triglycerides, apoA-I, apoB, and Lp(a).

Baseline LDL-C value can be calculated as the average of LDL-C levels obtained under fasting condition in 2 separate occasions before administration of AAV8.TBG.hLDLR to control for laboratory and biological variability and ensure a reliable efficacy assessment.

6.5.1. Pharmacodynamic/ Efficacy Assessments

The following efficacy laboratory tests can be evaluated under fasting conditions:

- LDL-C directly measured
- Lipid panel: total cholesterol, LDL-C, non-HDL-C, HDL-C, TG, Lp(a)
- Apolipoproteins: apoB and apoA-I.

Additionally, optional LDL apoB kinetics may be determined prior to and 12 weeks after treatment. Lipid lowering efficacy may be assessed as percent changes from baseline at about 12, 24 and 52 weeks post vector administration. Baseline LDL-C values are calculated by averaging the LDL-C levels obtained under fasting condition in 2 separate occasions before administration. The percent change from baseline in LDL-C at 12 weeks post vector administration is the primary measure of gene transfer efficacy.

- Change in LDL-apoB fractional catabolic rate from baseline to 12 weeks after vector administration. Additional apoB kinetic parameters will be also considered.
- Absolute LDL-C levels at 12 weeks, 24 weeks, 52 weeks and annually up to 260 weeks following administration of AAV8.hLDLR.
- Percent change in LDL-C and other lipid parameters from baseline at 24 weeks, 52 weeks and annually up to 260 weeks following administration of AAV8. hLDLR
- The percentage of subjects who achieve absolute LDL-C levels < 200 mg/dl at 12 weeks, 24 weeks, 52 weeks following administration of AAV8.hLDLR.
- The number of subjects at 12 weeks, 24 weeks, 36 weeks, 52 weeks who did not resume previously taken or did not initiate any new lipid lowering treatment, following administration of AAV8.hLDLR.

• For those subjects who received lipid apheresis prior to screening, the number of subjects who experienced a change in frequency of apheresis treatments any time during the study.

• For those subjects who received a PCSK9 inhibitor, the LDL-C achieved following administration of AAV8. hLDLR compared with the LDL-C achieved while on the PCSK9 inhibitor prior to administration of AAV8.hLDLR.

• For subjects with easy to describe xanthomas at baseline, the number who have documented improvement in number, size or extent of clinical presentation at 12 weeks and 52 weeks following administration of AAV8.hLDLR.

6.6. Lipoprotein Kinetics

Lipoprotein kinetic studies may be performed prior to vector administration and again 12 weeks after to assess the metabolic mechanism by which LDL-C is reduced. The primary parameter to be evaluated is the fractional catabolic rate (FCR) of LDL-apoB. Endogenous labeling of apoB is achieved by intravenous infusion of deuterated leucine, followed by blood sampling over a 48 hour period.

6.6.1. ApoB-100 isolation

VLDL, IDL and LDL are isolated by sequential ultracentrifugation of timed samples drawn after the D3-leucine infusion. Apo B-100 is isolated from these lipoproteins by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) using a Tris-glycine buffer system. ApoB concentrations within individual apoB species are determined by enzyme-linked immunosorbent assay (ELISA). The total apoB concentration is determined using an automated immunoturbidimetric assay.

6.6.2. Isotopic Enrichment Determinations

ApoB-100 bands are excised from polyacrylamide gels. Excised bands are hydrolyzed in 12N HCl at 100°C for 24 hours. Amino acids are converted to the N-isobutyl ester and N-heptafluorobutyramide derivatives before analysis using a gas chromatograph/mass spectrometer. Isotope enrichment (percentage) is calculated from the observed ion current ratios. Data in this format are analogous to specific radioactivity in radiotracer experiments. It is assumed that each subject remains in steady state with respect to apoB-100 metabolism during this procedure.

6.7. Pharmacokinetics and Immune Response to AAV8 Assessments

The following tests can be used to evaluate pharmacokinetics, pre-immunity to the AAV vector and immune response to the AAV vector:

• Immune response monitoring: AAV8 NAb titer; T-cell responses to AAV8 vector; T-cell responses to hLDLR.

• Vector concentration: AAV8 concentrations in plasma, measured as vector genomes by PCR.

• Human Leukocyte Antigen Typing (HLA type): HLA type is assessed in deoxyribonucleic acid (DNA) from peripheral blood mononuclear cells (PBMCs) by high resolution evaluation of HLA-A, HLA-B, HLA-C for Class I and HLA DRB1/DRB345, DQB1 and DPB1 for Class II. This information allows for correlation of the potential T cell immune response to AAV8 capsid or to LDLR transgene with a specific HLA allele, helping to explain individual variability in the intensity and timing of T cell responses.

6.8   Xanthoma Assessment

Physical exams include identification, examination and description of any xanthomas. Documentation of xanthoma location and type is determined, i.e., cutaneous, palpebral (eye), tuberous, and/or tendinous. Where possible, metric rulers or calipers are used to document size of xanthomas (largest and smallest extents) during physical exam. If possible, digital photographs of xanthomas that are most extensive and readily identifiable are made with placement of a tape ruler (metric with millimeters) next to the lesion.

7.   Example 2: Pre-Clinical Data

Nonclinical studies were undertaken to study the effects of AAV8.TBG.hLDLR on animal models for HoHF and pre-existing humoral immunity. Multiple single dose pharmacology studies were conducted in small and large animal models measuring decreases in cholesterol. Additionally, regression in atherosclerosis was measured in the Double Knock-Out LDLR-/- Apobec1-/- mouse model (DKO), which is deficient in both LDLR and Apobec1, develops severe hypercholesterolemia due to elevations in apoB-100-containing LDL even on a chow diet, and develops extensive atherosclerosis. These data were used to determine a minimally effective dose and to adequately justify dose selection for human studies. To further characterize the appropriate dose for human

studies and identify potential safety signals, toxicology studies were conducted in non-human primates (NHPs) and a mouse model of HoFH.

7.1 Pre-existing humoral immunity: Effect on AAV-mediated gene transfer to liver

5   The goal of this study was to evaluate the impact of pre-existing humoral immunity to AAV on liver directed gene transfer using AAV8 encapsidated vectors in rhesus and cynomolgus macaques. Twenty–one rhesus and cynomolgus macaques were selected from a larger population of animals who were pre-screened for levels of pre-existing immunity against AAV8. Animals represented a wide age distribution and all

10   were male. These studies focused on animals with low to undetectable levels of neutralizing antibodies (NAbs) while including a more limited number with AAV8 NAb titers up to 1:160. Animals were infused with 3×10^{12} GC/kg of AAV8 vector expressing enhanced green fluorescent protein (EGFP) from the liver-specific tyroxine binding globulin (TBG) promoter via a peripheral vein infusion. Animals were necropsied 7 days

15   later and tissues were evaluated for EGFP expression and liver targeting of AAV8 vector genomes (Figure 1). Pre-existing NAbs to AAV8 in NHP sera were assessed using an *in vitro* transduction inhibition assay, as well as in the context of passive transfer experiments, in which sera from NHP was infused into mice prior to and at the time of vector administration to evaluate the impact of pre-existing AAV8 NAbs on liver

20   directed gene transfer *in vivo* (Wang et al., 2010 Molecular Therapy 18(1): 126-134).

Animals with undetectable to low levels of pre-existing NAbs to AAV8 displayed high level transduction in liver, as evidenced by EGFP detection by fluorescent microscopy (Figure 1) and ELISA, as well as vector DNA quantification in the liver. The most useful measure of transduction in terms of efficacy in HoFH is percent of

25   hepatocytes transduced, which in the absence of pre-existing NAb was 17% (range of 4.4% to 40%). This is very close to the efficiency observed in mice at the same dose of vector. T threshold titer of pre-existing NAbs significantly impacting transduction of liver cells was $\leqslant$ 1:5 (i.e., titers of 1:10 or greater substantially reduced transduction). Antibody-mediated inhibition of liver transduction correlated directly with diminished AAV

30   genomes in liver. Human sera were screened for evidence of pre-existing NAb to AAV8 and results suggest that about 15% of adults have NAbs to AAV8 that are in excess of $\leqslant$

1:5. Also, it was shown that higher levels of NAb are associated with a change in the biodistribution of the vector, such that NAb decreases liver gene transfer while increasing deposition of the vector genome into the spleen, without increasing spleen transduction.

5   **7.2 Effect of AAV8.TBG.mLDLR on Serum Cholesterol in a Mouse Model of HoFH**

DKO mice (6 to 12 week old males) were injected IV with AAV8.TBG.mLDLR and followed for metabolic correction and reversal of pre-existing atherosclerosis lesions. Animals were also evaluated for gross clinical toxicity and

10   abnormalities in serum transaminases. The mouse version of LDLR was utilized for vector administration into the DKO mouse.

Mice that received 10^{11} GC/mouse (5×10^{12} GC/kg) showed a near complete normalization of hypercholesterolemia that was stable for 180 days (Figure 2). No elevation in ALT levels or abnormal liver biochemistry were observed for up to 6

15   months post-vector injection at the highest doses in rodents (Kassim et al., 2010, PLoS One 5(10): e13424).

7.3 Effect of AAV8.TBG.mLDLR on Atherosclerotic Lesions in a Mouse Model of HoFH on a High-fat Diet

Given that AAV8-mediated delivery of LDLR induced significant lowering of

20   total cholesterol, AAV8-mediated expression of mLDLR was examined in a proof-of-concept study to determine whether it had an effect on atherosclerotic lesions (Kassim et al., 2010, PLoS One 5(10): e13424). Three groups of male DKO mice were fed a high-fat diet to hasten the progression of atherosclerosis. After two months, one group of mice received a single IV injection of 5×10^{12} GC/kg of control AAV8.TBG.nLacZ vector, one

25   group received a single IV injection of 5×10^{12} GC/kg of AAV8.TBG.mLDLR vector, while a third non-intervention group were necropsied for atherosclerosis lesion quantification. The mice which received vectors were maintained on the high-fat diet for an additional 60 days at which time they were necropsied.

Animals that received the AAV8.TBG.mLDLR vector realized a rapid drop in

30   total cholesterol from 1555 ± 343 mg/dl at baseline to 266 ± 78 mg/dl at day 7 and to 67

± 13 mg/dl by day 60 after treatment. By contrast, the plasma cholesterol levels of AAV8.TBG.nLacZ treated mice remained virtually unchanged from 1566 ± 276 mg/dl at baseline to 1527 ± 67 mg/dl when measured 60 days after vector. All animals developed slight increases in serum transaminases following the two months on the high-fat diet,

5   which remained elevated following treatment with the AAV8.TBG.nLacZ vector but diminished three-fold to normal levels after treatment with the AAV8.TBG.mLDLR vector.

       Evolution of pre-existing atherosclerotic lesions was assessed by two independent methods. In the first method the aortas were opened from the arch to the iliac bifurcation

10   and stained with Oil Red O (Figure 3A); morphometric analyses quantified the percent of aorta stained with Oil Red O along the entire length of the aorta (Figure 3B). Oil Red O is a lysochrome (fat-soluble dye) diazo dye used for staining of neutral triglycerides and lipids on frozen sections. Staining of the aorta with this dye allows for the visualization of lipid laden plaques. As seen in Figure 3, two months of high fat diet resulted in

15   extensive atherosclerosis covering 20% of the aorta reflecting the baseline disease at the time of vector; this increased to 33% over an additional two month period following treatment with the AAV8.TBG.nLacZ vector, representing a 65% further progression in atherosclerosis. In contrast, treatment with the AAV8.TBG.mLDLR vector led to a regression of atherosclerosis by 87% over two months, from 20% of the aorta covered by

20   atherosclerosis at baseline to only 2.6% of the aorta covered by atherosclerosis 60 days after vector administration.

       In the second method, total lesion area was quantified in the aortic root (Figure 3C-F). This analysis revealed the same overall trends, with AAV8.TBG.nLacZ injected mice showing a 44% progression over 2 months compared to baseline mice, while

25   AAV8.TBG.mLDLR injected mice demonstrating a 64% regression in lesion compared with baseline mice. In summary, expression of LDLR via injection of AAV8.TBG.mLDLR induced marked reduction in cholesterol and substantial regression of atherosclerosis over two months as assessed by two independent methods of quantification at two different sites within the aorta.

30   7.4 Assessment of Minimal Effective Dose in a Mouse Model of HoFH

Extensive studies of the correlations between phenotypes and genotype in HoFH populations have demonstrated that differences in LDL and total cholesterol of only 25-30% translate to substantial differences in clinical outcome (Bertolini et al. 2013, Atherosclerosis 227(2): 342-348; Kolansky et al. 2008, Am J Cardiol 102(11): 1438-1443; Moorjani et al. 1993, The Lancet 341(8856): 1303-1306). Furthermore, lipid-lowering treatment associated with LDL-C reduction lower than 30%, translates to delayed cardiovascular events and prolonged survival in patients with HoFH (Raal et al. 2011, Circulation 124(20): 2202-2207). Recently, the FDA approved the drug mipomersen for the treatment of HoFH in which the primary endpoint was a reduction of LDL-C of 20 to 25% from baseline (Raal et al. 2010, Lancet 375(9719): 998-1006).

Against this background, the minimal effective dose (MED) in the gene therapy mouse studies discussed below was defined as the lowest dose of vector that lead to a statistically significant and stable reduction of total cholesterol in the serum that is at least 30% lower than baseline. The MED has been evaluated in a number of different studies and a brief description of each experiment is provided below.

7.4.1. POC Dose-ranging study of AAV8.TBG.mLDLR in DKO mice

A proof-of-concept dose-ranging study of AAV8.TBG.mLDLR and AAV8.TBG.hLDLR in DKO mice was conducted to identify suitable doses for further study. In these studies, DKO male mice were injected IV with different doses of AAV8.TBG.mLDLR ranging from 1.5 to 500×10^{11} GC/kg and followed for reductions in plasma cholesterol (Kassim et al., 2010, PLoS One 5(10): e13424). The GC doses used in these research experiments (1.5 to 500×10^{11}) were based on quantitative PCR (qPCR) titer. Statistically significant reductions of plasma cholesterol of up to 30% were observed at day 21 at a dose of AAV8.TBG.mLDLR of 1.5×10^{11} GC/kg, with greater reductions achieved in proportion to larger doses of vector (Kassim et al., 2010, PLoS One 5(10): e13424). Analyses of liver tissues harvested subsequent to metabolic correction revealed levels of mouse LDLR transgene and protein in proportion to the dose of vector. Thus, a dose-response correlation was observed.

7.4.2. Dose-ranging study of AAV8.TBG.hLDLR in DKO and LAHB mice

Similar proof-of-concept studies in the DKO mouse were performed with a vector that contained the human LDL receptor (hLDLR) gene rather than the mouse LDLR gene. The results with the hLDLR vector were very similar to those observed with the mLDLR in that the dose of vector was proportional to expression of the transgene and deposition of vector genomes in liver (Kassim et al. 2013, Hum Gene Ther 24(1): 19-26). The major difference was in its efficacy – the human LDLR vector was less potent in this model. Reductions of cholesterol close to at least 30% were achieved at $5x10^{12}$ GC/kg and $5x10^{11}$ GC/kg, (doses based on qPCR titer) although statistical significance was achieved only at the higher dose.

The reduced efficacy observed was attributable to the diminished affinity of human LDLR for the mouse ApoB. To by-pass this problem, studies were repeated using the LAHB mouse model that expresses the human ApoB100 and, therefore, more authentically models the interaction of human apoB100 with human LDLR relevant to human studies. Male mice of both strains (DKO vs. LAHB) received a tail vein injection of one of three vector doses of AAV8.TBG.hLDLR ($0.5x10^{11}$ GC/kg, $1.5x10^{11}$ GC/kg, and $5.0x10^{11}$ GC/kg based on qPCR titer). Animals from each cohort were bled on day 0 (prior to vector administration), day 7, and day 21 and evaluation of serum cholesterol level was performed. The human LDLR was much more effective in the LAHB mouse as compared to mLDLR in the DKO mouse: a 30% reduction of serum cholesterol was achieved at a dose of $1.5x10^{11}$ GC/kg, which is the same efficacy achieved with previous studies of the mouse LDLR construct in the DKO animals (Kassim et al. 2013, Hum Gene Ther 24(1): 19-26).

7.4.3. Non-clinical Pharmacology/Toxicology Study of AAV8.TBG.mLDLR and AAV8.TBG.hLDLR in a Mouse Model of HoFH

Male and female DKO mice (n = 280, 140 male and 140 female) 6-22 weeks of age received a tail vein injection of one of three vector doses of AAV8.TBG.mLDLR ($7.5x10^{11}$ GC/kg, $7.5x10^{12}$ GC/kg, $6.0x10^{13}$ GC/kg) or one dose of the intended gene therapy vector AAV8.TBG.hLDLR ($6.0x10^{13}$ GC/kg). Animals were

dosed based on genome copies (GC) per kilogram body weight using the oqPCR titration method, which is described herein at Section 8.4.1. An additional cohort of animals received PBS as a vehicle control. Animals from each cohort were sacrificed on day 3, day 14, day 90, and day 180 and blood was collected for evaluation of serum cholesterol

5   levels (Figure 4).

A rapid and significant reduction of cholesterol at all necropsy time points in all groups of treated mice was observed. This reduction appeared to be less in females than in males at low dose of vector at early time points, although this difference decreased with time and eventually there was no detectable difference between the sexes.

10   Each group demonstrated a statistically significant reduction in serum cholesterol of at least 30% relative to PBS controls at the same necropsy time point. Therefore, the determination of the MED based on this study is $\leq 7.5 \times 10^{11}$ GC/kg.

7.4.4. Efficacy Study of AAV8.TBG.hLDLR in a Mouse Model of Homozygous Familial Hypercholesterolemia

15   Male DKO mice (n=40) 12-16 weeks of age were administered IV with one of four doses (1.5×10^{11} GC/kg, 5.0×10^{11} GC/kg, 1.5×10^{12} GC/kg, 5.0×10^{12} GC/kg) of AAV8.TBG.hLDLR (doses based on the oqPCR titration method). Animals were bled on day 0 (prior to vector administration), day 7, and day 30 and evaluation of serum cholesterol (Figure 5). A rapid and significant reduction of cholesterol was observed on

20   days 7 and 30 in groups of mice treated with $\geq 5.0 \times 10^{11}$ GC/kg. The determination of the MED based on this study is between 1.5×10^{11} GC/kg and 5.0×10^{11} GC/kg.

7.5. Effects of AAV8.TBG.rhLDLR in LDLR+/- Rhesus Macaques on a High-fat Diet

Studies designed to evaluate AAV8-LDLR gene transfer in the FH

25   macaque were conducted. Following administration of 10^{13} GC/kg of AAV8.TBG.rhAFP (a control vector; dose based on qPCR titration method) into either fat-fed or chow fed wild type rhesus macaques, no elevations in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) values were seen. This suggests that AAV8 capsid itself is not responsible for triggering an inflammatory or

30   injurious hepatic process.

7.6.   Pilot Biodistribution Study of AAV8.TBG.hLDLR in a Mouse Model of HoFH

In order to assess the safety and pharmacodynamics properties of gene therapy for HoFH, pilot biodistribution (BD) studies were conducted in DKO mice. These studies examined vector distribution and persistence in five female DKO mice systemically administered $5x10^{12}$ GC/kg (dose based on qPCR titration method) of AAV8.TBG.hLDLR vector via one of two routes: 1) IV injection into the tail vein or 2) intra-portal injection. At two different time points (day 3 and day 28), a panel of tissues was harvested and total cellular DNA was extracted from harvested tissues. In these pilot studies, both the IV and intra-portal routes resulted in a comparable BD profile, supporting the rationale to infuse the gene therapy vector in patients and animals via peripheral vein.

7.7.   Toxicology

In order to assess the potential toxicity of gene therapy for HoFH, pharmacology/toxicology studies were conducted in DKO mice (a mouse model of HoFH), and wild type and LDLR+/- rhesus macaques. The studies include an examination of the role of LDLR transgene expression in vector associated toxicity in chow-fed wild type and LDLR+/- Rhesus Macaques, a pharmacology/toxicology study of AAV8.TBG.mLDLR and AAV8.TBG.hLDLR in a mouse model of HoFH, and an examination of the non-clinical biodistribution of AAV8.TGB.hLDLR in a mouse model of HoFH. These studies are described in detail below.

7.8.   Non-Clinical Study Examining the Role of LDLR Transgene Expression in Vector Associated Toxicity in Chow-fed Wild Type and LDLR+/- Rhesus Macaques

Four wild type and four LDLR+/- rhesus macaques were administered IV with $1.25x10^{13}$ GC/kg of AAV8.TBG.hLDLR (dose based on oqPCR titration method), Non human primates (NHPs) were monitored for up to one year post-vector administration. Four animals (two wild type and two LDLR+/-) were necropsied at day 28 post-vector administration to assess acute vector-associated toxicity and vector distribution and four animals (two wild type and two LDLR+/-) were necropsied at day

364/365 post-vector administration to assess long–term vector-associated pathology and vector distribution. Each cohort of wild type and LDLR+/- macaques had two males and two females.

The animals tolerated the infusion of vector well without long-term or short-term clinical sequelae. Biodistribution studies demonstrated high level and stable targeting of liver with far less, but still detectable, extrahepatic distribution, which declined over time. These data suggested that the target organ for efficacy, the liver, is also the most likely source of potential toxicity. A detailed review of tissues harvested at necropsy performed 28 and 364/365 days post-vector administration revealed some minimal to mild findings in liver and some evidence of atherosclerosis in the LDLR+/- macaques. The nature of the liver pathology and the fact that similar pathology was observed in one of the two untreated wild type animals suggested to the pathologist that they were unrelated to the test article.

One animal had persistent elevations in alanine aminotransferase (ALT) prior to vector administration, which continued after vector administration at levels that ranged from 58 to 169 U/L. The remaining animals demonstrated either no elevations in transaminases or only transient and low level increases in aspartate aminotransferase (AST) and ALT, never exceeding 103 U/L. The most consistent abnormalities were found after vector injection, suggesting they were related to the test article. Activation of T cells to human LDLR or to AAV8 capsid was assessed for correlation with AST/ALT increases. Figure 6 presents the AAV capsid ELISPOT data and serum AST levels in three selected animals that demonstrated relevant findings. Only one animal showed a correlation in which an increase in AST to 103 U/L corresponded to the appearance of T cells against capsid (Figure 6, animal 090-0263); the capsid T cell response persisted while the AST returned immediately to normal range.

Analysis of tissue-derived T cells for presence of capsid and transgene-specific T cells showed that liver derived T cells became responsive to capsid from both genotypes (wild type and LDLR+/-) by the late time point while T cells to human LDLR were detected in the LDLR+/- animals at this late time point. This suggests that PBMCs are not reflective of the T cell compartment in the target tissue. Liver tissue harvested at days 28 and at 364/365 was analyzed for expression of the

transgene by RT-PCR and did appear to be affected by the abnormalities in clinical pathology or the appearance of T cells.

Neither the wild type nor LDLR+/- animals developed hypercholesterolemia on chow diet. Dose-Limiting Toxicities (DLTs) were not observed at a dose of 1.25×10^{13} GC/kg (based on oqPCR), implying that the maximal tolerated dose (MTD) would be equal to or greater than this dose. Test article related elevations in transaminases were observed, which were low and transient but nevertheless present. Accordingly, the no-observed-adverse-effect-level (NOAEL) is less than the single high dose evaluated in Example 1 herein.

7.9. Non-Clinical Pharmacology/Toxicology Study of AAV8.TBG.mLDLR and AAV8.TBG.hLDLR in a Mouse Model of HoFH

This study was conducted in the DKO mice because using this strain would allow, 1) evaluation of proof-of-concept efficacy in parallel with toxicity, and 2) evaluation of vector-associated toxicity in the setting of any pathology associated with the defect in LDLR and the associated dyslipidemia and its sequelae, such as steatosis.

The study was designed to test AAV8.TBG.hLDLR at the highest dose, which is 8-fold higher than the highest dose for administration to human subjects with HoFH, as set forth in Example 1. A version of the vector that expresses the murine LDLR was tested at this high dose, as well as two lower doses, to provide an assessment of the effect of dose on toxicity parameters, as well as reduction in cholesterol. The dose-response experiment was performed with the vector expressing murine LDLR to be more reflective of the toxicity and efficacy that would be observed in humans using the human LDLR vector.

In this study, male and female DKO mice aged 6-22 weeks were administered with one of the doses of AAV8.TBG.mLDLR (7.5×10^{11} GC/kg, 7.5×10^{12} GC/kg and 6.0×10^{13} GC/kg) or 6.0×10^{13} GC/kg of the vector (AAV8.TBG.hLDLR) (doses based on the oqPCR titration method). Animals were necropsied at day 3, day 14, day 90, and day 180 post-vector administration; these times were selected to capture the vector expression profile of the test article as well as acute and chronic toxicity. Efficacy

of transgene expression was monitored by measurement of serum cholesterol levels. Animals were evaluated for comprehensive clinical pathology, immune reactions to the vector (cytokines, NAbs to AAV8 capsid, and T cell responses against both capsid and transgene), and tissues were harvested for a comprehensive histopathological

5   examination at the time of necropsy.

The key toxicology findings from this study are as follows:

• No clinical sequelae were observed in the treated groups

• Clinical pathology:

                    o *Transaminases:* Abnormalities were limited to elevations of the
10   liver function tests AST and ALT that ranged from 1-4x ULN and were
                    primarily found at day 90 of all doses of murine LDLR vector. There was
                    no elevation of transaminases in the group administered high dose human
                    LDLR vector, except for <2x ULN of ALT in a few male animals. The
                    abnormalities associated with the mouse vector were mild and not dose-
15   dependent and, therefore, were not believed to be related to vector. There
                    were essentially no findings associated with the high dose human vector.
                    There was no evidence of treatment related toxicity based on these
                    findings, meaning that the no adverse effect level (NOAEL) based on
                    these criteria is 6.0×10^{13} GC/kg.

20   • *Pathology:* There were no gross pathology findings. Histopathology was limited
        to minimal or mild findings in liver as follows:

                    o Animals administered with PBS had evidence of minimal and/or
                    mild abnormalities according to all criteria evaluated. In assessing
                    treatment related pathology we focused on any finding categorized as
25   mild that was above that found in PBS injected animals.

                    o Mild bile duct hyperplasia and sinusoidal cell hyperplasia was
                    observed in high dose female mice administered the mouse and human
                    LDLR vectors. This could represent vector related effects observed only
                    at the high dose.

30   o Centrilobular hypertrophy was mild, only in males and not at
                    high doses of vector arguing that it not vector related.

o Minimal necrosis was found in 1/7 males and 3/7 females at day

180 in the high dose human LDLR vector.

o Based on the finding of mild bile duct and sinusoidal hyperplasia

at the high dose of vector, and a few examples of minimal necrosis in the

high dose human LDLR vector, that the NOAEL based on these criteria is

between 7.5×10^{12} GC/kg and 6.0×10^{13} GC/kg.

• *Other findings*: The animals developed an increase in NAbs to AAV8 and

evidence of very low T cell response based on an IFN-γ ELISPOT to capsid and LDLR

following administration of the high dose of the human LDLR vector. There was little

evidence of an acute inflammatory response based on analysis of serum 3 and 14 days

after vector; a few cytokines did show modest and transient elevations although there

was no increase in IL6.

One notable finding was that toxicity was not worse in DKO mice treated with

the mouse LDLR vector than with the human LDLR vector, which could have been the

case if the human LDLR was more immunogenic in terms of T cells than the mouse

transgene. ELISPOT studies did show some activation of LDLR-specific T cells in mice

administered with the high dose vector expressing the human transgene, although they

were low and in a limited number of animals supporting the toxicity data, which

suggested this mechanism of host response would unlikely contribute to safety concerns.

In conclusion, there were no dose-limited toxicities, meaning the maximally

tolerated dose was higher than the highest dose tested which was 6.0×10^{13} GC/kg. Based

on mild and reversible findings in liver pathology at the highest dose, the NOAEL is

somewhere between 6.0×10^{13} GC/kg, where in liver mild reversible pathology was

observed, down to 7.5×10^{12} GC/kg, where there was no clear indication of vector related

findings.

7.10. Non-Clinical Biodistribution of AAV8.TGB.hLDLR in a Mouse Model of HoFH

Male and female DKO mice 6-22 weeks of age were administered IV

with 7.5×10^{12} GC/kg (dose measured by oqPCR titration method) of

AAV8.TBG.hLDLR, the highest dose for treating human subjects in Example 1 f.

Animals were necropsied for biodistribution assessment on day 3, day 14, day 90, and

day 180 post-vector administration. In addition to blood, 20 organs were harvested. The distribution of vector genomes in organs was assessed by quantitative, sensitive PCR analysis of total genomic DNA harvested. One sample of each tissue included a spike of control DNA, including a known amount of the vector sequences, in order to assess the adequacy of the PCR assay reaction.

The vector GC number in liver was substantially higher in liver than in other organs/tissues, which is consistent with the high hepatotropic properties of the AAV8 capsid. For example, vector genome copies in the liver were at least 100-fold greater than that found in any other tissue at day 90. There was no significant difference between male or female mice at the first three time points. GC number decreased over time in the liver until day 90, where it then stabilized. A similar trend of decline was observed in all tissues but the decline in vector copy number was more rapid in tissues with higher cell turnover rate. Low but detectable levels of vector genome copies were present in the gonads of both genders and the brain.

The biodistribution of AAV8.TBG.hLDLR in DKO mice was consistent with published results with AAV8. Liver is the target primary target of gene transfer following IV infusion and genome copies in liver do not decline significantly over time. Other organs are targeted for vector delivery, although the levels of gene transfer in these non-hepatic tissues are substantially lower and decline over time. Therefore, the data presented here suggest that the primary organ system to be evaluated is the liver.

7.11.   Conclusions from Non-clinical Safety Studies

The rhesus macaque and DKO mouse studies confirmed that high dose vector is associated with low level, transient, and asymptomatic liver pathology evident by transient elevations in transaminases in NHPs, and in mice by transient appearance of mild bile duct and sinusoidal hypertrophy. No other toxicity felt to be due to the vector was observed.

There were no DLTs observed at doses as high as 1.25×10^{13} GC/kg in macaques and 6×10^{13} GC/kg in DKO mice. Determination of the NOAEL focus primarily on liver toxicity as reflected in elevations in transaminases in macaques and histopathology in DKO mice. This translated to an NOAEL of less than 1.25×10^{13} GC/kg

in macaques and less than 6×10^{13} GC/kg but greater than 7.5×10^{12} GC/kg in DKO mice. The doses were based on the oqPCR titration method.

7.12. Overall assessment of non-clinical data to support human treatment

The key findings that emerged from the pharmacology and toxicology studies that have informed the dose selection and design for the clinical study, are the following:

• Minimal Effective Dose (MED): The MED was defined in nonclinical studies as a GC/kg dose that resulted in a 30% reduction in serum cholesterol. Two IND-enabling nonclinical studies established the MED to be between 1.5 to 5.0×10^{11} GC/kg . The mouse pharmacology/toxicology study demonstrated a statistically significant reduction in serum cholesterol of at least 30% relative to PBS controls, allowing estimation of a MED $\leqslant 7.5\times10^{11}$ GC/kg. The observed dose-response relationship allowed determination of the MED to be between 1.5 to 5.0×10^{11} GC/kg as determined by oqPCR.

• Maximum Tolerated Dose (MTD): The MTD was defined in nonclinical studies as the GC/kg dose that did not result in a dose limiting toxicity (DLT). DLTs were not observed in the toxicology studies at the highest doses tested, which were 6.0×10^{13} GC/kg in DKO mice and 1.25×10^{13} GC/kg in macaques as determined by oqPCR. Our results suggested that the actual MTD is higher than these doses.

• No Observed Adverse Event Level (NOAEL): This was determined to be 7.5×10^{12} GC/kg in the DKO mice. This was based on minimal to mild histopathologic findings, predominantly in the liver (bile duct and sinusoidal hyperplasia, minimal necrosis), observed at higher doses of the human LDLR (hLDLR) transgene. Only one dose was tested in macaques; however the toxicity at 1.25×10^{13} GC/kg was mild, including transient and low level increases in AST and ALT, suggesting the true NOAEL would be achieved at a dose lower than the dose tested.

Based on these data, we arrived at two doses: a single dose of 2.5×10^{12} GC/kg or a single dose of 7.5×10^{12} GC/kg (doses based on the oqPCR titration method). The

highest dose proposed to test in the clinic is lower than the highest dose tested in the macaque toxicology study and 8- fold lower than the highest dose tested in DKO mice – neither of which was considered to be the MTD. A dose that is at least 5-fold higher than the MED is proposed, suggesting that patients who participate in the low dose

5   cohort could potentially obtain some benefit. The lower dose is also approximately 3-fold lower than the NOAEL dose in DKO mice and 5-fold lower than the dose tested in macaques.

8.   Example 3: Manufacture of AAV8.TBG.hLDLR

The AAV8.TBG.hLDLR vector consists of the AAV vector active

10 ingredient and a formulation buffer. The external AAV vector component is a serotype 8, $T = 1$ icosahedral capsid consisting of 60 copies of three AAV viral proteins, VP1, VP2, and VP3, at a ratio of 1:1:18. The capsid contains a single-stranded DNA recombinant AAV (rAAV) vector genome (Figure 7). The genome contains a human low density lipoprotein receptor (LDLR) transgene flanked by the two AAV inverted terminal

15 repeats (ITRs). An enhancer, promoter, intron, human LDLR coding sequence and polyadenylation (polyA) signal comprise the human LDLR transgene. The ITRs are the genetic elements responsible for the replication and packaging of the genome during vector production and are the only viral *cis* elements required to generate rAAV. Expression of the human LDLR coding sequence is driven from the hepatocyte-specific

20 thyroxine-binding globulin (TBG) promoter. Two copies of the alpha 1 microglobulin/bikunin enhancer element precede the TBG promoter to stimulate promoter activity. A chimeric intron is present to further enhance expression and a rabbit beta globin polyA signal is included to mediate termination of human LDLR mRNA transcripts. The vector is supplied as a suspension of AAV8.TBG.hLDLR

25 vector in formulation buffer. The formulation buffer is 180 mM NaCl, 10 mM sodium phosphate, 0.001% Poloxamer 188, pH 7.3.

Details of the vector manufacturing and characterization of the vectors, are described in the sections below.

30

8.1.   Plasmids used to Produce AAV8.TBG.hLDLR

The plasmids used for production of AAV8.TBG.hLDLR are as follows:

8.1.1 Cis plasmid (vector genome expression construct):

pENN.AAV.TBG.hLDLR.RBG.KanR containing the human LDLR expression cassette (Figure 8). This plasmid encodes the rAAV vector genome. The

5   polyA signal for the expression cassette is from the rabbit β globin gene. Two copies of the alpha 1 microglobulin /bikunin enhancer element precede the TBG promoter.

To generate the *cis* plasmid used for production of AAV8.TBG.hLDLR, the human LDLR cDNA was cloned into an AAV2 ITR-containing construct, pENN.AAV.TBG.PI to create pENN.AAV.TBG.hLDLR.RBG. The plasmid backbone

10   in pENN.AAV.TBG.PI was originally from, pZac2.1, a pKSS-based plasmid. The ampicillin resistance gene in pENN.AAV.TBG.hLDLR.RBG was excised and replaced with the kanamycin gene to create pENN.AAV.TBG.hLDLR.RBG.KanR. Expression of the human LDLR cDNA is driven from the TBG promoter with a chimeric intron (Promega Corporation, Madison, Wisconsin). The polyA signal for the expression

15   cassette is from the rabbit β globin gene. Two copies of the alpha 1 microglobulin /bikunin enhancer element precede the TBG promoter.

Description of the Sequence Elements

1.   *Inverted terminal repeats (ITR)*: AAV ITRs (GenBank # NC001401) are sequences that are identical on both ends, but found in opposite orientation. The AAV2

20   ITR sequences function as both the origin of vector DNA replication and the packaging signal for the vector genome, when AAV and adenovirus (ad) helper functions are provided in *trans*. As such, the ITR sequences represent the only *cis* acting sequences required for vector genome replication and packaging.

2.   *Human alpha 1 microglobulin/bikunin enhancer* (2 copies; 0.1Kb) ; Genbank #

25   X67082) This liver specific enhancer element serves to lend liver-specificity and enhance expression from the TBG promoter.

3.   *Human thyroxine-binding globulin (TBG) promoter* (0.46Kb; Gen bank # L13470) This hepatocyte-specific promoter drives the expression of the human LDLR coding sequence

30   4.   *Human LDLR cDNA* (2.58Kb; Genbank # NM000527, complete CDS). The human LDLR cDNA encodes a low density lipoprotein receptor of 860 amino acids

with a predicted molecular weight of 95kD and an apparent molecular weight of 130 kD by SDS-PAGE.

5.   *Chimeric intron* (0.13Kb; Genbank # U47121; Promega Corporation, Madison, Wisconsin) The chimeric intron consists of a 5′-donor site from the first intron of the human β-globin gene and the branch and 3′-acceptor site from the intron located between the leader and body of an immunoglobulin gene heavy chain variable region. The presence of an intron in an expression cassette has been shown to facilitate the transport of mRNA from the nucleus to the cytoplasm, thus enhancing the accumulation of the steady level of mRNA for translation. This is a common feature in gene vectors intended to mediate increased levels of gene expression.

6.   *Rabbit beta-globin polyadenylation signal*: (0.13Kb; GenBank # V00882.1) The rabbit beta-globin polyadenylation signal provides *cis* sequences for efficient polyadenylation of the antibody mRNA. This element functions as a signal for transcriptional termination, a specific cleavage event at the 3' end of the nascent transcript followed by addition of a long polyadenyl tail.

8.1.2 Trans plasmid (packaging construct): pAAV2/8(Kan), containing the AAV2 *rep* gene and AAV8 *cap* gene (Figure 9).

The AAV8 *trans* plasmid pAAV2/8(Kan) expresses the AAV2 replicase (*rep*) gene and the AAV8 capsid (*cap*) gene encoding virion proteins, VP1, VP2 and VP3. The AAV8 capsid gene sequences were originally isolated from heart DNA of a rhesus monkey (GenBank accession AF513852). To create the chimeric packaging constructs, plasmid p5E18, containing AAV2 *rep* and *cap* genes, was digested with *Xba*I and *Xho*I to remove the AAV2 *cap* gene. The AAV2 *cap* gene was then replaced with a 2.27Kb *Spe*I/*Xho*I PCR fragment of the AAV8 *cap* gene to create plasmid p5E18VD2/8 (Figure 9a). The AAV p5 promoter, which normally drives *rep* expression is relocated in this construct from the 5' end of *rep* gene to the 3' end of the *cap* gene. This arrangement serves to down-regulate expression of *rep* in order to increase vector yields. The plasmid backbone in p5E18 is from pBluescript KS. As a final step, the ampicillin resistance gene was replaced by the kanamycin resistance gene to create pAAV2/8(Kan) (FIG 9B). The entire pAAV2/8(Kan) *trans* plasmid has been verified by direct sequencing.

8.1.3 Adenovirus helper plasmid: pAdΔF6(Kan)

Plasmid pAdΔF6(Kan) is 15.7Kb in size and contains regions of the adenoviral genome that are important for AAV replication, namely E2A, E4, and VA RNA. pAdΔF6(Kan) does not encode any additional adenoviral replication or structural genes and does not contain *cis* elements, such as the adenoviral ITRs, that are necessary

5   for replication, therefore, no infectious adenovirus is expected to be generated. Adenoviral E1 essential gene functions are supplied by the HEK293 cells in which the rAAV vectors are produced. pAdΔF6(Kan) was derived from an E1, E3 deleted molecular clone of Ad5 (pBHG10, a pBR322 based plasmid). Deletions were introduced in the Ad5 DNA to remove unnecessary adenoviral coding regions and

10   reduce the amount of adenoviral DNA from 32Kb to 12Kb in the resulting ad-helper plasmid. Finally, the ampicillin resistance gene was replaced by the kanamycin resistance gene to create pAdΔF6(Kan) (Figure 10). DNA plasmid sequencing was performed by Qiagen Sequencing Services, Germany and revealed 100% homology between the reference sequence for pAdDeltaF6(Kan) and the following adenoviral

15   elements: p1707FH-Q: E4 ORF6 3.69-2.81Kb; E2A DNA binding protein 11.8–10.2Kb; VA RNA region 12.4–13.4Kb.

Each of the *cis, trans* and ad-helper plasmids described above contains a kanamycin-resistance cassette, therefore, β -lactam antibiotics are not used in their production.

8.1.4 Plasmid Manufacturing

20   All plasmids used for the production of vectors were produced by Puresyn Inc. (Malvern, PA). All growth media used in the process is animal free. All components used in the process, including fermentation flasks, containers, membranes, resin, columns, tubing, and any component that comes into contact with the plasmid, are dedicated to a single plasmid and are certified BSE-free. There are no shared components

25   and disposables are used when appropriate.

8.2. Cell Banks

AAV8.TBG.hLDLR vector was produced from a HEK293 working cell bank which was derived from a fully characterized master cell bank. The manufacturing and

30   testing details of both cell banks appears below.

8.2.1 HEK293 Master Cell Bank

HEK293 Master Cell Bank (MCB) is a derivative of primary human embryonic kidney cells (HEK) 293. The HEK293 cell line is a permanent line transformed by sheared human adenovirus type 5 (Ad5) DNA (Graham et al., 1977, Journal of General Virology 36(1): 59-72). The HEK293 MCB has been tested extensively for microbial and viral contamination. The HEK293 MCB is currently stored in liquid nitrogen. Additional testing was performed on the HEK293 MCB to demonstrate the absence of specific pathogens of human, simian, bovine, and porcine origin. The human origin of the HEK293 MCB was demonstrated by isoenzyme analysis.

Tumorigenicity testing was also performed on the HEK293 MCB by evaluating tumor formation in nude (nu/nu) athymic mice following subcutaneous injection of the cell suspension. In this study, fibrosarcoma was diagnosed at the injection site in ten of ten positive control mice and carcinoma was diagnosed at the injection site in ten of ten test article mice. No neoplasms were diagnosed in any of the negative control mice. The HEK293 MCB L/N 3006-105679 was also tested for the presence of Porcine Circovirus (PCV) Types 1 and 2.The MCB was found negative for PCV types 1 and 2.

8.2.2 HEK293 Working Cell Bank

The HEK293 Working Cell Bank (WCB) was manufactured using New Zealand sourced Fetal Bovine Serum, FBS (Hyclone PN SH30406.02) certified for suitability in accordance with the European Pharmacopea monograph. The HEK293 WCB was established using one vial (1mL) of the MCB as seed material. Characterization tests were performed and the test results are listed in

Table 4.1.

Table 4.1 Characterization of HEK293 WCB.

Test	Method	Study Number	Result
Test for the presence of agar-cultivable and non-agar	*In vivo*	BioReliance AD61FS.102063GMP.BSV	No mycoplasma detected

Test	Method	Study Number	Result
cultivable mycoplasma USP, EP, 1993 PTC			
Qualification of the test for agar-cultivable and non-agar cultivable mycoplasma USP, EP, 1993 PTC/JP	*In vivo*	BioReliance AD61FS.102062GMP.BSV	No Mycoplasmastasis observed
Isolator sterility testing, USP <71>, 21 CFR 610.12	Direct inoculation	BioReliance AD61FS.510120GMP.BSV	No bacterial or fungal growth
Test for presence of inapparent viruses	*In vivo*	BioReliance AD61FS.005002GMP.BSV	Negative
28-day assay for the presence of viral contaminants	*In vitro*	BioReliance AD61FS.003800.BSV	Negative
Cell culture identification and characterization	Isoenzyme analysis	BioReliance AD61FS.380801.BSV	Human

8.3. Vector Manufacturing

General descriptions of the vector manufacturing processes are given below and are also reflected in a flow diagram in Figure 11.

8.3.1 Vector Generation Process (Upstream Process)

8.3.1.1 Initiation of HEK293 WCB cell culture into a T-flask (75cm²)

One vial of HEK293 cells from the WCB containing 10^7 cells in 1mL is thawed
at 37 °C and seeded in a 75 cm² tissue culture flask containing DMEM High Glucose
5   supplemented with 10% fetal bovine serum (DMEM HG/10% FBS). The cells are then
placed in a 37 °C /5% CO2 incubator, and grown to ~70% confluence with daily direct
visual and microscopic inspection to assess cell growth. These cells are designated
Passage 1 and are passaged to generate a cell seed train for vector biosynthesis for up to
~10 weeks as described below. The passage number is recorded at each passage and the
10   cells are discontinued after passage 20. If additional cells are required for vector
biosynthesis, a new HEK293 cell seed train is initiated from another vial of the HEK293
WCB.

8.3.1.2 Passage of cells into ~2 T-flasks (225 cm²)

When the HEK293 cells growing in the T75 flask are ~70% confluent, the cells
15   are detached from the surface of the flask using recombinant trypsin (TrypLE) and
seeded in two T225 flasks containing DMEM HG/10% FBS. Cells are placed in the
incubator and grown to ~70% confluence. Cells are monitored for cell growth, absence
of contamination, and consistency by visual inspection and using a microscope.

8.3.1.3 Passage of cells into ~10 T-flasks (225 cm²)

20   When the HEK293 cells growing in the two T225 flask are ~70% confluent, the
cells are detached using recombinant trypsin (TrypLE), and seeded at a density of ~3×10^6
cells per flask in ten 225cm2 T-flasks containing DMEM HG/10% FBS. Cells are placed
in a 37°C/5% CO2 incubator and grown to ~70% confluence. Cells are monitored for cell
growth, absence of contamination, and consistency by direct visual inspection and using
25   a microscope. Cells are maintained by serial passaging in T225 flasks to maintain the cell
seed train and to provide cells for expansion to support manufacture of subsequent vector
batches.

8.3.1.4 Passage of cells into ~10 roller bottles

When the HEK293 cells growing in ten T225 flasks are ~70% confluent, the cells
30   are detached using recombinant trypsin (TrypLE), counted and seeded in 850cm² roller

bottles (RB) containing DMEM HG/10% FBS. The RBs are then placed in the RB
incubator and the cells grown to ~70% confluence. RBs are monitored for cell growth,
absence of contamination, and consistency by direct visual inspection and using a
microscope.

8.3.1.5 Passage of cells into ~100 roller bottles

When the HEK293 cells growing in RBs prepared as described in the previous
process step are ~70% confluent, they are detached using recombinant trypsin (TrypLE),
counted and seeded in 100 RBs containing DMEM/10% FBS. The RBs are then placed
in the RB incubator (37 °C, 5% CO_2) and grown to ~70% confluence. Cells are
monitored for cell growth, absence of contamination, and consistency by direct visual
inspection and using a microscope.

8.3.1.6 Transfection of cells with plasmid DNA

When the HEK293 cells growing in 100 RBs are ~70% confluent, the cells are
transfected with each of the three plasmids: the AAV serotype-specific packaging (*trans*)
plasmid, the ad-helper plasmid, and vector *cis* plasmid containing the expression cassette
for the human LDLR gene flanked by AAV inverted terminal repeats (ITRs).
Transfection is carried out using the calcium phosphate method (For plasmid details, see
Section 4.1.1). The RBs are placed in the RB incubator (37°C, 5% CO_2) overnight.

8.3.1.7. Medium exchange to serum free medium

After overnight incubation of 100 RBs following transfection, the DMEM/10%
FBS culture medium containing transfection reagents is removed from each RB by
aspiration and replaced with DMEM-HG (without FBS). The RBs are returned to the RB
incubator and incubated at 37 °C, 5% CO_2 until harvested.

8.3.1.8. Vector Harvest

RBs are removed from the incubator and examined for evidence of transfection
(transfection-induced changes in cell morphology, detachment of the cell monolayer) and
for any evidence of contamination. Cells are detached from the RB surface by agitation
of each RB, and then harvested by decanting into a sterile disposable funnel connected to
a BioProcess Container (BPC). The combined harvest material in the BPC is labeled

'Product Intermediate: Crude Cell Harvest' and samples are taken for (1) in-process bioburden testing and (2) bioburden, mycoplasma, and adventitious agents product release testing. The Product Intermediate batch labeled as Crude Cell Harvest (CH) is stored at 2–8°C until further processed.

5 ## 8.3.2 Vector Purification Process (Downstream Process)

While a common, 'platform' purification process is used for all of the AAV serotypes (i.e. incorporating the same series and order of steps), each serotype requires unique conditions for the chromatography step, a requirement that also impacts some details (buffer composition and pH) of the steps used to prepare the clarified cell lysate

10 applied to the chromatography resin.

8.3.2.1 AAV8 Vector Harvest Concentration and Diafiltration by TFF

The BPC containing Crude CH is connected to the inlet of the sanitized reservoir of a hollow fiber (100k MW cut-off) TFF apparatus equilibrated with phosphate-buffered saline. The Crude CH is applied to the TFF apparatus using a peristaltic pump and

15 concentrated to 1-2 L. The vector is retained (retentate) while small molecular weight moieties and buffer pass through the TFF filter pore and are discarded. The harvest is then diafiltered using the AAV8 diafiltration buffer. Following diafiltration, the concentrated vector is recovered into a 5L BPC. The material is labeled 'Product Intermediate: Post Harvest TFF', and a sample taken for in-process bioburden testing.

20 The concentrated harvest is further processed immediately or stored at 2-8C until further processing.

8.3.2.2 Microfluidization and Nuclease Digestion of Harvest

The concentrated and diafiltered harvest is subjected to shear that breaks open intact HEK293 cells using a microfluidizer. The microfluidizer is sanitized with 1N

25 NaOH for a minimum of 1h after each use, stored in 20% ethyl alcohol until the next run, and rinsed with WFI prior to each use. The crude vector contained in the BPC is attached to the sanitized inlet port of the microfluidizer, and a sterile empty BPC is attached to the outlet port. Using air pressure generated by the microfluidizer, vector-containing cells are passed through the microfluidizer interaction chamber (a convoluted 300μm diameter

30 pathway) to lyse cells and release vector. The microfluidization process is repeated to

ensure complete lysis of cells and high recovery of vector. Following the repeat passage of the product intermediate through the microfluidizer, the flowpath is rinsed with ~500mL of AAV8 Benzonase Buffer. The 5L BPC containing microfluidized vector is detached from the outlet port of the microfluidizer. The material is labeled 'Product Intermediate: Final Microfluidized', and samples are taken for in-process bioburden testing. The microfluidized product intermediate is further processed immediately or stored at 2-8 °C until further processing. Nucleic acid impurities are removed from AAV8 particles by additional of 100 U/mL Benzonase®. The contents of the BPC are mixed and incubated at room temperature for at least 1 hour. Nuclease digested product intermediate is processed further.

8.3.2.3 Filtration of Microfluidized Intermediate

The BPC containing microfluidized and digested product intermediate is connected to a cartridge filter with a gradient pore size starting at 3μm going down to 0.45μm. The filter is conditioned with AAV Benzonase Buffer. Using the peristaltic pump, the microfluidized product intermediate is passed through the cartridge filter and collected in the BPC connected to the filter outlet port. Sterile AAV8 Benzonase Buffer is pumped through the filter cartridge to rinse the filter. The filtered product intermediate is then connected to a 0.2μm final pore size capsule filter conditioned with AAV8 Benzonase Buffer. Using the peristaltic pump, the filtered intermediate is passed through the cartridge filter and collected in the BPC connected to the filter outlet port. A volume of sterile AAV8 Benzonase Buffer is pumped through the filter cartridge to rinse the filter. The material is labeled 'Product Intermediate: Post MF 0.2μm Filtered', and samples taken for in-process bioburden testing. The material is stored overnight at 2-8°C until further processing. An additional filtration step may be performed on the day of chromatography prior to application of the clarified cell lysate to the chromatography column.

8.3.2.4 Purification by Anion-Exchange Chromatography

The 0.2 μm filtered Product Intermediate is adjusted for NaCl concentration by adding Dilution Buffer AAV8. The cell lysate containing vector is next purified by ion exchange chromatography using ion exchange resin. The GE Healthcare AKTA Pilot

chromatography system is fitted with a BPG column containing approximately 1L resin
bed volume. The column is packed using continuous flow conditions and meets
established asymmetry specifications. The system is sanitized according to the
established procedure and is stored in 20% ethyl alcohol until the next run. Immediately
5   prior to use, the system is equilibrated with sterile AAV8 Wash Buffer. Using aseptic
techniques and sterile materials and components, the BPC containing clarified cell lysate
is connected to the sanitized sample inlet port, and BPC's containing bioprocessing
buffers listed below are connected to sanitized inlet ports on the AKTA Pilot. All
connections during the chromatography procedure are performed aseptically. The
10   clarified cell lysate is applied to the column and rinsed using AAV8 Wash Buffer. Under
these conditions, vector is bound to the column, and impurities are rinsed from the resin.
AAV8 particles are eluted from the column by application of AAV8 Elution buffer and
collected into a sterile plastic bottle. The material is labeled 'Product Intermediate and
samples are taken for in-process bioburden testing. The material is further processed
15   immediately.

8.3.2.5 Purification by CsCl Gradient Ultracentrifugation

The AAV8 particles purified by anion exchange column chromatography as
described above contain empty capsids and other product related impurities. Empty
capsids are separated from vector particles by cesium chloride gradient
20   ultracentrifugation. Using aseptic techniques, cesium chloride is added to the vector
'Product Intermediate' with gentle mixing to a final concentration corresponding to a
density of 1.35 g/mL. The solution is filtered through a 0.2μm filter, distributed into
ultracentrifugation tubes, and subjected to ultracentrifugation in a Ti50 rotor for
approximately 24h at 15°C. Following centrifugation, the tubes are removed from the
25   rotor, wiped with Septihol, and brought into the BSC. Each tube is clamped in a stand
and subjected to focused illumination to assist in visualization of bands. Two major
bands are typically observed, the upper band corresponding to empty capsids, and the
lower band corresponding to vector particles. The lower band is recovered from each
tube with a sterile needle attached to a sterile syringe. Vector recovered from each tube is
30   combined, and samples are taken for in-process bioburden, endotoxin, and vector titer.
The pooled material is distributed into sterile 50mL polypropylene conical tubes labeled

'Product Intermediate: Post CsCl Gradient', and stored immediately at -80°C until the next process step.

8.3.2.6 Buffer Exchange by Tangential Flow Filtration

After testing and release for pooling, batches of vector purified through the CsCl banding process step are combined and subjected to diafiltration by TFF to produce the Bulk Vector. Based on titering of samples obtained from individual batches, the volume of the pooled vectors is adjusted using calculated volume of sterile diafiltration buffer. Depending on the available volume, aliquots of the pooled, concentration adjusted vector are subjected to TFF with single use, TFF devices. Devices are sanitized prior to use and then equilibrated in Diafiltration buffer. Once diafiltration process is complete, the vector is recovered from the TFF apparatus in a sterile bottle. The material is labeled "Pre-0.2 μm Filtration Bulk". The material is further processed immediately.

8.3.2.7 Formulation and 0.2μm Filtration to Prepare Bulk Vector

Batches prepared by individual TFF units are pooled together and mixed by gentle swirling in a 500mL sterile bottle. The pooled material is then passed through a 0.22μm filter to prepare the Bulk Vector. The pooled material is sampled for Bulk Vector and reserved QC testing, and then aliquoted into sterile 50mL polypropylene tubes, labeled 'Bulk Vector', and stored at -80°C until the next step.

8.4. Testing of Vector

Characterization assays including serotype identity, empty particle content and transgene product identity are performed. Descriptions of all the assays appear below.

8.4.1 Genomic Copy (GC) Titer

An optimized quantitative PCR (oqPCR) assay is used to determine genomic copy titer by comparison with a cognate plasmid standard. The oqPCR assay utilizes sequential digestion with DNase I and Proteinase K, followed by qPCR analysis to measure encapsidated vector genomic copies. DNA detection is accomplished using sequence specific primers targeting the RBG polyA region in combination with a fluorescently tagged probe hybridizing to this same region. Comparison to the plasmid DNA standard curve allows titer determination without the need of any post-PCR sample

manipulation. A number of standards, validation samples and controls (for background and DNA contamination) have been introduced into the assay. This assay has been qualified by establishing and defining assay parameters including sensitivity, limit of detection, range of qualification and intra and inter assay precision. An internal AAV8 reference lot was established and used to perform the qualification studies.

8.4.2 Potency Assay

An *in vivo* potency assay was designed to detect human LDLR vector-mediated reduction of total cholesterol levels in the serum of a double knock-out (DKO) LDLR-/-Apobec-/- mouse model of HoFH. The basis for the development of the *in vivo* potency assay is described in section 4.3.5.11. To determine the potency of the AAV8.TBG.hLDLR vector, 6-20 week old DKO mice are injected IV (via tail vein) with 5×10^{11}GC/kg per mouse of the vector diluted in PBS. Animals are bled by retroorbital bleeds and serum total cholesterol levels are evaluated before and after vector administration (day 14 and 30) by Antech GLP. Total cholesterol levels in vector-administered animals are expected to decline by 25% - 75% of baseline by day 14 based on previous experience with vector administration at this dose. The 5×10^{11}GC/kg per mouse dose was chosen for the clinical assay based on the anticipated range of total cholesterol reduction which would allow for the evaluation of changes in vector potency over the course of stability testing.

8.4.3 Vector Capsid Identity: AAV Capsid Mass spectrometry of VP3

Confirmation of the AAV2/8 serotype of the vector is achieved by an assay based upon analysis of peptides of the VP3 capsid protein by mass spectrometry (MS). The method involves multi-enzyme digestion (trypsin, chymotrypsin and endoproteinase Glu-C) of the VP3 protein band excised from SDS-PAGE gels followed by characterization on a UPLC-MS/MS on a Q-Exactive Orbitrap mass spectrometer to sequence the capsid protein. A tandem mass spectra (MS) method was developed that allows for identification of certain contaminant proteins and deriving peptide sequence from mass spectra.

8.4.4 Empty to Full Particle Ratio

Vector particle profiles using analytical ultracentrifugation (AUC) Sedimentation velocity as measured in an analytical ultracentrifuge are an excellent method for obtaining information about macromolecular structure heterogeneity, difference in confirmation and the state of association or aggregation. Sample was loaded into cells and sedimented at 12000 RPM in a Beckman Coulter Proteomelab XL-I analytical ultracentrifuge. Refractive index scans were recorded every two minutes for 3.3 hours. Data are analyzed by a c(s) model (Sedfit program) and calculated sedimentation coefficients plotted versus normalized c(s) values. A major peak representing the monomeric vector should be observed. The appearance of peaks migrating slower than the major monomeric peak indicate empty/misassembled particles. The sedimentation coefficient of the empty particle peak is established using empty AAV8 particle preparations. Direct quantitation of the major monomeric peak and preceding peaks allow for the determination of the empty to full particle ratio.

8.4.5 Infectious Titer

The infectious unit (IU) assay is used to determine the productive uptake and replication of vector in RC32 cells (rep2 expressing HeLa cells). Briefly, RC32 cell in 96 well plates are co-infected by serial dilutions of vector and a uniform dilution of Ad5 with 12 replicates at each dilution of rAAV. Seventy-two hours after infection the cells are lysed, and qPCR performed to detect rAAV vector amplification over input. An end-point dilution $TCID_{50}$ calculation (Spearman-Karber) is performed to determine a replicative titer expressed as IU/ml. Since "infectivity" values are dependent on particles coming into contact with cells, receptor binding, internalization, transport to the nucleus and genome replication, they are influenced by assay geometry and the presence of appropriate receptors and post-binding pathways in the cell line used. Receptors and post-binding pathways critical for AAV vector import are usually maintained in immortalized cell lines and thus infectivity assay titers are not an absolute measure of the number of "infectious" particles present. However, the ratio of encapsidated GC to "infectious units" (described as GC/IU ratio) can be used as a

measure of product consistency from lot to lot. The variability of this *in vitro* bioassay is high (30-60 % CV) likely due to the low infectivity of AAV8 vectors *in vitro*.

8.4.6 Transgene Expression Assay

Transgene expression is evaluated in livers harvested from LDLR-/- Apobec-/-
5   mice that receive $1x10^{10}$ GC ($5x10^{11}$ GC/kg) of the AAV8.TBG.hLDLR vector. Animals dosed 30 days earlier with vector are euthanized, livers harvested and homogenized in RIPA buffer. 25-100 ug of total liver homogenate is electrophoresed on a 4-12% denaturing SDS-PAGE gel and probed using antibodies against human LDLR to determine transgene expression. Animals that receive no vector or an irrelevant vector is
10   used as controls for the assay. Animals treated with vector are expected to show a band migrating anywhere from 90- 160 kDa due to post-translational modifications. Relative expression levels are determined by quantifying the integrated intensity of the bands.

(Sequence Listing Free Text)

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

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

1	<221> misc_feature
	<222> (188)..(2770)
	<223> LDLR isoform 1 encoded by full-length CDS, 188-2770; other
	     variants encoded by alternative splice variants missing an exon;
	     most common variant missing fourth exon or twelfth exon
	
	<220>
	<221> misc_signal
	<222> (188)..(250)
	
	<220>
	<221> misc_feature
	<222> (251)..(2767)
	<223> Mature protein of isoform 1

4	<223> Artificial hLDLR

SEQ ID NO: (containing free text)	Free text under <223>
	<220> <221> misc_feature <222> (1)..(2583) <223> Artificial hLDLR coding sequence
5	<223> Adeno-associated virus 8 vp1 capsid protein
6	<223> pAAV.TBG.PI.hLDLRco.RGB <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 <220> <221> TATA_signal <222> (885)..(888) <223> TATA <220> <221> CDS <222> (969)..(3551) <223> codon optimized hLDLR <220> <221> polyA_signal <222> (3603)..(3729)

SEQ ID NO: (containing free text)	Free text under <223>
	<223> Rabbit globin poly A <220> <221> repeat_region <222> (3818)..(3947) <223> 3' ITR <220> <221> rep_origin <222> (4124)..(4579) <223> f1 ori <220> <221> misc_feature <222> (4710)..(5567) <223> AP(R) <220> <221> rep_origin <222> (5741)..(6329) <223> Origin of replication
7	<223> Synthetic Construct

All publications cited in this specification are incorporated herein by reference in their entirety, as are US Patent Application 62/461,015, filed February 20, 2017, International Patent Application No. PCT/US16/65984, filed December 6, 2016, US Provisional Patent Application No. 62/269,440, filed December 18, 2015 and US Provisional Patent Application No. 62/266,383, filed December 11, 2015. Similarly, the SEQ ID NOs which are referenced herein and which appear in the appended Sequence Listing are incorporated by reference. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS:

1.   A method for reducing the need for apheresis in a patient having familial hypercholesterolemia, the method comprising administering to the patients a pharmaceutical composition suitable for peripheral vein infusion in human subjects, comprising a suspension of replication deficient recombinant adeno-associated virus (rAAV) in a formulation buffer, wherein:

      (a)   the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid;

      (b)   the formulation buffer comprises an aqueous solution of phosphate buffered saline and a Poloxamer; and

      (c)   one or more of:

            (i)   the rAAV Genome Copy (GC) titer is at least 1×10^{13} GC/ml;

            (ii)   the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR;

            (iii)   the Empty:Full particle ratio is between 0:4 to 1:4; and

            (iv)   a dose of 5×10^{11} GC/kg of the rAAV suspension decreases baseline cholesterol levels in a double knockout (DKO) LDLR-/-Apobec-/- mouse model of Homozygous Familial Hypercholesterolemia (HoFH) by 25% to 75%.

2.   The method according to claim 1, wherein the patients have a neutralizing antibody titer of less than or equal to 1:10 against the AAV8 capsid.

3.   The method according to claim 2, wherein the patients are co-treated with an immunosuppressive regimen.

4.   The method according to claim 3, wherein the patients have a neutralizing antibody titer of at least about 1:5 prior to co-treatment with the immunosuppressive regimen.

5.   The method according to claim 1, wherein the patients have a neutralizing antibody titer of less than or equal to 1:5 against the AAV8 capsid.

6.   The method according to claim 1, wherein the rAAV is *AAV8.TBG.hLDLR*.

7.   The method according to claim 1, wherein the formulation buffer is 180mM NaCl, 10mM Na phosphate, 0.001% Poloxamer 188, pH 7.3.

8.   The method according to claim 1, wherein the composition is administrable to the human subject via a peripheral vein by infusion of a suspension of replication deficient recombinant adeno-associated virus (rAAV) at a dose of (a) at least about 5×10^{11} Genome Copies/kg or (b) 2.5×10^{12} Genome Copy (GC)/kg to 7.5×10^{12} Genome Copy (GC)/kg body weight of the human subject as determined by oqPCR or ddPCR

9.   A pharmaceutical composition suitable comprising a suspension of replication deficient recombinant adeno-associated virus (rAAV) in a formulation buffer suitable for use in reducing the need for apheresis in a patient having familial hypercholesterolemia, wherein:

        (a)   the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid;

        (b)   the formulation buffer comprises an aqueous solution of phosphate buffered saline and a Poloxamer; and

        (c)   one or more of:

                (i)   the rAAV Genome Copy (GC) titer is at least 1×10^{13} GC/ml;

                (ii)   the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR;

                (iii)   the Empty:Full particle ratio is between 0:4 to 1:4; and

                (iv)   a dose of 5×10^{11} GC/kg of the rAAV suspension decreases baseline cholesterol levels in a double knockout (DKO) LDLR-/-Apobec-/- mouse model of Homozygous Familial Hypercholesterolemia (HoFH) by 25% to 75%.

10. Use of a pharmaceutical composition comprising a suspension of replication deficient recombinant adeno-associated virus (rAAV) in a formulation buffer suitable for use in reducing the need for apheresis in a patient having familial hypercholesterolemia, wherein:

(a) the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid;

(b) the formulation buffer comprises an aqueous solution of phosphate buffered saline and a Poloxamer; and

(c) one or more of:

(i) the rAAV Genome Copy (GC) titer is at least 1 x 10^{13} GC/ml;

(ii) the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR;

(iii) the Empty:Full particle ratio is between 0:4 to 1:4; and

(iv) a dose of 5 x 10^{11} GC/kg of the rAAV suspension decreases baseline cholesterol levels in a double knockout (DKO) LDLR-/-Apobec-/- mouse model of Homozygous Familial Hypercholesterolemia (HoFH) by 25% to 75%.

11. The pharmaceutical composition or according to claim 9 or claim 10, wherein the patient has a neutralizing antibody titer of less than or equal to 1:10 against the AAV8 capsid.

12. The pharmaceutical composition or use according any one of claims 9 to 11, wherein the patient is co-treated with an immunosuppressive regimen.

13. The pharmaceutical composition or use according to any one of claims 9 to 12, wherein the patient has a neutralizing antibody titer of at least about 1:5 prior to co-treatment with the immunosuppressive regimen.

14.   The pharmaceutical composition or use according to any one of claims 9 to 12, wherein the patient has a neutralizing antibody titer of less than or equal to 1:5 against the AAV8 capsid.

15.   The pharmaceutical composition or use according to any one of claims 9 to 12, wherein the rAAV is AAV8.TBG.hLDLR.

16.   The pharmaceutical composition or use according to any one of claims 9 to 15, wherein the formulation buffer is 180mM NaCl, 10mM Na phosphate, 0.001% Poloxamer 188, pH 7.3.

17.   The pharmaceutical composition or use according to any one of claims 9 to 16, wherein the composition is administrable to the human subject via a peripheral vein by infusion of a suspension of replication deficient recombinant adeno-associated virus (rAAV) at a dose of (a) at least about 5×10^{11} Genome Copies/kg or (b) 2.5×10^{12} Genome Copy (GC)/kg to 7.5×10^{12} Genome Copy (GC)/kg body weight of the human subject as determined by oqPCR or ddPCR

18.   The pharmaceutical composition or use according to any one of claims 9 to 17, wherein the patient has been diagnosed with Homozygous FH (HoFH).

19.   The pharmaceutical composition or use according to any one of claims 9 to 17, wherein the patient has been diagnosed with Heterozygous FH (HeFH).

20.   A method for reducing the need for treatment with a PCSK9 inhibitor in a human subject diagnosed with Familial Hypercholesterolemia (FH), comprising administering to the human subject via a peripheral vein by infusion of a suspension of replication deficient recombinant adeno-associated virus (rAAV) at a dose of (a) at least about 5×10^{11} Genome Copies/kg or (b) 2.5×10^{12} Genome Copy (GC)/kg to 7.5×10^{12} Genome Copy (GC)/kg body weight of the human subject as determined by oqPCR or ddPCR, wherein:

(a)   the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a wild-type human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid, and

(b)   the rAAV suspension has a potency such that a dose of 5×10^{11} GC/Kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%; and

(c)   one or more of:

(i)   the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR; and

(ii)   the rAAV Empty:Full particle ratio is between 0:4 to 1:4.

21.   The method according to claim 20, wherein the patients have a neutralizing antibody titer of less than or equal to 1:10 against the AAV8 capsid.

22.   The method according to claim 20, wherein the patients are co-treated with an immunosuppressive regimen.

23.   The method according to claim 20, wherein the patients have a neutralizing antibody titer of at least about 1:5 prior to co-treatment with the immunosuppressive regimen.

24.   The method according to claim 20, wherein the patients have a neutralizing antibody titer of less than or equal to 1:5 against the AAV8 capsid.

25.   The method according to claim 20, wherein the rAAV is *AAV8.TBG.hLDLR*.

26.   The method according to claim 20, wherein the formulation buffer is 180mM NaCl, 10mM Na phosphate, 0.001% Poloxamer 188, pH 7.3.

27.   The method according to claim 20, wherein the subject has been diagnosed with Homozygous FH (HoFH).

28.   The method according to claim 20, wherein the subject has been diagnosed with Heterozygous FH (HeFH).

29.   A recombinant adeno-associated virus (rAAV) at a dose of (a) at least about 5 x 10^{11} Genome Copies/kg or (b) 2.5 x 10^{12} Genome Copy (GC)/kg to 7.5 x 10^{12} Genome Copy (GC)/kg body weight of the human subject as determined by oqPCR or ddPCR useful for reducing the need for treatment with a PCSK9 inhibitor in a human subject diagnosed with Familial Hypercholesterolemia (FH), comprising administering to the human subject via a peripheral vein by infusion of a suspension of replication deficient, wherein:

          (a)   the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a wild-type human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid, and

          (b)   the rAAV suspension has a potency such that a dose of 5 x 10^{11} GC/Kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%; and

          (c)   one or more of:

          (i)   the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR; and

          (ii)   the rAAV Empty:Full particle ratio is between 0:4 to 1:4.

30.   Use of a recombinant adeno-associated virus (rAAV) at a dose of (a) at least about 5 x 10^{11} Genome Copies/kg or (b) 2.5 x 10^{12} Genome Copy (GC)/kg to 7.5 x 10^{12} Genome Copy (GC)/kg body weight of the human subject as determined by oqPCR or ddPCR for reducing the need for treatment with a PCSK9 inhibitor in a human subject diagnosed with Familial Hypercholesterolemia (FH), comprising administering to the human subject via a peripheral vein by infusion of a suspension of replication deficient, wherein:

(a)   the rAAV comprises a vector genome comprising AAV ITRs and a nucleic acid sequence encodes a wild-type human LDL receptor (hLDLR) operably linked to a liver specific promoter, said vector genome packaged in an AAV8 capsid, and

(b)   the rAAV suspension has a potency such that a dose of 5×10^{11} GC/Kg administered to a double knockout LDLR-/-Apobec-/- mouse model of HoFH (DKO mouse) decreases baseline cholesterol levels in the DKO mouse by 25% to 75%; and

(c)   one or more of:

(i)   the rAAV is at least about 95% free of empty capsids as determined by oqPCR or ddPCR; and

(ii)   the rAAV Empty:Full particle ratio is between 0:4 to 1:4.

31.   The rAAV or use according to claim 30, wherein the patients have a neutralizing antibody titer of less than or equal to 1:10 against the AAV8 capsid.

32.   The rAAV or use according to claim 30 or 31, wherein the patients are co-treated with an immunosuppressive regimen.

33.   The rAAV or use according to any one of claims 30 to 32, wherein the patients have a neutralizing antibody titer of at least about 1:5 prior to co-treatment with the immunosuppressive regimen.

34.   The rAAV or use according to any one of claims 30 to 32, wherein the patients have a neutralizing antibody titer of less than or equal to 1:5 against the AAV8 capsid.

35.   The rAAV or use according to any one of claims 30 to 34, wherein the rAAV is *AAV8.TBG.hLDLR*.

36.   The rAAV or use according to any one of claims 30 to 35, wherein the formulation buffer is 180mM NaCl, 10mM Na phosphate, 0.001% Poloxamer 188, pH 7.3.

37. The rAAV or use according to any one of claims 30 to 36, wherein the subject has been diagnosed with Homozygous FH (HoFH).

38. The rAAV or use according to any one of claims 30 to 36, wherein the subject has been diagnosed with Heterozygous FH (HeFH).
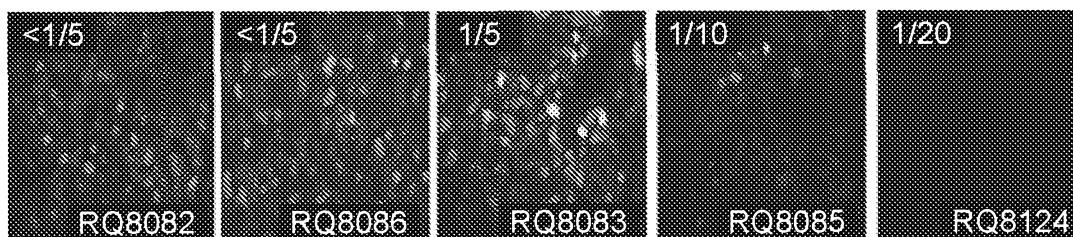
FIG. 1A   FIG. 1B   FIG. 1C   FIG. 1D   FIG. 1E
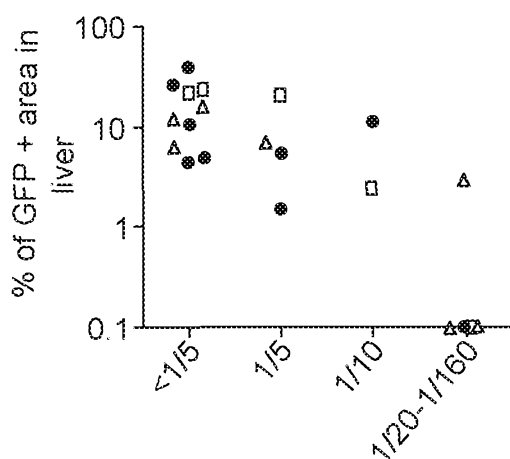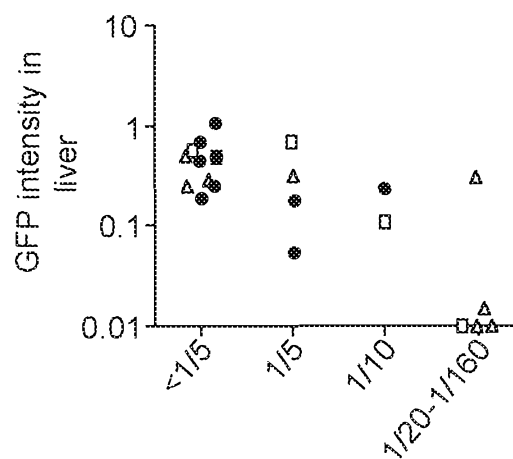
FIG. 1F   FIG. 1G

FIG. 1H

FIG. 2

FIG. 3A

FIG. 3B

Baseline   nLacZ   mLDLR

FIG. 3C   FIG. 3D   FIG. 3E

Baseline   nLacZ   mLDLR

FIG. 3F   FIG. 3G   FIG. 3H

Baseline   nLacZ   mLDLR
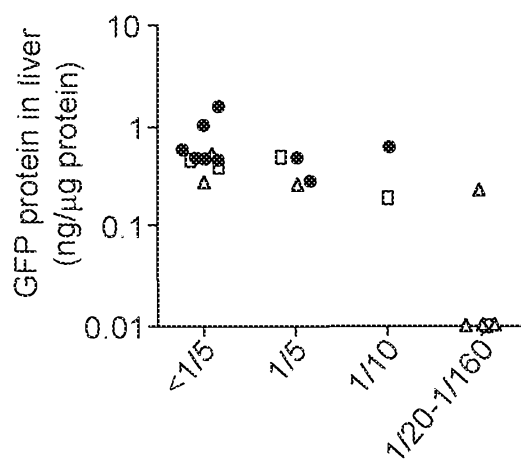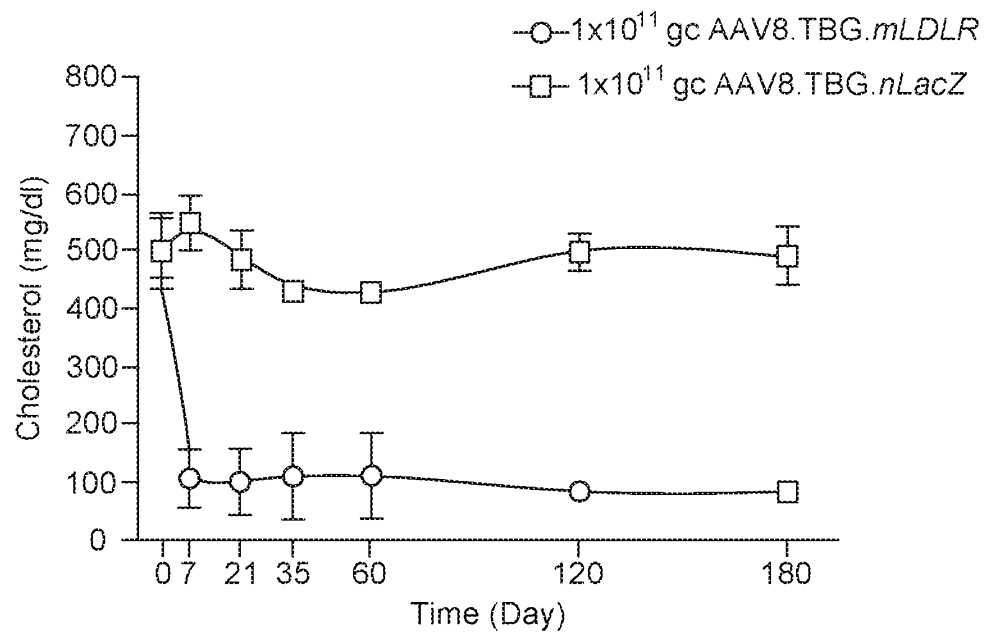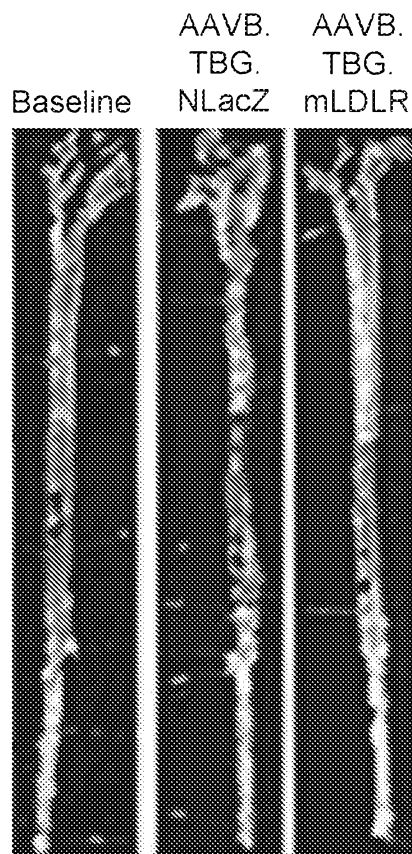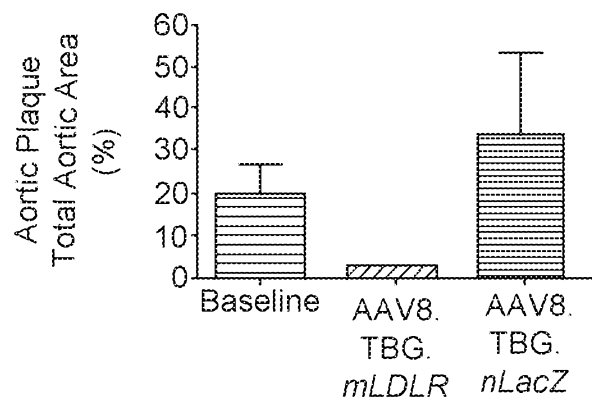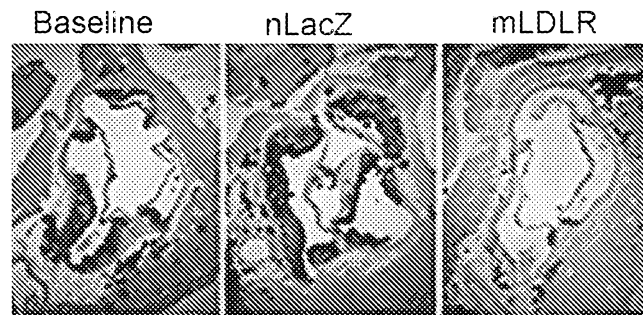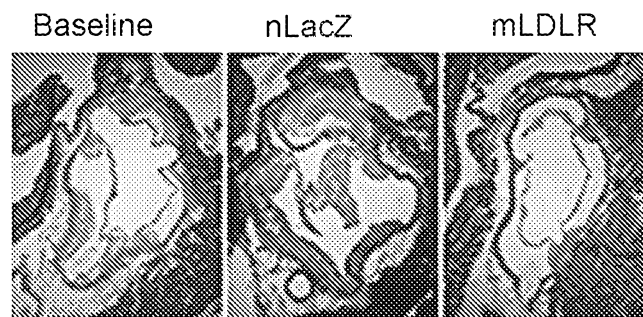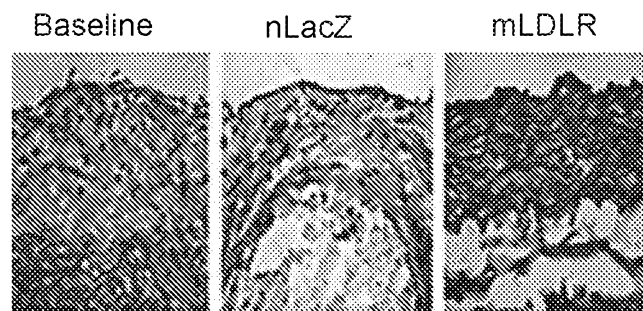
FIG. 3I   FIG. 3J   FIG. 3K
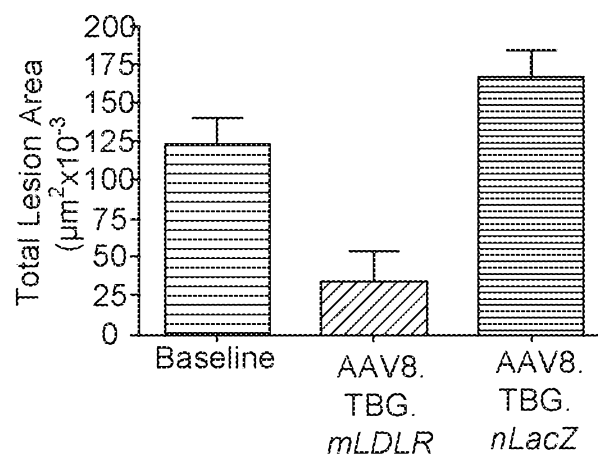
FIG. 3L
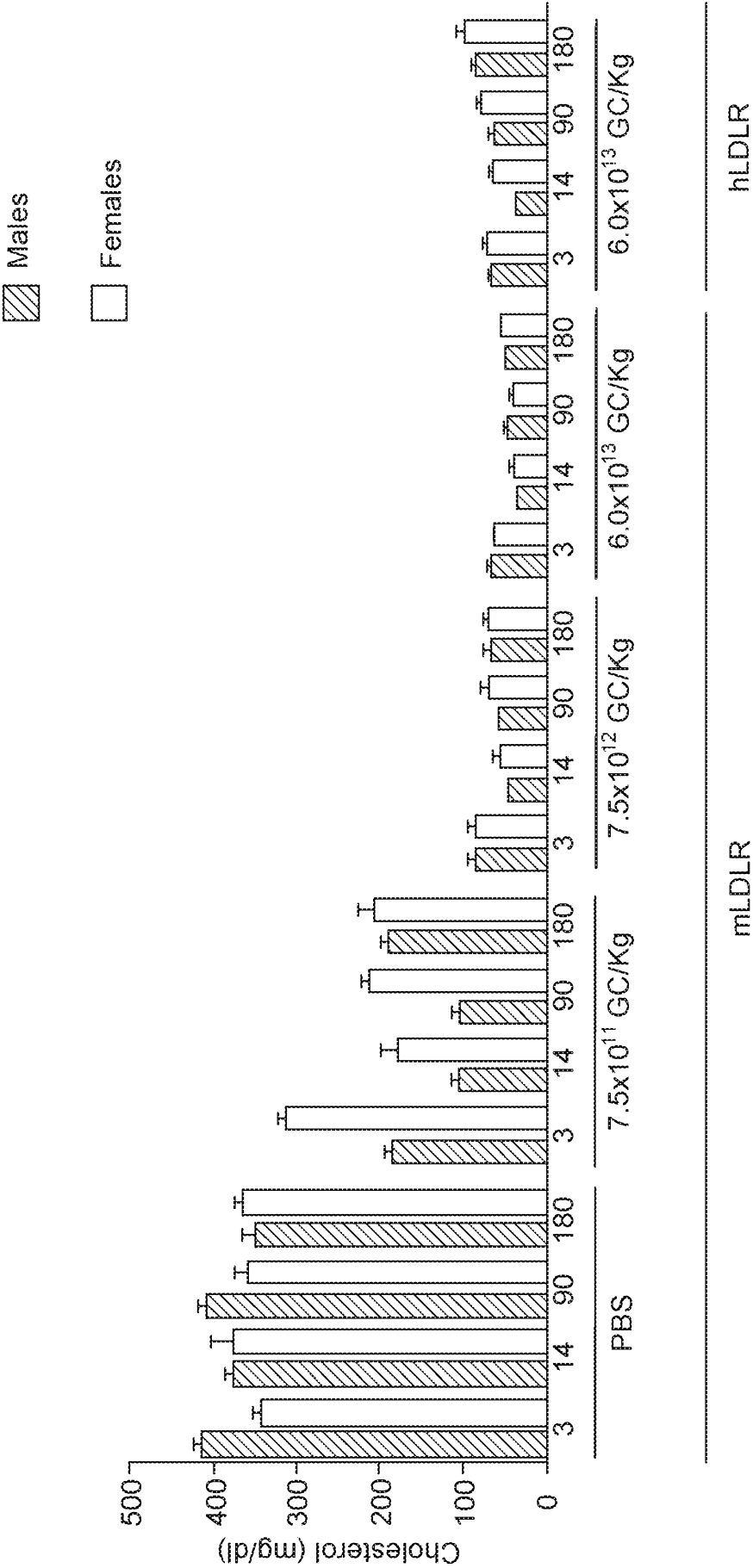
FIG. 4
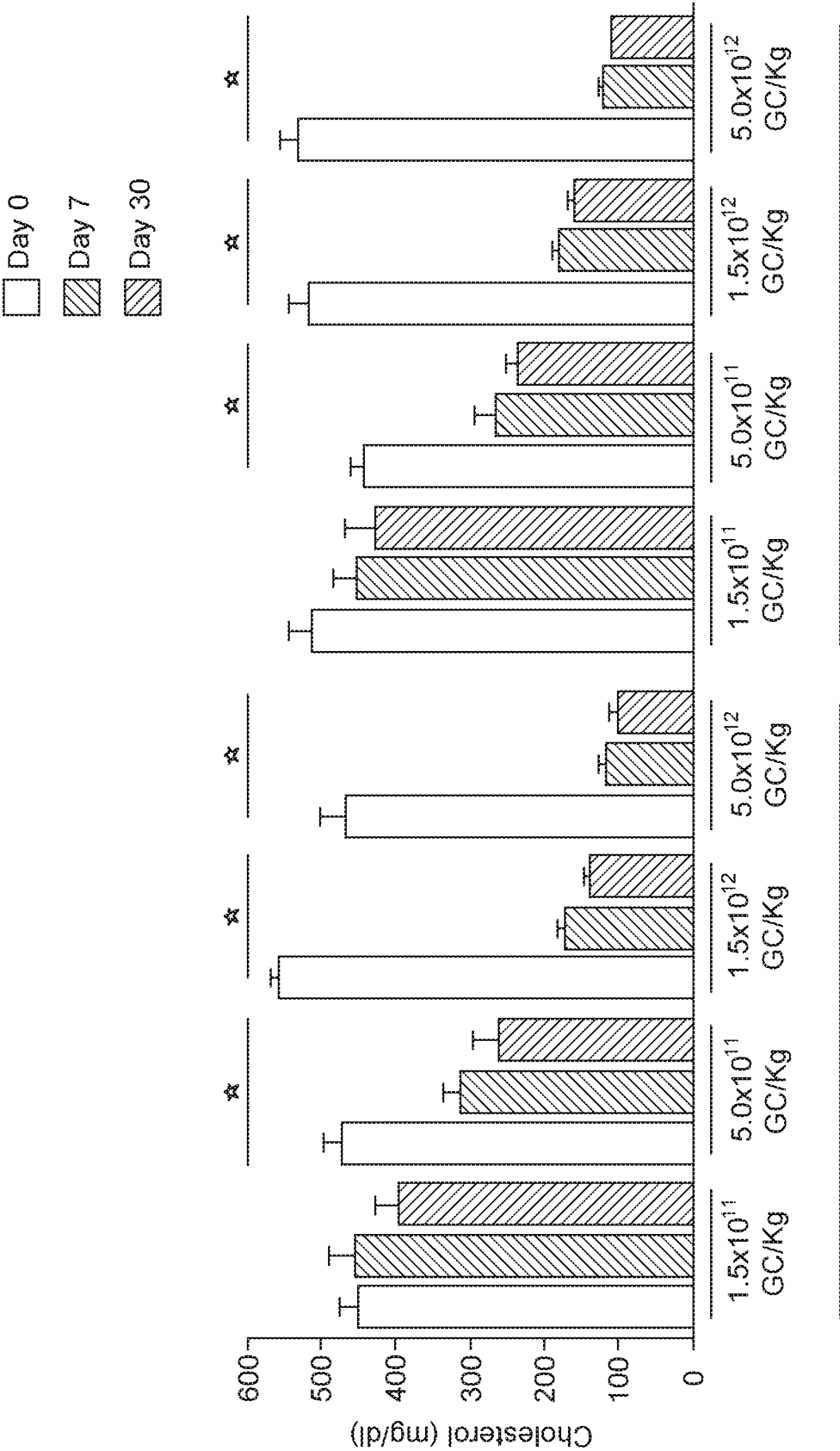
FIG 5A

FIG 5B
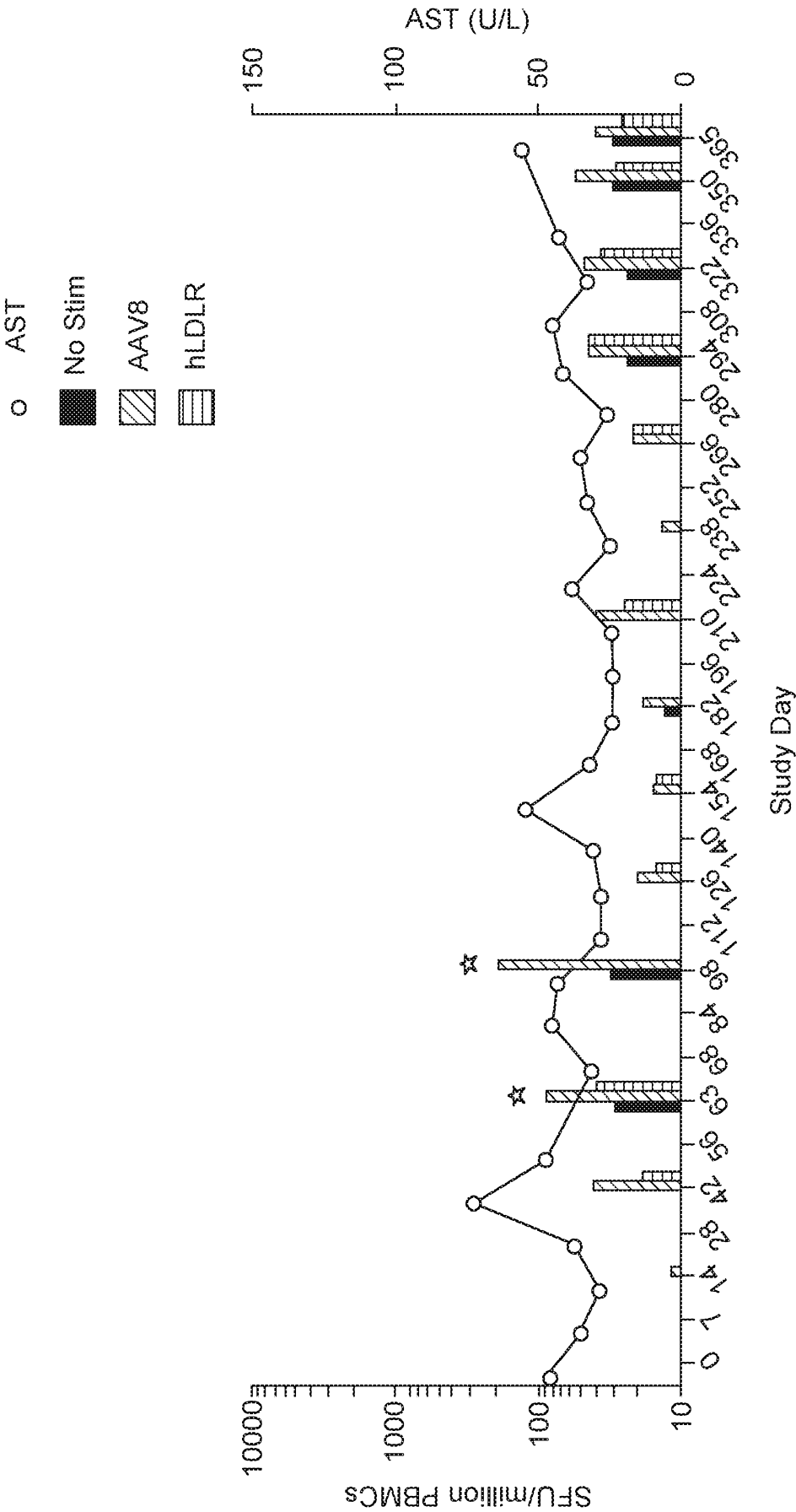
FIG. 6A
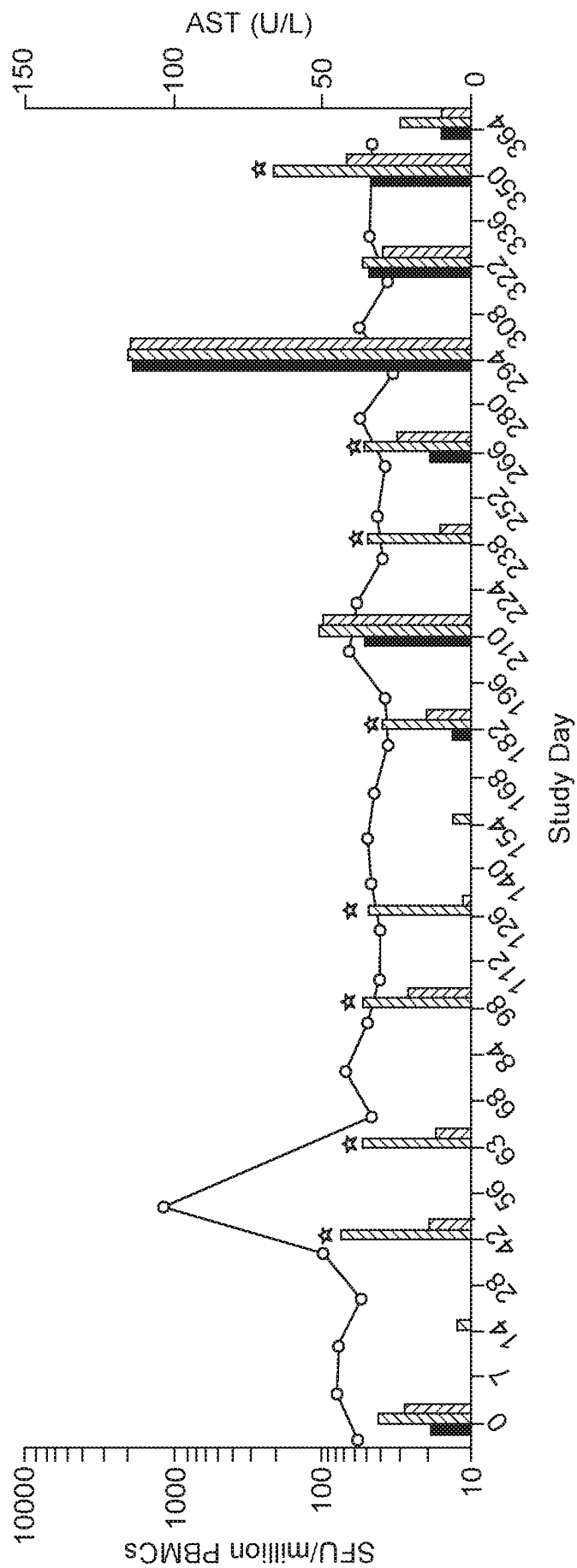
FIG.6B
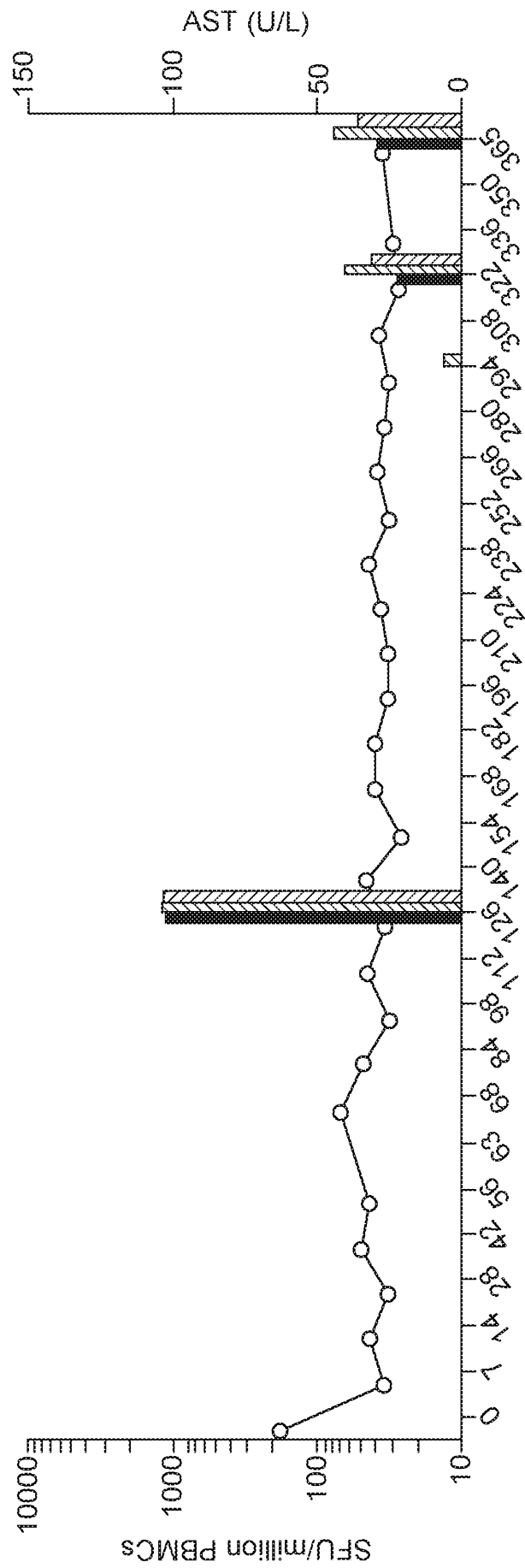
FIG. 6C
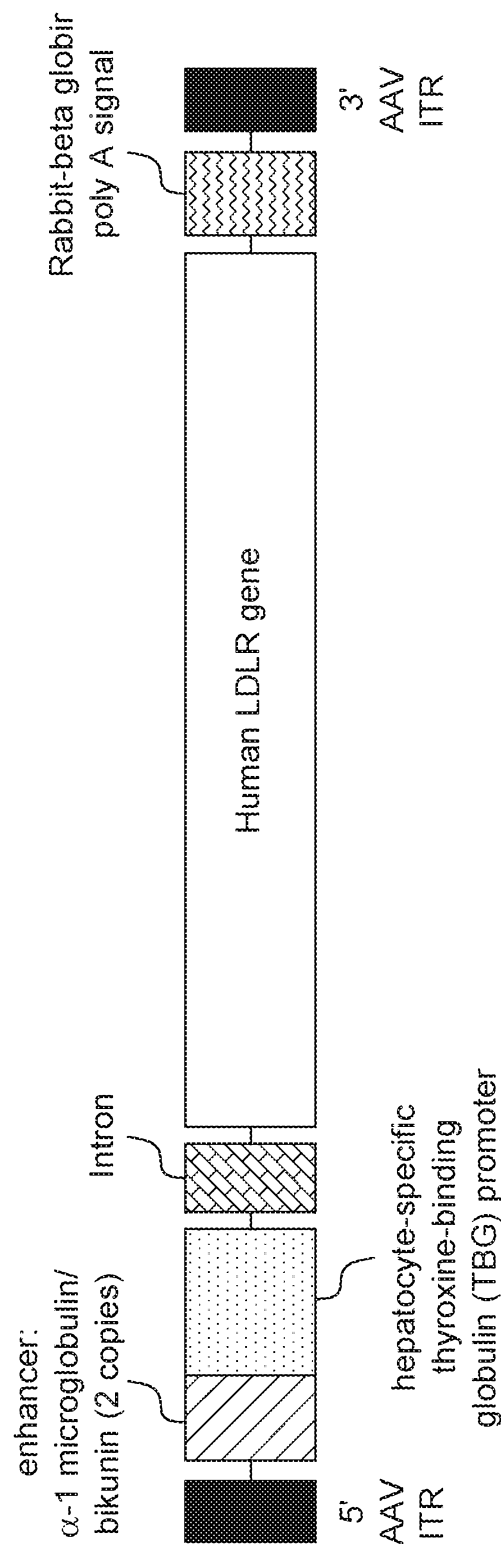
FIG. 7
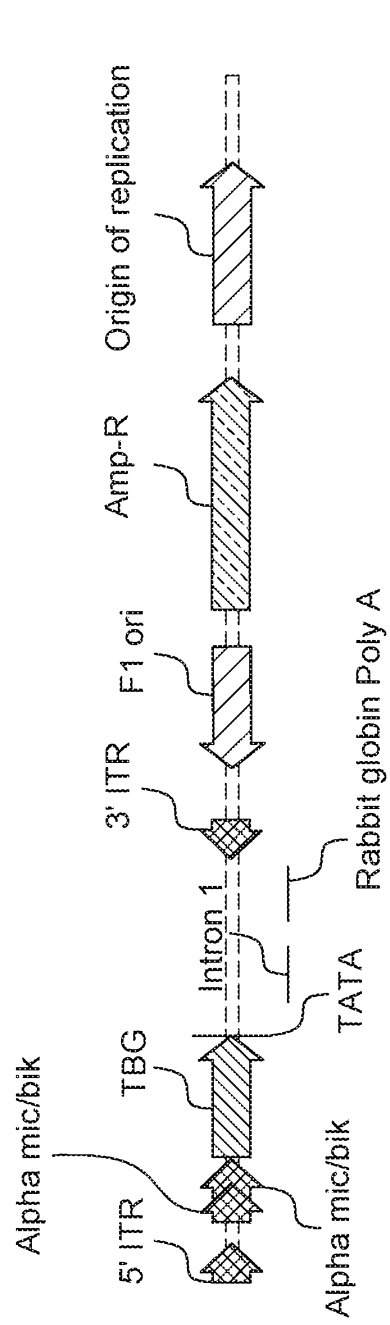
FIG. 8A
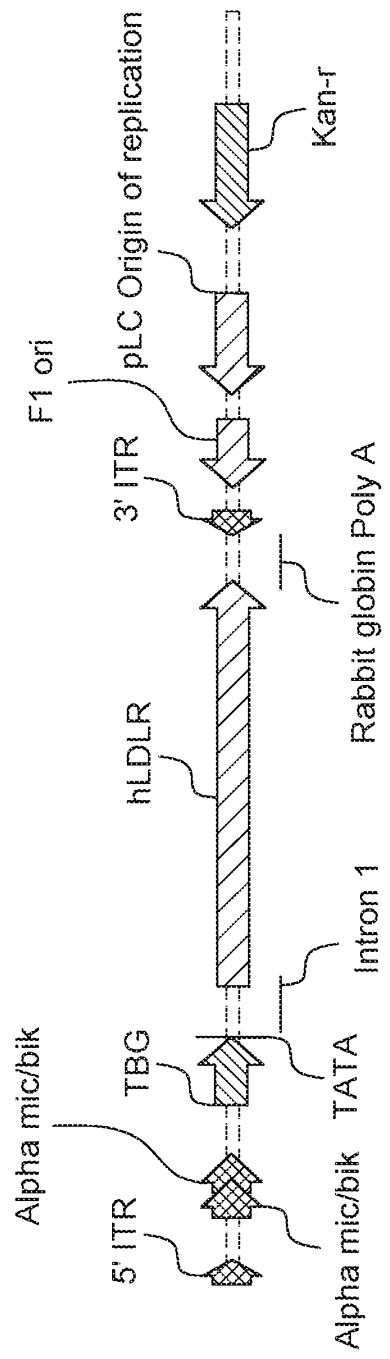
FIG. 8B
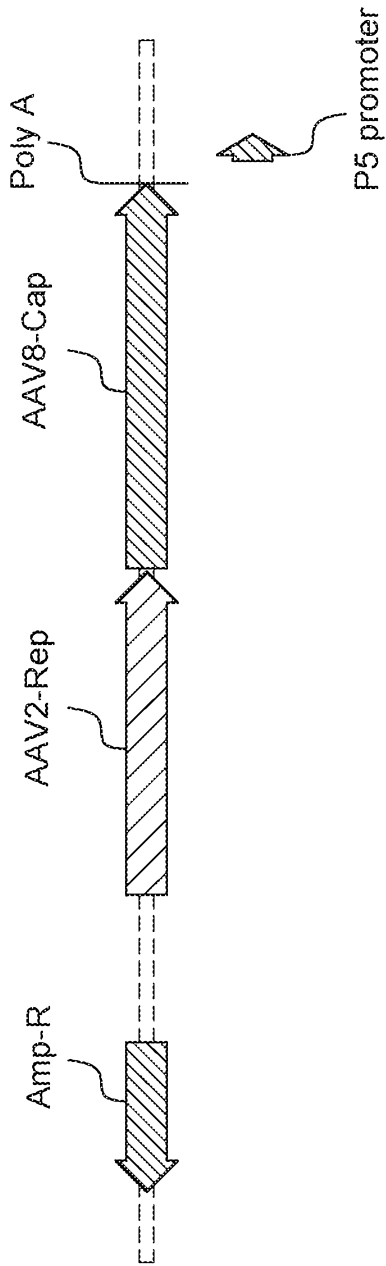
FIG. 9A
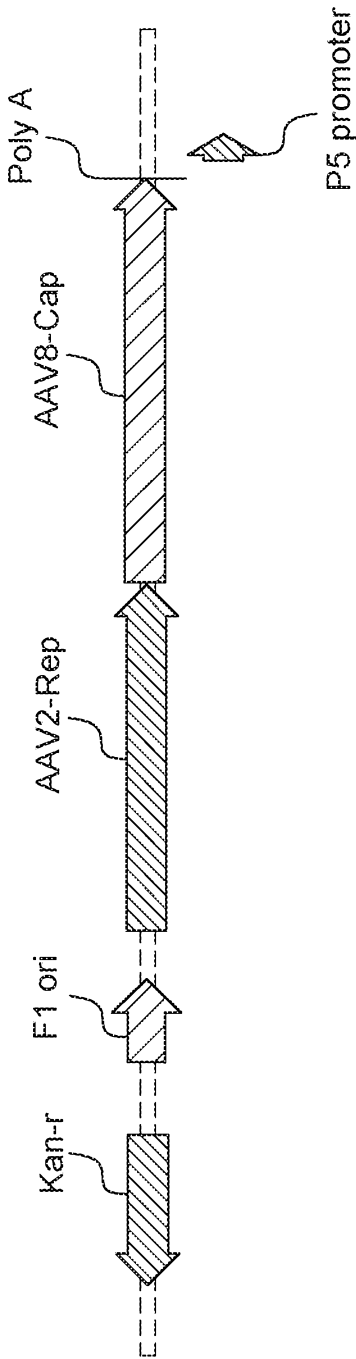
FIG. 9B
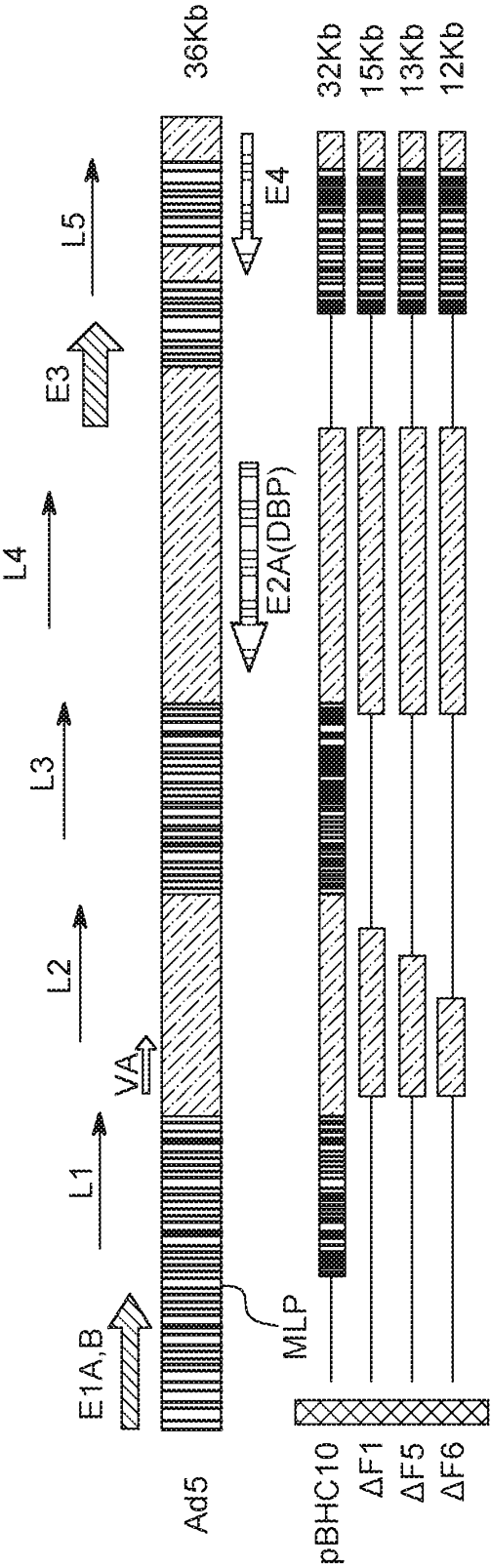
ΔF1 = pBHC10 minus MLP to SalI,and PmcVSgn fragments

ΔF5 = ΔF1 minus NRUL/NRUL(2.3Kb) fragment

ΔF6 = ΔF5 minus RsrII/Nrul(0.5Kb) fragment

FIG. 10A
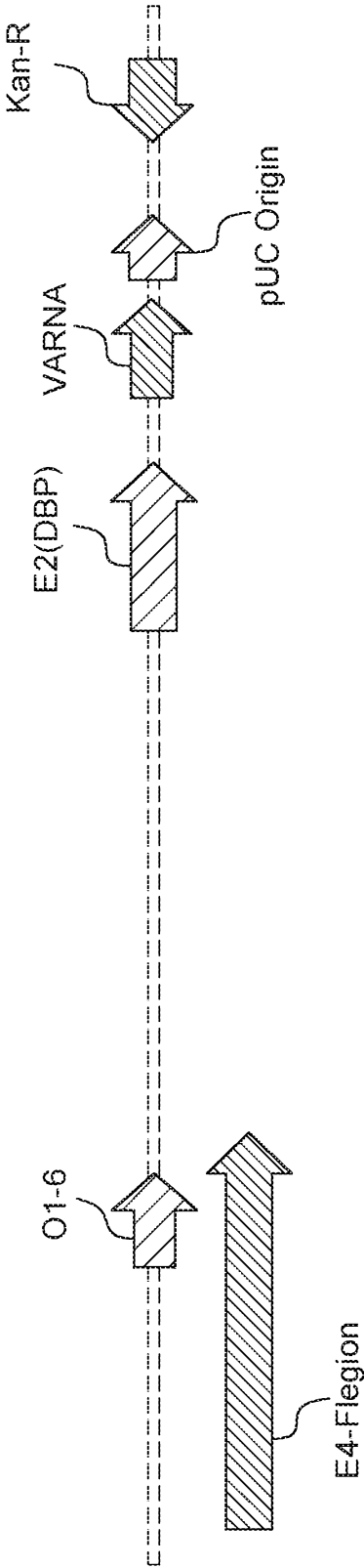
FIG. 10B

Vector Generation Process (upstream process)

Brief Description:

1.Initation of HEK293 from a Cell Bank vial into T75 flask

↓

2. Passage of cells into~2T225 flasks

↓

3. Passage of cells into~10T225 flasks

↓

4. Seeding of HEK293 cells in roller bottles

↓

5. Passage of cells into ~100 roller bottles

↓

6. Transfection of HEK203 cells for vector generation

↓

7. Post- transfection medium exchange to serum free medium

↓

8. Harvest of vector-containing cells and culture media
(CRUDE CELL HARVEST)

FIG. 11A

Vector Purification Process (downstream process)

Brief Description:

9. AAV8 vector harvest concentration and diafiltration by TFF

↓

10. Microfluidizied and nuclease digestion of harvest

↓

11. Filtration of microfluidizied intermediate (final 0.2m)

↓

12. AAV8 vector purification by ion exchange chromatography

↓

13. AAV8 vector purification by gradient ultracentrifugation

↓

14. Buffer exchange by tangential flow to prepare Bulk Vector

↓

15. Formulation and 0.2 m filtration to prepare Bulk Vector

Vector Purification Process (downstream process)

Brief Description:

16. Final Vector Product Fill and Finish

FIG. 11B

SEQUENCE LISTING

<110> The Trustees of the University of Pennsylvania

<120> GENE THERAPY FOR TREATING FAMILIAL HYPERCHOLESTEROLEMIA

<130> UPN-16-7717C1PCT

<150> US 62/461,015
<151> 2017-02-20

<160> 7

<170> PatentIn version 3.5

<210> 1
<211> 5292
<212> DNA
<213> Homo sapiens

<220>
<221> exon
<222> (1)..(254)

<220>
<221> misc_feature
<222> (188)..(2770)
<223> LDLR isoform 1 encoded by full-length CDS, 188-2770; other
       variants encoded by alternative splice variants missing an exon;
       most common variant missing fourth exon or twelfth exon

<220>
<221> misc_signal
<222> (188)..(250)

<220>
<221> misc_feature
<222> (251)..(2767)
<223> Mature protein of isoform 1

<220>
<221> exon
<222> (255)..(377)

<220>
<221> exon
<222> (378)..(500)

<220>
<221> exon
<222> (501)..(881)

```
<220>
<221>    exon
<222>    (882)..(1004)

<220>
<221>    exon
<222>    (1005)..(1127)

<220>
<221>    exon
<222>    (1128)..(1247)

<220>
<221>    exon
<222>    (1248)..(1373)

<220>
<221>    exon
<222>    (1374)..(1545)

<220>
<221>    exon
<222>    (1546)..(1773)

<220>
<221>    exon
<222>    (1774)..(1892)

<220>
<221>    exon
<222>    (1893)..(2032)

<220>
<221>    exon
<222>    (2033)..(2174)

<220>
<221>    exon
<222>    (2175)..(2327)

<220>
<221>    exon
<222>    (2328)..(2498)

<220>
<221>    polyA_signal
<222>    (5252)..(5257)

<220>
<221>    polyA_site
<222>    (5284)..(5284)
```

<400> 1

ctc	ttg	cag	tga	ggt	gaa	gac	att	tga	aaa	tca	ccc	cac	tgc	aaa	ctc	48
Leu	Leu	Gln		Gly	Glu	Asp	Ile		Lys	Ser	Pro	His	Cys	Lys	Leu	
1				5						10						

ctc	ccc	ctg	cta	gaa	acc	tca	cat	tga	aat	gct	gta	aat	gac	gtg	ggc	96
Leu	Pro	Leu	Leu	Glu	Thr	Ser	His		Asn	Ala	Val	Asn	Asp	Val	Gly	
15				20						25						

ccc	gag	tgc	aat	cgc	ggg	aag	cca	ggg	ttt	cca	gct	agg	aca	cag	cag	144
Pro	Glu	Cys	Asn	Arg	Gly	Lys	Pro	Gly	Phe	Pro	Ala	Arg	Thr	Gln	Gln	
30					35					40					45	

gtc	gtg	atc	cgg	gtc	ggg	aca	ctg	cct	ggc	aga	ggc	tgc	gag	cat	ggg	192
Val	Val	Ile	Arg	Val	Gly	Thr	Leu	Pro	Gly	Arg	Gly	Cys	Glu	His	Gly	
				50					55					60		

gcc	ctg	ggg	ctg	gaa	att	gcg	ctg	gac	cgt	cgc	ctt	gct	cct	cgc	cgc	240
Ala	Leu	Gly	Leu	Glu	Ile	Ala	Leu	Asp	Arg	Arg	Leu	Ala	Pro	Arg	Arg	
			65					70					75			

ggc	ggg	gac	tgc	ag	t	ggg	cga	cag	atg	cga	aag	aaa	cga	gtt	cca	gtg	288
Gly	Gly	Asp	Cys	Ser		Gly	Arg	Gln	Met	Arg	Lys	Lys	Arg	Val	Pro	Val	
		80						85					90				

cca	aga	cgg	gaa	atg	cat	ctc	cta	caa	gtg	ggt	ctg	cga	tgg	cag	cgc		336
Pro	Arg	Arg	Glu	Met	His	Leu	Leu	Gln	Val	Gly	Leu	Arg	Trp	Gln	Arg		
	95					100					105						

tga	gtg	cca	gga	tgg	ctc	tga	tga	gtc	cca	gga	gac	gtg	ct	t	gtc	tgt	384
	Val	Pro	Gly	Trp	Leu			Val	Pro	Gly	Asp	Val	Leu		Val	Cys	
	110							115				120					

cac	ctg	caa	atc	cgg	gga	ctt	cag	ctg	tgg	ggg	ccg	tgt	caa	ccg	ctg	432
His	Leu	Gln	Ile	Arg	Gly	Leu	Gln	Leu	Trp	Gly	Pro	Cys	Gln	Pro	Leu	
		125					130					135				

cat	tcc	tca	gtt	ctg	gag	gtg	cga	tgg	cca	agt	gga	ctg	cga	caa	cgg	480
His	Ser	Ser	Val	Leu	Glu	Val	Arg	Trp	Pro	Ser	Gly	Leu	Arg	Gln	Arg	
	140					145					150					

ctc	aga	cga	gca	agg	ctg	tc	c	ccc	caa	gac	gtg	ctc	cca	gga	cga	gtt	528
Leu	Arg	Arg	Ala	Arg	Leu	Ser		Pro	Gln	Asp	Val	Leu	Pro	Gly	Arg	Val	
155					160					165						170	

tcg	ctg	cca	cga	tgg	gaa	gtg	cat	ctc	tcg	gca	gtt	cgt	ctg	tga	ctc	576
Ser	Leu	Pro	Arg	Trp	Glu	Val	His	Leu	Ser	Ala	Val	Arg	Leu		Leu	
			175					180						185		

aga	ccg	gga	ctg	ctt	gga	cgg	ctc	aga	cga	ggc	ctc	ctg	ccc	ggt	gct	624
Arg	Pro	Gly	Leu	Leu	Gly	Arg	Leu	Arg	Arg	Gly	Leu	Leu	Pro	Gly	Ala	

```
                190                          195                          200

cac ctg tgg tcc cgc cag ctt cca gtg caa cag ctc cac ctg cat ccc        672
His Leu Trp Ser Arg Gln Leu Pro Val Gln Gln Leu His Leu His Pro
            205                      210                      215

cca gct gtg ggc ctg cga caa cga ccc cga ctg cga aga tgg ctc gga        720
Pro Ala Val Gly Leu Arg Gln Arg Pro Arg Leu Arg Arg Trp Leu Gly
            220                      225                      230

tga gtg gcc gca gcg ctg tag ggg tct tta cgt gtt cca agg gga cag        768
    Val Ala Ala Ala Leu     Gly Ser Leu Arg Val Pro Arg Gly Gln
            235                      240                      245

tag ccc ctg ctc ggc ctt cga gtt cca ctg cct aag tgg cga gtg cat        816
    Pro Leu Leu Gly Leu Arg Val Pro Leu Pro Lys Trp Arg Val His
            250                      255                      260

cca ctc cag ctg gcg ctg tga tgg tgg ccc cga ctg caa gga caa atc        864
Pro Leu Gln Leu Ala Leu     Trp Trp Pro Arg Leu Gln Gly Gln Ile
            265                      270                      275

tga cga gga aaa ctg cg  c tgt ggc cac ctg tcg ccc tga cga att cca      912
    Arg Gly Lys Leu Arg     Cys Gly His Leu Ser Pro     Arg Ile Pro
            280                      285                      290

gtg ctc tga tgg aaa ctg cat cca tgg cag ccg gca gtg tga ccg gga        960
Val Leu     Trp Lys Leu His Pro Trp Gln Pro Ala Val     Pro Gly
            295                      300                      305

ata tga ctg caa gga cat gag cga tga agt tgg ctg cgt taa tg  t gac      1008
Ile     Leu Gln Gly His Glu Arg     Ser Trp Leu Arg     Cys     Asp
            310                      315

act ctg cga ggg acc caa caa gtt caa gtg tca cag cgg cga atg cat        1056
Thr Leu Arg Gly Thr Gln Gln Val Gln Val Ser Gln Arg Arg Met His
   320                      325                      330

cac cct gga caa agt ctg caa cat ggc tag aga ctg ccg gga ctg gtc        1104
His Pro Gly Gln Ser Leu Gln His Gly     Arg Leu Pro Gly Leu Val
335                      340                      345

aga tga acc cat caa aga gtg cg  g gac caa cga atg ctt gga caa caa      1152
Arg     Thr His Gln Arg Val Arg     Asp Gln Arg Met Leu Gly Gln Gln
350                      355                      360

cgg cgg ctg ttc cca cgt ctg caa tga cct taa gat cgg cta cga gtg        1200
Arg Arg Leu Phe Pro Arg Leu Gln     Pro     Asp Arg Leu Arg Val
365                      370                      375

cct gtg ccc cga cgg ctt cca gct ggt ggc cca gcg aag atg cga ag  a      1248
Pro Val Pro Arg Arg Leu Pro Ala Gly Gly Pro Ala Lys Met Arg Arg
```

```
         380                    385                        390

tat cga tga gtg tca gga tcc cga cac ctg cag cca gct ctg cgt gaa      1296
Tyr Arg     Val Ser Gly Ser Arg His Leu Gln Pro Ala Leu Arg Glu
395                     400                    405

cct gga ggg tgg cta caa gtg cca gtg tga gga agg ctt cca gct gga      1344
Pro Gly Gly Trp Leu Gln Val Pro Val     Gly Arg Leu Pro Ala Gly
410                     415                    420

ccc cca cac gaa ggc ctg caa ggc tgt gg  g ctc cat cgc cta cct ctt    1392
Pro Pro His Glu Gly Leu Gln Gly Cys Gly   Leu His Arg Leu Pro Leu
425                     430                    435                440

ctt cac caa ccg gca cga ggt cag gaa gat gac gct gga ccg gag cga      1440
Leu His Gln Pro Ala Arg Gly Gln Glu Asp Asp Ala Gly Pro Glu Arg
                445                    450                    455

gta cac cag cct cat ccc caa cct gag gaa cgt ggt cgc tct gga cac      1488
Val His Gln Pro His Pro Gln Pro Glu Glu Arg Gly Arg Ser Gly His
                460                    465                    470

gga ggt ggc cag caa tag aat cta ctg gtc tga cct gtc cca gag aat      1536
Gly Gly Gly Gln Gln     Asn Leu Leu Val     Pro Val Pro Glu Asn
                475                    480                    485

gat ctg cag cac cca gct tga cag agc cca cgg cgt ctc ttc cta tga      1584
Asp Leu Gln His Pro Ala     Gln Ser Pro Arg Arg Leu Phe Leu
                490                    495                    500

cac cgt cat cag cag aga cat cca ggc ccc cga cgg gct ggc tgt gga      1632
His Arg His Gln Gln Arg His Pro Gly Pro Arg Arg Ala Gly Cys Gly
                505                    510                    515

ctg gat cca cag caa cat cta ctg gac cga ctc tgt cct ggg cac tgt      1680
Leu Asp Pro Gln Gln His Leu Leu Asp Arg Leu Cys Pro Gly His Cys
                520                    525                    530

ctc tgt tgc gga tac caa ggg cgt gaa gag gaa aac gtt att cag gga      1728
Leu Cys Cys Gly Tyr Gln Gly Arg Glu Glu Glu Asn Val Ile Gln Gly
                535                    540                    545

gaa cgg ctc caa gcc aag ggc cat cgt ggt gga tcc tgt tca tgg ctt      1776
Glu Arg Leu Gln Ala Lys Gly His Arg Gly Gly Ser Cys Ser Trp Leu
                550                    555                    560

cat gta ctg gac tga ctg ggg aac tcc cgc caa gat caa gaa agg ggg      1824
His Val Leu Asp     Leu Gly Asn Ser Arg Gln Asp Gln Glu Arg Gly
565                     570                    575

cct gaa tgg tgt gga cat cta ctc gct ggt gac tga aaa cat tca gtg      1872
Pro Glu Trp Cys Gly His Leu Leu Ala Gly Asp     Lys His Ser Val
```

Page 5

```
                580                    585                    590


gcc caa tgg cat cac cct ag  a tct cct cag tgg ccg cct cta ctg ggt      1920
Ala Gln Trp His His Pro Arg    Ser Pro Gln Trp Pro Pro Leu Leu Gly
595                    600                    605                    610


tga ctc caa act tca ctc cat ctc aag cat cga tgt caa cgg ggg caa      1968
    Leu Gln Thr Ser Leu His Leu Lys His Arg Cys Gln Arg Gly Gln
                           615                    620                    625


ccg gaa gac cat ctt gga gga tga aaa gag gct ggc cca ccc ctt ctc      2016
Pro Glu Asp His Leu Gly Gly     Lys Glu Ala Gly Pro Pro Leu Leu
                       630                    635                    640


ctt ggc cgt ctt tga g ga  caa agt att ttg gac aga tat cat caa cga      2064
Leu Gly Arg Leu         Gly Gln Ser Ile Leu Asp Arg Tyr His Gln Arg
                           645                    650                    655


agc cat ttt cag tgc caa ccg cct cac agg ttc cga tgt caa ctt gtt      2112
Ser His Phe Gln Cys Gln Pro Pro His Arg Phe Arg Cys Gln Leu Val
                       660                    665                    670


ggc tga aaa cct act gtc ccc aga gga tat ggt tct ctt cca caa cct      2160
Gly     Lys Pro Thr Val Pro Arg Gly Tyr Gly Ser Leu Pro Gln Pro
                       675                    680                    685


cac cca gcc aag ag  g agt gaa ctg gtg tga gag gac cac cct gag caa      2208
His Pro Ala Lys Arg    Ser Glu Leu Val     Glu Asp His Pro Glu Gln
                       690                    695                    700


tgg cgg ctg cca gta tct gtg cct ccc tgc ccc gca gat caa ccc cca      2256
Trp Arg Leu Pro Val Ser Val Pro Pro Cys Pro Ala Asp Gln Pro Pro
                       705                    710                    715


ctc gcc caa gtt tac ctg cgc ctg ccc gga cgg cat gct gct ggc cag      2304
Leu Ala Gln Val Tyr Leu Arg Leu Pro Gly Arg His Ala Ala Gly Gln
                   720                    725                    730


gga cat gag gag ctg cct cac ag  a ggc tga ggc tgc agt ggc cac cca      2352
Gly His Glu Glu Leu Pro His Arg    Gly     Gly Cys Ser Gly His Pro
        735                    740                    745


gga gac atc cac cgt cag gct aaa ggt cag ctc cac agc cgt aag gac      2400
Gly Asp Ile His Arg Gln Ala Lys Gly Gln Leu His Ser Arg Lys Asp
        750                    755                    760


aca gca cac aac cac ccg acc tgt tcc cga cac ctc ccg gct gcc tgg      2448
Thr Ala His Asn His Pro Thr Cys Ser Arg His Leu Pro Ala Ala Trp
765                    770                    775                    780


ggc cac ccc tgg gct cac cac ggt gga gat agt gac aat gtc tca cca      2496
Gly His Pro Trp Ala His His Gly Gly Asp Ser Asp Asn Val Ser Pro
```

ag ctctgggcga cgttgctggc agaggaaatg agaagaagcc cagtagcgtg   2548

agggctctgt ccattgtcct ccccatcgtg ctcctcgtct tcctttgcct gggggtcttc   2608

cttctatgga agaactggcg gcttaagaac atcaacagca tcaactttga caaccccgtc   2668

tatcagaaga ccacagagga tgaggtccac atttgccaca accaggacgg ctacagctac   2728

ccctcgagac agatggtcag tctggaggat gacgtggcgt gaacatctgc ctggagtccc   2788

gtccctgccc agaacccttc ctgagacctc gccggccttg ttttattcaa agacagagaa   2848

gaccaaagca ttgcctgcca gagctttgtt ttatatattt attcatctgg gaggcagaac   2908

aggcttcgga cagtgcccat gcaatggctt gggttgggat tttggtttct tcctttcctc   2968

gtgaaggata agagaaacag gcccggggggg accaggatga cacctccatt tctctccagg   3028

aagttttgag tttctctcca ccgtgacaca atcctcaaac atggaagatg aaaggggagg   3088

ggatgtcagg cccagagaag caagtggctt tcaacacaca acagcagatg gcaccaacgg   3148

gaccccctgg ccctgcctca tccaccaatc tctaagccaa acccctaaac tcaggagtca   3208

acgtgtttac ctcttctatg caagccttgc tagacagcca ggttagcctt tgccctgtca   3268

cccccgaatc atgacccacc cagtgtcttt cgaggtgggt ttgtaccttc cttaagccag   3328

gaaagggatt catggcgtcg gaaatgatct ggctgaatcc gtggtggcac cgagaccaaa   3388

ctcattcacc aaatgatgcc acttcccaga ggcagagcct gagtcactgg tcacccttaa   3448

tatttattaa gtgcctgaga cacccggtta ccttggccgt gaggacacgt ggcctgcacc   3508

caggtgtggc tgtcaggaca ccagcctggt gcccatcctc ccgacccta cccacttcca   3568

ttcccgtggt ctccttgcac tttctcagtt cagagttgta cactgtgtac atttggcatt   3628

tgtgttatta ttttgcactg ttttctgtcg tgtgtgttgg gatgggatcc caggccaggg   3688

aaagcccgtg tcaatgaatg ccggggacag agaggggcag gttgaccggg acttcaaagc   3748

cgtgatcgtg aatatcgaga actgccattg tcgtctttat gtccgcccac ctagtgcttc   3808

cacttctatg caaatgcctc caagccattc acttccccaa tcttgtcgtt gatgggtatg   3868

tgtttaaaac atgcacggtg aggccgggcg cagtggctca cgcctgtaat cccagcactt   3928

tgggaggccg aggcgggtgg atcatgaggt caggagatcg agaccatcct ggctaacacg   3988

```
tgaaaccccg tctctactaa aaatacaaaa aattagccgg gcgtggtggc gggcacctgt      4048

agtcccagct actcgggagg ctgaggcagg agaatggtgt gaacccggga agcggagctt      4108

gcagtgagcc gagattgcgc cactgcagtc cgcagtctgg cctgggcgac agagcgagac      4168

tccgtctcaa aaaaaaaaaa caaaaaaaaa ccatgcatgg tgcatcagca gcccatggcc      4228

tctggccagg catggcgagg ctgaggtggg aggatggttt gagctcaggc atttgaggct      4288

gtcgtgagct atgattatgc cactgctttc cagcctgggc aacatagtaa gaccccatct      4348

cttaaaaaat gaatttggcc agacacaggt gcctcacgcc tgtaatccca gcactttggg      4408

aggctgagct ggatcacttg agttcaggag ttggagacca ggcctgagca acaaagcgag      4468

atcccatctc tacaaaaacc aaaaagttaa aaatcagctg ggtacggtgg cacgtgcctg      4528

tgatcccagc tacttgggag gctgaggcag gaggatcgcc tgagcccagg aggtggaggt      4588

tgcagtgagc catgatcgag ccactgcact ccagcctggg caacagatga agaccctatt      4648

tcagaaatac aactataaaa aaataaataa atcctccagt ctggatcgtt tgacgggact      4708

tcaggttctt tctgaaatcg ccgtgttact gttgcactga tgtccggaga gacagtgaca      4768

gcctccgtca gactcccgcg tgaagatgtc acaagggatt ggcaattgtc cccagggaca      4828

aaacactgtg tccccccccag tgcagggaac cgtgataagc ctttctggtt tcggagcacg      4888

taaatgcgtc cctgtacaga tagtggggat tttttgttat gtttgcactt tgtatattgg      4948

ttgaaactgt tatcacttat atatatatat atacacacat atatataaaa tctatttatt      5008

tttgcaaacc ctggttgctg tatttgttca gtgactattc tcggggccct gtgtaggggg      5068

ttattgcctc tgaaatgcct cttctttatg tacaaagatt atttgcacga actggactgt      5128

gtgcaacgct ttttgggaga atgatgtccc cgttgtatgt atgagtggct tctgggagat      5188

gggtgtcact ttttaaacca ctgtatagaa ggttttttgta gcctgaatgt cttactgtga      5248

tcaattaaat ttcttaaatg aaccaatttg tctaaaaaaa aaaa                       5292
```

```
<210>  2
<211>  2583
<212>  DNA
<213>  Homo sapiens

<220>
```

<221> CDS
<222> (1)..(2583)

<400> 2

```
atg  ggg  ccc  tgg  ggc  tgg  aaa  ttg  cgc  tgg  acc  gtc  gcc  ttg  ctc  ctc          48
Met  Gly  Pro  Trp  Gly  Trp  Lys  Leu  Arg  Trp  Thr  Val  Ala  Leu  Leu  Leu
1              5                        10                       15

gcc  gcg  gcg  ggg  act  gca  gtg  ggc  gac  aga  tgt  gaa  aga  aac  gag  ttc          96
Ala  Ala  Ala  Gly  Thr  Ala  Val  Gly  Asp  Arg  Cys  Glu  Arg  Asn  Glu  Phe
                   20                       25                       30

cag  tgc  caa  gac  ggg  aaa  tgc  atc  tcc  tac  aag  tgg  gtc  tgc  gat  ggc         144
Gln  Cys  Gln  Asp  Gly  Lys  Cys  Ile  Ser  Tyr  Lys  Trp  Val  Cys  Asp  Gly
          35                       40                       45

agc  gct  gag  tgc  cag  gat  ggc  tct  gat  gag  tcc  cag  gag  acg  tgc  ttg         192
Ser  Ala  Glu  Cys  Gln  Asp  Gly  Ser  Asp  Glu  Ser  Gln  Glu  Thr  Cys  Leu
     50                       55                       60

tct  gtc  acc  tgc  aaa  tcc  ggg  gac  ttc  agc  tgt  ggg  ggc  cgt  gtc  aac         240
Ser  Val  Thr  Cys  Lys  Ser  Gly  Asp  Phe  Ser  Cys  Gly  Gly  Arg  Val  Asn
65                       70                       75                       80

cgc  tgc  att  cct  cag  ttc  tgg  agg  tgc  gat  ggc  caa  gtg  gac  tgc  gac         288
Arg  Cys  Ile  Pro  Gln  Phe  Trp  Arg  Cys  Asp  Gly  Gln  Val  Asp  Cys  Asp
                   85                       90                       95

aac  ggc  tca  gac  gag  caa  ggc  tgt  ccc  ccc  aag  acg  tgc  tcc  cag  gac         336
Asn  Gly  Ser  Asp  Glu  Gln  Gly  Cys  Pro  Pro  Lys  Thr  Cys  Ser  Gln  Asp
                   100                      105                      110

gag  ttt  cgc  tgc  cac  gat  ggg  aag  tgc  atc  tct  cgg  cag  ttc  gtc  tgt         384
Glu  Phe  Arg  Cys  His  Asp  Gly  Lys  Cys  Ile  Ser  Arg  Gln  Phe  Val  Cys
               115                      120                      125

gac  tca  gac  cgg  gac  tgc  ttg  gac  ggc  tca  gac  gag  gcc  tcc  tgc  ccg         432
Asp  Ser  Asp  Arg  Asp  Cys  Leu  Asp  Gly  Ser  Asp  Glu  Ala  Ser  Cys  Pro
          130                      135                      140

gtg  ctc  acc  tgt  ggt  ccc  gcc  agc  ttc  cag  tgc  aac  agc  tcc  acc  tgc         480
Val  Leu  Thr  Cys  Gly  Pro  Ala  Ser  Phe  Gln  Cys  Asn  Ser  Ser  Thr  Cys
145                      150                      155                      160

atc  ccc  cag  ctg  tgg  gcc  tgc  gac  aac  gac  ccc  gac  tgc  gaa  gat  ggc         528
Ile  Pro  Gln  Leu  Trp  Ala  Cys  Asp  Asn  Asp  Pro  Asp  Cys  Glu  Asp  Gly
                   165                      170                      175

tcg  gat  gag  tgg  ccg  cag  cgc  tgt  agg  ggt  ctt  tac  gtg  ttc  caa  ggg         576
Ser  Asp  Glu  Trp  Pro  Gln  Arg  Cys  Arg  Gly  Leu  Tyr  Val  Phe  Gln  Gly
                   180                      185                      190
```

```
gac agt agc ccc tgc tcg gcc ttc gag ttc cac tgc cta agt ggc gag        624
Asp Ser Ser Pro Cys Ser Ala Phe Glu Phe His Cys Leu Ser Gly Glu
        195             200             205

tgc atc cac tcc agc tgg cgc tgt gat ggt ggc ccc gac tgc aag gac        672
Cys Ile His Ser Ser Trp Arg Cys Asp Gly Gly Pro Asp Cys Lys Asp
    210             215             220

aaa tct gac gag gaa aac tgc gct gtg gcc acc tgt cgc cct gac gaa        720
Lys Ser Asp Glu Glu Asn Cys Ala Val Ala Thr Cys Arg Pro Asp Glu
225             230             235             240

ttc cag tgc tct gat gga aac tgc atc cat ggc agc cgg cag tgt gac        768
Phe Gln Cys Ser Asp Gly Asn Cys Ile His Gly Ser Arg Gln Cys Asp
            245             250             255

cgg gaa tat gac tgc aag gac atg agc gat gaa gtt ggc tgc gtt aat        816
Arg Glu Tyr Asp Cys Lys Asp Met Ser Asp Glu Val Gly Cys Val Asn
            260             265             270

gtg aca ctc tgc gag gga ccc aac aag ttc aag tgt cac agc ggc gaa        864
Val Thr Leu Cys Glu Gly Pro Asn Lys Phe Lys Cys His Ser Gly Glu
        275             280             285

tgc atc acc ctg gac aaa gtc tgc aac atg gct aga gac tgc cgg gac        912
Cys Ile Thr Leu Asp Lys Val Cys Asn Met Ala Arg Asp Cys Arg Asp
    290             295             300

tgg tca gat gaa ccc atc aaa gag tgc ggg acc aac gaa tgc ttg gac        960
Trp Ser Asp Glu Pro Ile Lys Glu Cys Gly Thr Asn Glu Cys Leu Asp
305             310             315             320

aac aac ggc ggc tgt tcc cac gtc tgc aat gac ctt aag atc ggc tac       1008
Asn Asn Gly Gly Cys Ser His Val Cys Asn Asp Leu Lys Ile Gly Tyr
            325             330             335

gag tgc ctg tgc ccc gac ggc ttc cag ctg gtg gcc cag cga aga tgc       1056
Glu Cys Leu Cys Pro Asp Gly Phe Gln Leu Val Ala Gln Arg Arg Cys
            340             345             350

gaa gat atc gat gag tgt cag gat ccc gac acc tgc agc cag ctc tgc       1104
Glu Asp Ile Asp Glu Cys Gln Asp Pro Asp Thr Cys Ser Gln Leu Cys
            355             360             365

gtg aac ctg gag ggt ggc tac aag tgc cag tgt gag gaa ggc ttc cag       1152
Val Asn Leu Glu Gly Gly Tyr Lys Cys Gln Cys Glu Glu Gly Phe Gln
    370             375             380

ctg gac ccc cac acg aag gcc tgc aag gct gtg ggc tcc atc gcc tac       1200
Leu Asp Pro His Thr Lys Ala Cys Lys Ala Val Gly Ser Ile Ala Tyr
385             390             395             400
```

```
ctc ttc ttc acc aac cgg cac gag gtc agg aag atg acg ctg gac cgg          1248
Leu Phe Phe Thr Asn Arg His Glu Val Arg Lys Met Thr Leu Asp Arg
                405                 410                 415

agc gag tac acc agc ctc atc ccc aac ctg agg aac gtg gtc gct ctg          1296
Ser Glu Tyr Thr Ser Leu Ile Pro Asn Leu Arg Asn Val Val Ala Leu
                420                 425                 430

gac acg gag gtg gcc agc aat aga atc tac tgg tct gac ctg tcc cag          1344
Asp Thr Glu Val Ala Ser Asn Arg Ile Tyr Trp Ser Asp Leu Ser Gln
                435                 440                 445

aga atg atc tgc agc acc cag ctt gac aga gcc cac ggc gtc tct tcc          1392
Arg Met Ile Cys Ser Thr Gln Leu Asp Arg Ala His Gly Val Ser Ser
            450                 455                 460

tat gac acc gtc atc agc agg gac atc cag gcc ccc gac ggg ctg gct          1440
Tyr Asp Thr Val Ile Ser Arg Asp Ile Gln Ala Pro Asp Gly Leu Ala
465                 470                 475                 480

gtg gac tgg atc cac agc aac atc tac tgg acc gac tct gtc ctg ggc          1488
Val Asp Trp Ile His Ser Asn Ile Tyr Trp Thr Asp Ser Val Leu Gly
                485                 490                 495

act gtc tct gtt gcg gat acc aag ggc gtg aag agg aaa acg tta ttc          1536
Thr Val Ser Val Ala Asp Thr Lys Gly Val Lys Arg Lys Thr Leu Phe
                500                 505                 510

agg gag aac ggc tcc aag cca agg gcc atc gtg gtg gat cct gtt cat          1584
Arg Glu Asn Gly Ser Lys Pro Arg Ala Ile Val Val Asp Pro Val His
                515                 520                 525

ggc ttc atg tac tgg act gac tgg gga act ccc gcc aag atc aag aaa          1632
Gly Phe Met Tyr Trp Thr Asp Trp Gly Thr Pro Ala Lys Ile Lys Lys
                530                 535                 540

ggg ggc ctg aat ggt gtg gac atc tac tcg ctg gtg act gaa aac att          1680
Gly Gly Leu Asn Gly Val Asp Ile Tyr Ser Leu Val Thr Glu Asn Ile
545                 550                 555                 560

cag tgg ccc aat ggc atc acc cta gat ctc ctc agt ggc cgc ctc tac          1728
Gln Trp Pro Asn Gly Ile Thr Leu Asp Leu Leu Ser Gly Arg Leu Tyr
                565                 570                 575

tgg gtt gac tcc aaa ctt cac tcc atc tca agc atc gat gtc aat ggg          1776
Trp Val Asp Ser Lys Leu His Ser Ile Ser Ser Ile Asp Val Asn Gly
                580                 585                 590

ggc aac cgg aag acc atc ttg gag gat gaa aag agg ctg gcc cac ccc          1824
Gly Asn Arg Lys Thr Ile Leu Glu Asp Glu Lys Arg Leu Ala His Pro
                595                 600                 605
```

ttc	tcc	ttg	gcc	gtc	ttt	gag	gac	aaa	gta	ttt	tgg	aca	gat	atc	atc	1872
Phe	Ser	Leu	Ala	Val	Phe	Glu	Asp	Lys	Val	Phe	Trp	Thr	Asp	Ile	Ile	
	610					615					620					

aac	gaa	gcc	att	ttc	agt	gcc	aac	cgc	ctc	aca	ggt	tcc	gat	gtc	aac	1920
Asn	Glu	Ala	Ile	Phe	Ser	Ala	Asn	Arg	Leu	Thr	Gly	Ser	Asp	Val	Asn	
625					630					635					640	

ttg	ttg	gct	gaa	aac	cta	ctg	tcc	cca	gag	gat	atg	gtc	ctc	ttc	cac	1968
Leu	Leu	Ala	Glu	Asn	Leu	Leu	Ser	Pro	Glu	Asp	Met	Val	Leu	Phe	His	
				645					650					655		

aac	ctc	acc	cag	cca	aga	gga	gtg	aac	tgg	tgt	gag	agg	acc	acc	ctg	2016
Asn	Leu	Thr	Gln	Pro	Arg	Gly	Val	Asn	Trp	Cys	Glu	Arg	Thr	Thr	Leu	
			660					665					670			

agc	aat	ggc	ggc	tgc	cag	tat	ctg	tgc	ctc	cct	gcc	ccg	cag	atc	aac	2064
Ser	Asn	Gly	Gly	Cys	Gln	Tyr	Leu	Cys	Leu	Pro	Ala	Pro	Gln	Ile	Asn	
		675					680					685				

ccc	cac	tcg	ccc	aag	ttt	acc	tgc	gcc	tgc	ccg	gac	ggc	atg	ctg	ctg	2112
Pro	His	Ser	Pro	Lys	Phe	Thr	Cys	Ala	Cys	Pro	Asp	Gly	Met	Leu	Leu	
	690					695					700					

gcc	agg	gac	atg	agg	agc	tgc	ctc	aca	gag	gct	gag	gct	gca	gtg	gcc	2160
Ala	Arg	Asp	Met	Arg	Ser	Cys	Leu	Thr	Glu	Ala	Glu	Ala	Ala	Val	Ala	
705					710					715					720	

acc	cag	gag	aca	tcc	acc	gtc	agg	cta	aag	gtc	agc	tcc	aca	gcc	gta	2208
Thr	Gln	Glu	Thr	Ser	Thr	Val	Arg	Leu	Lys	Val	Ser	Ser	Thr	Ala	Val	
				725					730					735		

agg	aca	cag	cac	aca	acc	acc	cgg	cct	gtt	ccc	gac	acc	tcc	cgg	ctg	2256
Arg	Thr	Gln	His	Thr	Thr	Thr	Arg	Pro	Val	Pro	Asp	Thr	Ser	Arg	Leu	
			740					745					750			

cct	ggg	gcc	acc	cct	ggg	ctc	acc	acg	gtg	gag	ata	gtg	aca	atg	tct	2304
Pro	Gly	Ala	Thr	Pro	Gly	Leu	Thr	Thr	Val	Glu	Ile	Val	Thr	Met	Ser	
		755					760					765				

cac	caa	gct	ctg	ggc	gac	gtt	gct	ggc	aga	gga	aat	gag	aag	aag	ccc	2352
His	Gln	Ala	Leu	Gly	Asp	Val	Ala	Gly	Arg	Gly	Asn	Glu	Lys	Lys	Pro	
	770					775					780					

agt	agc	gtg	agg	gct	ctg	tcc	att	gtc	ctc	ccc	atc	gtg	ctc	ctc	gtc	2400
Ser	Ser	Val	Arg	Ala	Leu	Ser	Ile	Val	Leu	Pro	Ile	Val	Leu	Leu	Val	
785					790					795					800	

ttc	ctt	tgc	ctg	ggg	gtc	ttc	ctt	cta	tgg	aag	aac	tgg	cgg	ctt	aag	2448
Phe	Leu	Cys	Leu	Gly	Val	Phe	Leu	Leu	Trp	Lys	Asn	Trp	Arg	Leu	Lys	
				805					810					815		

```
aac atc aac agc atc aac ttt gac aac ccc gtc tat cag aag acc aca         2496
Asn Ile Asn Ser Ile Asn Phe Asp Asn Pro Val Tyr Gln Lys Thr Thr
            820             825             830

gag gat gag gtc cac att tgc cac aac cag gac ggc tac agc tac ccc         2544
Glu Asp Glu Val His Ile Cys His Asn Gln Asp Gly Tyr Ser Tyr Pro
        835             840             845

tcg aga cag atg gtc agt ctg gag gat gac gtg gcg tag                     2583
Ser Arg Gln Met Val Ser Leu Glu Asp Asp Val Ala
    850             855             860
```

<210> 3
<211> 860
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Gly Pro Trp Gly Trp Lys Leu Arg Trp Thr Val Ala Leu Leu Leu
1               5               10              15

Ala Ala Ala Gly Thr Ala Val Gly Asp Arg Cys Glu Arg Asn Glu Phe
            20              25              30

Gln Cys Gln Asp Gly Lys Cys Ile Ser Tyr Lys Trp Val Cys Asp Gly
        35              40              45

Ser Ala Glu Cys Gln Asp Gly Ser Asp Glu Ser Gln Glu Thr Cys Leu
    50              55              60

Ser Val Thr Cys Lys Ser Gly Asp Phe Ser Cys Gly Gly Arg Val Asn
65              70              75              80

Arg Cys Ile Pro Gln Phe Trp Arg Cys Asp Gly Gln Val Asp Cys Asp
                85              90              95

Asn Gly Ser Asp Glu Gln Gly Cys Pro Pro Lys Thr Cys Ser Gln Asp
            100             105             110

Glu Phe Arg Cys His Asp Gly Lys Cys Ile Ser Arg Gln Phe Val Cys
        115             120             125
```

Asp Ser Asp Arg Asp Cys Leu Asp Gly Ser Asp Glu Ala Ser Cys Pro
    130   135   140

Val Leu Thr Cys Gly Pro Ala Ser Phe Gln Cys Asn Ser Ser Thr Cys
145   150   155   160

Ile Pro Gln Leu Trp Ala Cys Asp Asn Asp Pro Asp Cys Glu Asp Gly
            165   170   175

Ser Asp Glu Trp Pro Gln Arg Cys Arg Gly Leu Tyr Val Phe Gln Gly
            180   185   190

Asp Ser Ser Pro Cys Ser Ala Phe Glu Phe His Cys Leu Ser Gly Glu
    195   200   205

Cys Ile His Ser Ser Trp Arg Cys Asp Gly Gly Pro Asp Cys Lys Asp
    210   215   220

Lys Ser Asp Glu Glu Asn Cys Ala Val Ala Thr Cys Arg Pro Asp Glu
225   230   235   240

Phe Gln Cys Ser Asp Gly Asn Cys Ile His Gly Ser Arg Gln Cys Asp
            245   250   255

Arg Glu Tyr Asp Cys Lys Asp Met Ser Asp Glu Val Gly Cys Val Asn
            260   265   270

Val Thr Leu Cys Glu Gly Pro Asn Lys Phe Lys Cys His Ser Gly Glu
            275   280   285

Cys Ile Thr Leu Asp Lys Val Cys Asn Met Ala Arg Asp Cys Arg Asp
    290   295   300

Trp Ser Asp Glu Pro Ile Lys Glu Cys Gly Thr Asn Glu Cys Leu Asp
305   310   315   320

Asn Asn Gly Gly Cys Ser His Val Cys Asn Asp Leu Lys Ile Gly Tyr
            325   330   335

```
Glu Cys Leu Cys Pro Asp Gly Phe Gln Leu Val Ala Gln Arg Arg Cys
        340             345             350


Glu Asp Ile Asp Glu Cys Gln Asp Pro Asp Thr Cys Ser Gln Leu Cys
        355             360             365


Val Asn Leu Glu Gly Gly Tyr Lys Cys Gln Cys Glu Glu Gly Phe Gln
        370             375             380


Leu Asp Pro His Thr Lys Ala Cys Lys Ala Val Gly Ser Ile Ala Tyr
385             390             395             400


Leu Phe Phe Thr Asn Arg His Glu Val Arg Lys Met Thr Leu Asp Arg
            405             410             415


Ser Glu Tyr Thr Ser Leu Ile Pro Asn Leu Arg Asn Val Val Ala Leu
        420             425             430


Asp Thr Glu Val Ala Ser Asn Arg Ile Tyr Trp Ser Asp Leu Ser Gln
        435             440             445


Arg Met Ile Cys Ser Thr Gln Leu Asp Arg Ala His Gly Val Ser Ser
    450             455             460


Tyr Asp Thr Val Ile Ser Arg Asp Ile Gln Ala Pro Asp Gly Leu Ala
465             470             475             480


Val Asp Trp Ile His Ser Asn Ile Tyr Trp Thr Asp Ser Val Leu Gly
            485             490             495


Thr Val Ser Val Ala Asp Thr Lys Gly Val Lys Arg Lys Thr Leu Phe
        500             505             510


Arg Glu Asn Gly Ser Lys Pro Arg Ala Ile Val Val Asp Pro Val His
        515             520             525


Gly Phe Met Tyr Trp Thr Asp Trp Gly Thr Pro Ala Lys Ile Lys Lys
    530             535             540
```

Gly Gly Leu Asn Gly Val Asp Ile Tyr Ser Leu Val Thr Glu Asn Ile
545   550   555   560

Gln Trp Pro Asn Gly Ile Thr Leu Asp Leu Leu Ser Gly Arg Leu Tyr
                565   570   575

Trp Val Asp Ser Lys Leu His Ser Ile Ser Ser Ile Asp Val Asn Gly
            580   585   590

Gly Asn Arg Lys Thr Ile Leu Glu Asp Glu Lys Arg Leu Ala His Pro
        595   600   605

Phe Ser Leu Ala Val Phe Glu Asp Lys Val Phe Trp Thr Asp Ile Ile
    610   615   620

Asn Glu Ala Ile Phe Ser Ala Asn Arg Leu Thr Gly Ser Asp Val Asn
625   630   635   640

Leu Leu Ala Glu Asn Leu Leu Ser Pro Glu Asp Met Val Leu Phe His
            645   650   655

Asn Leu Thr Gln Pro Arg Gly Val Asn Trp Cys Glu Arg Thr Thr Leu
            660   665   670

Ser Asn Gly Gly Cys Gln Tyr Leu Cys Leu Pro Ala Pro Gln Ile Asn
        675   680   685

Pro His Ser Pro Lys Phe Thr Cys Ala Cys Pro Asp Gly Met Leu Leu
    690   695   700

Ala Arg Asp Met Arg Ser Cys Leu Thr Glu Ala Glu Ala Ala Val Ala
705   710   715   720

Thr Gln Glu Thr Ser Thr Val Arg Leu Lys Val Ser Ser Thr Ala Val
            725   730   735

Arg Thr Gln His Thr Thr Thr Arg Pro Val Pro Asp Thr Ser Arg Leu
        740   745   750

```
Pro Gly Ala Thr Pro Gly Leu Thr Thr Val Glu Ile Val Thr Met Ser
        755                 760                 765


His Gln Ala Leu Gly Asp Val Ala Gly Arg Gly Asn Glu Lys Lys Pro
        770                 775                 780


Ser Ser Val Arg Ala Leu Ser Ile Val Leu Pro Ile Val Leu Leu Val
785                 790                 795                 800


Phe Leu Cys Leu Gly Val Phe Leu Leu Trp Lys Asn Trp Arg Leu Lys
                805                 810                 815


Asn Ile Asn Ser Ile Asn Phe Asp Asn Pro Val Tyr Gln Lys Thr Thr
                820                 825                 830


Glu Asp Glu Val His Ile Cys His Asn Gln Asp Gly Tyr Ser Tyr Pro
        835                 840                 845


Ser Arg Gln Met Val Ser Leu Glu Asp Asp Val Ala
        850                 855                 860
```

```
<210>  4
<211>  2583
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Artificial hLDLR


<220>
<221>  misc_feature
<222>  (1)..(2583)
<223>  Artificial hLDLR coding sequence

<400>  4
atgggacctt ggggttggaa actccgctgg acagtggctc tgctcctggc agcagcagga       60

acagccgtgg agatcgctg cgaaaggaac gagttccagt gccaggacgg caagtgcatc      120

agctacaagt gggtctgcga cggtagcgca gagtgtcagg acggaagcga cgaaagccag      180

gagacttgcc tgagcgtgac ttgcaagtcc ggcgacttct cttgcggagg cagagtgaac      240

cgctgcatcc ctcagttttg gcggtgcgac ggccaggtgg attgcgataa cggaagcgac      300
```

gagcagggtt gccctcctaa gacttgcagc caggacgaat tccgctgtca cgacggcaag   360

tgcatcagca ggcagttcgt ctgcgacagc gacagggatt gtctggacgg aagcgacgag   420

gcctcttgtc ctgtgctgac ttgtggccca gccagcttcc agtgcaactc cagcacttgc   480

atcccacagc tctgggcttg cgacaacgac ccagattgcg aggacggatc agacgagtgg   540

ccacagcgct gcagaggcct gtacgtgttt cagggcgatt ccagcccttg cagcgctttt   600

gagttccact gcctgagcgg cgagtgcatt cactcttctt ggaggtgcga cggtggccca   660

gattgcaagg acaagagcga cgaggagaat tgcgccgtgg ctacttgcag accagacgaa   720

ttccagtgca gcgacggcaa ttgcatccac ggctctaggc agtgcgacag ggagtacgat   780

tgcaaggaca tgagcgacga agtcggttgc gtgaacgtca ccctctgcga gggtcccaat   840

aagttcaagt gccacagcgg cgagtgcatt accctggaca aggtctgcaa catggccagg   900

gattgccggg attggagcga cgagcctatc aaggagtgcg gcaccaacga gtgcctggat   960

aacaacggcg gctgcagcca cgtgtgcaat gacctgaaga tcggctacga gtgcctctgc   1020

ccagacggat tccagctggt ggctcagaga cgctgcgaag acatcgacga gtgccaggat   1080

cccgacactt gcagccagct gtgcgtgaat ctggagggag gctacaagtg ccagtgcgaa   1140

gagggattcc agctggaccc tcacaccaag gcttgtaaag ccgtgggcag catcgcctac   1200

ctgttcttca ccaacagaca cgaagtgcgg aagatgaccc tggatcggag cgagtacacc   1260

agcctgatcc ctaacctgcg gaacgtggtg gccctggata cagaagtggc cagcaacagg   1320

atctattgga gcgacctgag ccagcggatg atttgcagca cccagctgga cagagcccac   1380

ggagtgtcca gctacgacac cgtgatcagc agagacatcc aggctccaga cggactggca   1440

gtggattgga tccacagcaa catctactgg accgactcag tgctgggaac agtgtccgtg   1500

gccgatacaa agggcgtgaa gcggaagacc ctgttcagag agaacggcag caagcccagg   1560

gctattgtgg tggatcccgt gcacggcttc atgtattgga ccgattgggg cacccccgct   1620

aagatcaaga agggcggcct gaacggcgtg gacatctaca gcctggtgac cgagaacatc   1680

cagtggccca acggaattac cctggacctg ctgagcggca gactgtattg ggtggacagc   1740

aagctgcaca gcatcagcag catcgacgtg aacggcggaa accggaagac catcctggag   1800

gacgagaaga gactggccca cccctttcagc ctggccgtgt cgaggacaa ggtcttctgg   1860

```
accgacatca tcaacgaggc catcttcagc gccaacaggc tgacaggaag cgacgtgaac    1920

ctgctggcag agaatctgct gtctcccgag gacatggtgc tgttccacaa cctgacccag    1980

cccagaggcg tcaattggtg cgagagaacc accctgagca acggaggttg ccagtacctg    2040

tgcctgccag cccctcagat taaccctcac agccccaagt tcacttgcgc ttgcccagac    2100

ggcatgctgc tggccagaga tatgcggtct tgtctgacag aagccgaagc cgctgtggct    2160

acacaggaga caagcaccgt gcggctgaag gtgtctagca gccgtgag aacccagcac    2220

acaaccacca gacccgtgcc agataccagc agactgccag agctacacc aggactgacc    2280

accgtggaga tcgtgaccat gagccaccag gctctgggag acgtggcagg aagaggaaac    2340

gagaagaagc ctagcagcgt gagagccctg tctatcgtgc tgcccatcgt gctgctggtg    2400

ttcctctgtc tgggcgtgtt cctcctctgg aagaattggc ggctgaagaa catcaacagc    2460

atcaacttcg acaaccccgt gtaccagaag accaccgagg acgaggtgca catttgccac    2520

aaccaggacg ctacagcta ccctagcagg cagatggtgt ccctggaaga cgacgtggct    2580

tga                                                                  2583
```

<210> 5
<211> 738
<212> PRT
<213> Artificial Sequence

<220>
<223> Adeno-associated virus 8 vp1 capsid protein

<400> 5

```
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15


Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Lys Pro
                20                  25                  30


Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
        35                  40                  45


Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60
```

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65   70   75   80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
            85   90   95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100   105   110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
            115   120   125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130   135   140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145   150   155   160

Gly Lys Lys Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln
            165   170   175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro
            180   185   190

Pro Ala Ala Pro Ser Gly Val Gly Pro Asn Thr Met Ala Ala Gly Gly
            195   200   205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210   215   220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225   230   235   240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
            245   250   255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ala Thr Asn Asp
            260   265   270

Page 20

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
      275   280   285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
      290   295   300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Ser Phe Lys Leu Phe Asn
305   310   315   320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
            325   330   335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
          340   345   350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
      355   360   365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
      370   375   380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385   390   395   400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Thr Tyr
          405   410   415

Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
          420   425   430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
          435   440   445

Ser Arg Thr Gln Thr Thr Gly Gly Thr Ala Asn Thr Gln Thr Leu Gly
      450   455   460

Phe Ser Gln Gly Gly Pro Asn Thr Met Ala Asn Gln Ala Lys Asn Trp
465   470   475   480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Thr Gly
            485   490   495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Ala Gly Thr Lys Tyr His
            500   505   510

Leu Asn Gly Arg Asn Ser Leu Ala Asn Pro Gly Ile Ala Met Ala Thr
            515   520   525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Asn Gly Ile Leu Ile
    530   535   540

Phe Gly Lys Gln Asn Ala Ala Arg Asp Asn Ala Asp Tyr Ser Asp Val
545   550   555   560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
            565   570   575

Glu Glu Tyr Gly Ile Val Ala Asp Asn Leu Gln Gln Gln Asn Thr Ala
            580   585   590

Pro Gln Ile Gly Thr Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
            595   600   605

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    610   615   620

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
625   630   635   640

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
            645   650   655

Pro Ala Asp Pro Pro Thr Thr Phe Asn Gln Ser Lys Leu Asn Ser Phe
            660   665   670

Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
            675   680   685

Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
    690   695   700

Ser Asn Tyr Tyr Lys Ser Thr Ser Val Asp Phe Ala Val Asn Thr Glu
705   710   715   720

Gly Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
                725   730   735

Asn Leu

<210> 6
<211> 6768
<212> DNA
<213> Artificial Sequence

<220>
<223> pAAV.TBG.PI.hLDLRco.RGB

<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

<220>
<221> TATA_signal
<222> (885)..(888)
<223> TATA

<220>
<221> CDS
<222> (969)..(3551)
<223> codon optimized hLDLR

<220>
<221> polyA_signal
<222> (3603)..(3729)
<223> Rabbit globin poly A

<220>
<221> repeat_region
<222> (3818)..(3947)
<223> 3' ITR

<220>
<221> rep_origin
<222> (4124)..(4579)
<223> f1 ori

<220>
<221> misc_feature
<222> (4710)..(5567)
<223> AP(R)

<220>
<221> rep_origin
<222> (5741)..(6329)
<223> Origin of replication

<400> 6
ctgcgcgctc gctcgctcac tgaggccgcc cgggcaaagc ccgggcgtcg ggcgaccttt   60

ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact   120

aggggttcct tgtagttaat gattaacccg ccatgctact tatctaccag ggtaatgggg   180

atcctctaga actatagcta gaattcgccc ttaagctagc aggttaattt ttaaaaagca   240

gtcaaaagtc caagtggccc ttggcagcat ttactctctc tgtttgctct ggttaataat   300

ctcaggagca caaacattcc agatccaggt taattttttaa aaagcagtca aaagtccaag   360

tggcccttgg cagcatttac tctctctgtt tgctctggtt aataatctca ggagcacaaa   420

cattccagat ccggcgcgcc agggctggaa gctacctttg acatcatttc ctctgcgaat   480

gcatgtataa tttctacaga acctattaga aaggatcacc cagcctctgc ttttgtacaa   540

ctttcccctta aaaaactgcc aattccactg ctgtttggcc caatagtgag aacttttttcc   600

tgctgcctct tggtgctttt gcctatggcc cctattctgc ctgctgaaga cactcttgcc   660

agcatggact taaaccccctc cagctctgac aatcctcttt ctcttttgtt ttacatgaag   720

ggtctggcag ccaaagcaat cactcaaagt tcaaacctta tcattttttg ctttgttcct   780

cttggccttg gttttgtaca tcagctttga aaataccatc ccagggttaa tgctggggtt   840

aatttataac taagagtgct ctagttttgc aatacaggac atgctataaa aatggaaaga   900

tgttgctttc tgagagacag ctttattgcg gtagtttatc acagttaaat tgctaacgca   960

gacgttgc atg gga cct tgg ggt tgg aaa ctc cgc tgg aca gtg gct ctg   1010
         Met Gly Pro Trp Gly Trp Lys Leu Arg Trp Thr Val Ala Leu
         1   5   10

ctc ctg gca gca gca gga aca gcc gtg gga gat cgc tgc gaa agg aac   1058
Leu Leu Ala Ala Ala Gly Thr Ala Val Gly Asp Arg Cys Glu Arg Asn
15   20   25   30

gag ttc cag tgc cag gac ggc aag tgc atc agc tac aag tgg gtc tgc   1106
Glu Phe Gln Cys Gln Asp Gly Lys Cys Ile Ser Tyr Lys Trp Val Cys
              35   40   45

gac ggt agc gca gag tgt cag gac gga agc gac gaa agc cag gag act   1154
Asp Gly Ser Ala Glu Cys Gln Asp Gly Ser Asp Glu Ser Gln Glu Thr
         50   55   60

tgc ctg agc gtg act tgc aag tcc ggc gac ttc tct tgc gga ggc aga   1202
Cys Leu Ser Val Thr Cys Lys Ser Gly Asp Phe Ser Cys Gly Gly Arg
         65   70   75

gtg aac cgc tgc atc cct cag ttt tgg cgg tgc gac ggc cag gtg gat   1250
Val Asn Arg Cys Ile Pro Gln Phe Trp Arg Cys Asp Gly Gln Val Asp
         80   85   90

tgc gat aac gga agc gac gag cag ggt tgc cct cct aag act tgc agc   1298
Cys Asp Asn Gly Ser Asp Glu Gln Gly Cys Pro Pro Lys Thr Cys Ser
95   100   105   110

cag gac gaa ttc cgc tgt cac gac ggc aag tgc atc agc agg cag ttc   1346
Gln Asp Glu Phe Arg Cys His Asp Gly Lys Cys Ile Ser Arg Gln Phe
             115   120   125

gtc tgc gac agc gac agg gat tgt ctg gac gga agc gac gag gcc tct   1394
Val Cys Asp Ser Asp Arg Asp Cys Leu Asp Gly Ser Asp Glu Ala Ser
             130   135   140

tgt cct gtg ctg act tgt ggc cca gcc agc ttc cag tgc aac tcc agc   1442
Cys Pro Val Leu Thr Cys Gly Pro Ala Ser Phe Gln Cys Asn Ser Ser
         145   150   155

act tgc atc cca cag ctc tgg gct tgc gac aac gac cca gat tgc gag   1490

Thr Cys Ile Pro Gln Leu Trp Ala Cys Asp Asn Asp Pro Asp Cys Glu
    160   165   170

gac gga tca gac gag tgg cca cag cgc tgc aga ggc ctg tac gtg ttt   1538
Asp Gly Ser Asp Glu Trp Pro Gln Arg Cys Arg Gly Leu Tyr Val Phe
175   180   185   190

cag ggc gat tcc agc cct tgc agc gct ttt gag ttc cac tgc ctg agc   1586
Gln Gly Asp Ser Ser Pro Cys Ser Ala Phe Glu Phe His Cys Leu Ser
                195   200   205

ggc gag tgc att cac tct tct tgg agg tgc gac ggt ggc cca gat tgc   1634
Gly Glu Cys Ile His Ser Ser Trp Arg Cys Asp Gly Gly Pro Asp Cys
                210   215   220

aag gac aag agc gac gag gag aat tgc gcc gtg gct act tgc aga cca   1682
Lys Asp Lys Ser Asp Glu Glu Asn Cys Ala Val Ala Thr Cys Arg Pro
                225   230   235

gac gaa ttc cag tgc agc gac ggc aat tgc atc cac ggc tct agg cag   1730
Asp Glu Phe Gln Cys Ser Asp Gly Asn Cys Ile His Gly Ser Arg Gln
        240   245   250

tgc gac agg gag tac gat tgc aag gac atg agc gac gaa gtc ggt tgc   1778
Cys Asp Arg Glu Tyr Asp Cys Lys Asp Met Ser Asp Glu Val Gly Cys
255   260   265   270

gtg aac gtc acc ctc tgc gag ggt ccc aat aag ttc aag tgc cac agc   1826
Val Asn Val Thr Leu Cys Glu Gly Pro Asn Lys Phe Lys Cys His Ser
                275   280   285

ggc gag tgc att acc ctg gac aag gtc tgc aac atg gcc agg gat tgc   1874
Gly Glu Cys Ile Thr Leu Asp Lys Val Cys Asn Met Ala Arg Asp Cys
                290   295   300

cgg gat tgg agc gac gag cct atc aag gag tgc ggc acc aac gag tgc   1922
Arg Asp Trp Ser Asp Glu Pro Ile Lys Glu Cys Gly Thr Asn Glu Cys
                305   310   315

ctg gat aac aac ggc ggc tgc agc cac gtg tgc aat gac ctg aag atc   1970
Leu Asp Asn Asn Gly Gly Cys Ser His Val Cys Asn Asp Leu Lys Ile
        320   325   330

ggc tac gag tgc ctc tgc cca gac gga ttc cag ctg gtg gct cag aga   2018
Gly Tyr Glu Cys Leu Cys Pro Asp Gly Phe Gln Leu Val Ala Gln Arg
335   340   345   350

cgc tgc gaa gac atc gac gag tgc cag gat ccc gac act tgc agc cag   2066
Arg Cys Glu Asp Ile Asp Glu Cys Gln Asp Pro Asp Thr Cys Ser Gln
                355   360   365

ctg tgc gtg aat ctg gag gga ggc tac aag tgc cag tgc gaa gag gga   2114

```
Leu Cys Val Asn Leu Glu Gly Gly Tyr Lys Cys Gln Cys Glu Glu Gly
        370             375             380
```

```
ttc cag ctg gac cct cac acc aag gct tgt aaa gcc gtg ggc agc atc    2162
Phe Gln Leu Asp Pro His Thr Lys Ala Cys Lys Ala Val Gly Ser Ile
        385             390             395
```

```
gcc tac ctg ttc ttc acc aac aga cac gaa gtg cgg aag atg acc ctg    2210
Ala Tyr Leu Phe Phe Thr Asn Arg His Glu Val Arg Lys Met Thr Leu
    400             405             410
```

```
gat cgg agc gag tac acc agc ctg atc cct aac ctg cgg aac gtg gtg    2258
Asp Arg Ser Glu Tyr Thr Ser Leu Ile Pro Asn Leu Arg Asn Val Val
415             420             425             430
```

```
gcc ctg gat aca gaa gtg gcc agc aac agg atc tat tgg agc gac ctg    2306
Ala Leu Asp Thr Glu Val Ala Ser Asn Arg Ile Tyr Trp Ser Asp Leu
            435             440             445
```

```
agc cag cgg atg att tgc agc acc cag ctg gac aga gcc cac gga gtg    2354
Ser Gln Arg Met Ile Cys Ser Thr Gln Leu Asp Arg Ala His Gly Val
        450             455             460
```

```
tcc agc tac gac acc gtg atc agc aga gac atc cag gct cca gac gga    2402
Ser Ser Tyr Asp Thr Val Ile Ser Arg Asp Ile Gln Ala Pro Asp Gly
        465             470             475
```

```
ctg gca gtg gat tgg atc cac agc aac atc tac tgg acc gac tca gtg    2450
Leu Ala Val Asp Trp Ile His Ser Asn Ile Tyr Trp Thr Asp Ser Val
    480             485             490
```

```
ctg gga aca gtg tcc gtg gcc gat aca aag ggc gtg aag cgg aag acc    2498
Leu Gly Thr Val Ser Val Ala Asp Thr Lys Gly Val Lys Arg Lys Thr
495             500             505             510
```

```
ctg ttc aga gag aac ggc agc aag ccc agg gct att gtg gtg gat ccc    2546
Leu Phe Arg Glu Asn Gly Ser Lys Pro Arg Ala Ile Val Val Asp Pro
            515             520             525
```

```
gtg cac ggc ttc atg tat tgg acc gat tgg ggc acc ccc gct aag atc    2594
Val His Gly Phe Met Tyr Trp Thr Asp Trp Gly Thr Pro Ala Lys Ile
        530             535             540
```

```
aag aag ggc ggc ctg aac ggc gtg gac atc tac agc ctg gtg acc gag    2642
Lys Lys Gly Gly Leu Asn Gly Val Asp Ile Tyr Ser Leu Val Thr Glu
        545             550             555
```

```
aac atc cag tgg ccc aac gga att acc ctg gac ctg ctg agc ggc aga    2690
Asn Ile Gln Trp Pro Asn Gly Ile Thr Leu Asp Leu Leu Ser Gly Arg
    560             565             570
```

```
ctg tat tgg gtg gac agc aag ctg cac agc atc agc agc atc gac gtg    2738
```

Page 27

| Leu | Tyr | Trp | Val | Asp | Ser | Lys | Leu | His | Ser | Ile | Ser | Ser | Ile | Asp | Val |
| 575 | | | | | 580 | | | | 585 | | | | | | 590 |

aac	ggc	gga	aac	cgg	aag	acc	atc	ctg	gag	gac	gag	aag	aga	ctg	gcc	2786
Asn	Gly	Gly	Asn	Arg	Lys	Thr	Ile	Leu	Glu	Asp	Glu	Lys	Arg	Leu	Ala	
				595					600					605		

cac	cct	ttc	agc	ctg	gcc	gtg	ttc	gag	gac	aag	gtc	ttc	tgg	acc	gac	2834
His	Pro	Phe	Ser	Leu	Ala	Val	Phe	Glu	Asp	Lys	Val	Phe	Trp	Thr	Asp	
			610					615					620			

atc	atc	aac	gag	gcc	atc	ttc	agc	gcc	aac	agg	ctg	aca	gga	agc	gac	2882
Ile	Ile	Asn	Glu	Ala	Ile	Phe	Ser	Ala	Asn	Arg	Leu	Thr	Gly	Ser	Asp	
		625					630					635				

gtg	aac	ctg	ctg	gca	gag	aat	ctg	ctg	tct	ccc	gag	gac	atg	gtg	ctg	2930
Val	Asn	Leu	Leu	Ala	Glu	Asn	Leu	Leu	Ser	Pro	Glu	Asp	Met	Val	Leu	
	640					645					650					

ttc	cac	aac	ctg	acc	cag	ccc	aga	ggc	gtc	aat	tgg	tgc	gag	aga	acc	2978
Phe	His	Asn	Leu	Thr	Gln	Pro	Arg	Gly	Val	Asn	Trp	Cys	Glu	Arg	Thr	
655					660					665					670	

acc	ctg	agc	aac	gga	ggt	tgc	cag	tac	ctg	tgc	ctg	cca	gcc	cct	cag	3026
Thr	Leu	Ser	Asn	Gly	Gly	Cys	Gln	Tyr	Leu	Cys	Leu	Pro	Ala	Pro	Gln	
			675					680					685			

att	aac	cct	cac	agc	ccc	aag	ttc	act	tgc	gct	tgc	cca	gac	ggc	atg	3074
Ile	Asn	Pro	His	Ser	Pro	Lys	Phe	Thr	Cys	Ala	Cys	Pro	Asp	Gly	Met	
			690					695					700			

ctg	ctg	gcc	aga	gat	atg	cgg	tct	tgt	ctg	aca	gaa	gcc	gaa	gcc	gct	3122
Leu	Leu	Ala	Arg	Asp	Met	Arg	Ser	Cys	Leu	Thr	Glu	Ala	Glu	Ala	Ala	
		705					710					715				

gtg	gct	aca	cag	gag	aca	agc	acc	gtg	cgg	ctg	aag	gtg	tct	agc	aca	3170
Val	Ala	Thr	Gln	Glu	Thr	Ser	Thr	Val	Arg	Leu	Lys	Val	Ser	Ser	Thr	
	720					725					730					

gcc	gtg	aga	acc	cag	cac	aca	acc	acc	aga	ccc	gtg	cca	gat	acc	agc	3218
Ala	Val	Arg	Thr	Gln	His	Thr	Thr	Thr	Arg	Pro	Val	Pro	Asp	Thr	Ser	
735					740					745					750	

aga	ctg	cca	gga	gct	aca	cca	gga	ctg	acc	acc	gtg	gag	atc	gtg	acc	3266
Arg	Leu	Pro	Gly	Ala	Thr	Pro	Gly	Leu	Thr	Thr	Val	Glu	Ile	Val	Thr	
			755					760					765			

atg	agc	cac	cag	gct	ctg	gga	gac	gtg	gca	gga	aga	gga	aac	gag	aag	3314
Met	Ser	His	Gln	Ala	Leu	Gly	Asp	Val	Ala	Gly	Arg	Gly	Asn	Glu	Lys	
			770					775					780			

| aag | cct | agc | agc | gtg | aga | gcc | ctg | tct | atc | gtg | ctg | ccc | atc | gtg | ctg | 3362 |

```
Lys Pro Ser Ser Val Arg Ala Leu Ser Ile Val Leu Pro Ile Val Leu
        785             790                 795
```

```
ctg gtg ttc ctc tgt ctg ggc gtg ttc ctc ctc tgg aag aat tgg cgg        3410
Leu Val Phe Leu Cys Leu Gly Val Phe Leu Leu Trp Lys Asn Trp Arg
    800             805                 810
```

```
ctg aag aac atc aac agc atc aac ttc gac aac ccc gtg tac cag aag        3458
Leu Lys Asn Ile Asn Ser Ile Asn Phe Asp Asn Pro Val Tyr Gln Lys
815             820                 825                 830
```

```
acc acc gag gac gag gtg cac att tgc cac aac cag gac ggc tac agc        3506
Thr Thr Glu Asp Glu Val His Ile Cys His Asn Gln Asp Gly Tyr Ser
                835                 840                 845
```

```
tac cct agc agg cag atg gtg tcc ctg gaa gac gac gtg gct tga        3551
Tyr Pro Ser Arg Gln Met Val Ser Leu Glu Asp Asp Val Ala
                850             855                 860
```

```
taagtcgacc cgggcggcct cgaggacggg gtgaactacg cctgaggatc cgatcttttt        3611

ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac ttctggctaa        3671

taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct ctcactcgga        3731

agcaattcgt tgatctgaat ttcgaccacc cataataccc attaccctgg tagataagta        3791

gcatggcggg ttaatcatta actacaagga acccctagtg atggagttgg ccactccctc        3851

tctgcgcgct cgctcgctca ctgaggccgg gcgaccaaag gtcgcccgac gcccgggctt        3911

tgcccgggcg gcctcagtga gcgagcgagc gcgcagcctt aattaaccta attcactggc        3971

cgtcgtttta caacgtcgtg actgggaaaa ccctggcgtt acccaactta atcgccttgc        4031

agcacatccc cctttcgcca gctggcgtaa tagcgaagag gcccgcaccg atcgcccttc        4091

ccaacagttg cgcagcctga atggcgaatg ggacgcgccc tgtagcggcg cattaagcgc        4151

ggcgggtgtg gtggttacgc gcagcgtgac cgctacactt gccagcgccc tagcgcccgc        4211

tcctttcgct ttcttccctt cctttctcgc cacgttcgcc ggctttcccc gtcaagctct        4271

aaatcggggg ctccctttag ggttccgatt tagtgcttta cggcacctcg accccaaaaa        4331

acttgattag ggtgatggtt cacgtagtgg gccatcgccc tgatagacgg tttttcgccc        4391

tttgacgttg gagtccacgt tctttaatag tggactcttg ttccaaactg gaacaacact        4451

caaccctatc tcggtctatt cttttgattt ataagggatt ttgccgattt cggcctattg        4511

gttaaaaaat gagctgattt aacaaaaatt taacgcgaat tttaacaaaa tattaacgct        4571
```

tacaatttag gtggcacttt tcggggaaat gtgcgcggaa cccctatttg tttattttttc   4631

taaatacatt caaatatgta tccgctcatg agacaataac cctgataaat gcttcaataa   4691

tattgaaaaa ggaagagtat gagtattcaa catttccgtg tcgcccttat tcccttttttt   4751

gcggcatttt gccttcctgt ttttgctcac ccagaaacgc tggtgaaagt aaaagatgct   4811

gaagatcagt tgggtgcacg agtgggttac atcgaactgg atctcaacag cggtaagatc   4871

cttgagagtt ttcgccccga agaacgtttt ccaatgatga gcactttttaa agttctgcta   4931

tgtggcgcgg tattatcccg tattgacgcc gggcaagagc aactcggtcg ccgcatacac   4991

tattctcaga atgacttggt tgagtactca ccagtcacag aaaagcatct tacggatggc   5051

atgacagtaa gagaattatg cagtgctgcc ataaccatga gtgataacac tgcggccaac   5111

ttacttctga caacgatcgg aggaccgaag gagctaaccg cttttttgca caacatgggg   5171

gatcatgtaa ctcgccttga tcgttgggaa ccggagctga atgaagccat accaaacgac   5231

gagcgtgaca ccacgatgcc tgtagcaatg gcaacaacgt tgcgcaaact attaactggc   5291

gaactactta ctctagcttc ccggcaacaa ttaatagact ggatggaggc ggataaagtt   5351

gcaggaccac ttctgcgctc ggcccttccg gctggctggt ttattgctga taaatctgga   5411

gccggtgagc gtgggtctcg cggtatcatt gcagcactgg ggccagatgg taagccctcc   5471

cgtatcgtag ttatctacac gacggggagt caggcaacta tggatgaacg aaatagacag   5531

atcgctgaga taggtgcctc actgattaag cattggtaac tgtcagacca agtttactca   5591

tatatacttt agattgattt aaaacttcat ttttaattta aaaggatcta ggtgaagatc   5651

ctttttgata atctcatgac caaaatccct taacgtgagt tttcgttcca ctgagcgtca   5711

gaccccgtag aaaagatcaa aggatcttct tgagatcctt tttttctgcg cgtaatctgc   5771

tgcttgcaaa caaaaaaacc accgctacca gcggtggttt gtttgccgga tcaagagcta   5831

ccaactcttt ttccgaaggt aactggcttc agcagagcgc agataccaaa tactgttctt   5891

ctagtgtagc cgtagttagg ccaccacttc aagaactctg tagcaccgcc tacatacctc   5951

gctctgctaa tcctgttacc agtggctgct gccagtggcg ataagtcgtg tcttaccggg   6011

ttggactcaa gacgatagtt accggataag gcgcagcggt cgggctgaac ggggggttcg   6071

tgcacacagc ccagcttgga gcgaacgacc tacaccgaac tgagatacct acagcgtgag   6131

ctatgagaaa gcgccacgct tcccgaaggg agaaaggcgg acaggtatcc ggtaagcggc   6191

agggtcggaa caggagagcg cacgagggag cttccagggg gaaacgcctg gtatctttat   6251

agtcctgtcg ggtttcgcca cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg   6311

gggcggagcc tatggaaaaa cgccagcaac gcggcctttt tacggttcct ggccttttgc   6371

tggccttttg ctcacatgtt ctttcctgcg ttatcccctg attctgtgga taaccgtatt   6431

accgcctttg agtgagctga taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca   6491

gtgagcgagg aagcggaaga gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg   6551

attcattaat gcagctggca cgacaggttt cccgactgga aagcgggcag tgagcgcaac   6611

gcaattaatg tgagttagct cactcattag gcaccccagg ctttacactt tatgcttccg   6671

gctcgtatgt tgtgtggaat tgtgagcgga taacaatttc acacaggaaa cagctatgac   6731

catgattacg ccagatttaa ttaaggcctt aattagg   6768

<210> 7
<211> 860
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 7

Met Gly Pro Trp Gly Trp Lys Leu Arg Trp Thr Val Ala Leu Leu Leu
1   5   10   15

Ala Ala Ala Gly Thr Ala Val Gly Asp Arg Cys Glu Arg Asn Glu Phe
          20   25   30

Gln Cys Gln Asp Gly Lys Cys Ile Ser Tyr Lys Trp Val Cys Asp Gly
          35   40   45

Ser Ala Glu Cys Gln Asp Gly Ser Asp Glu Ser Gln Glu Thr Cys Leu
      50   55   60

Ser Val Thr Cys Lys Ser Gly Asp Phe Ser Cys Gly Gly Arg Val Asn
65   70   75   80

Arg Cys Ile Pro Gln Phe Trp Arg Cys Asp Gly Gln Val Asp Cys Asp
              85   90   95

Asn Gly Ser Asp Glu Gln Gly Cys Pro Pro Lys Thr Cys Ser Gln Asp
            100   105   110

Glu Phe Arg Cys His Asp Gly Lys Cys Ile Ser Arg Gln Phe Val Cys
            115   120   125

Asp Ser Asp Arg Asp Cys Leu Asp Gly Ser Asp Glu Ala Ser Cys Pro
    130   135   140

Val Leu Thr Cys Gly Pro Ala Ser Phe Gln Cys Asn Ser Ser Thr Cys
145   150   155   160

Ile Pro Gln Leu Trp Ala Cys Asp Asn Asp Pro Asp Cys Glu Asp Gly
            165   170   175

Ser Asp Glu Trp Pro Gln Arg Cys Arg Gly Leu Tyr Val Phe Gln Gly
            180   185   190

Asp Ser Ser Pro Cys Ser Ala Phe Glu Phe His Cys Leu Ser Gly Glu
            195   200   205

Cys Ile His Ser Ser Trp Arg Cys Asp Gly Gly Pro Asp Cys Lys Asp
    210   215   220

Lys Ser Asp Glu Glu Asn Cys Ala Val Ala Thr Cys Arg Pro Asp Glu
225   230   235   240

Phe Gln Cys Ser Asp Gly Asn Cys Ile His Gly Ser Arg Gln Cys Asp
            245   250   255

Arg Glu Tyr Asp Cys Lys Asp Met Ser Asp Glu Val Gly Cys Val Asn
            260   265   270

Val Thr Leu Cys Glu Gly Pro Asn Lys Phe Lys Cys His Ser Gly Glu
            275   280   285

```
Cys Ile Thr Leu Asp Lys Val Cys Asn Met Ala Arg Asp Cys Arg Asp
    290             295             300
```

```
Trp Ser Asp Glu Pro Ile Lys Glu Cys Gly Thr Asn Glu Cys Leu Asp
305             310             315             320
```

```
Asn Asn Gly Gly Cys Ser His Val Cys Asn Asp Leu Lys Ile Gly Tyr
            325             330             335
```

```
Glu Cys Leu Cys Pro Asp Gly Phe Gln Leu Val Ala Gln Arg Arg Cys
        340             345             350
```

```
Glu Asp Ile Asp Glu Cys Gln Asp Pro Asp Thr Cys Ser Gln Leu Cys
        355             360             365
```

```
Val Asn Leu Glu Gly Gly Tyr Lys Cys Gln Cys Glu Glu Gly Phe Gln
    370             375             380
```

```
Leu Asp Pro His Thr Lys Ala Cys Lys Ala Val Gly Ser Ile Ala Tyr
385             390             395             400
```

```
Leu Phe Phe Thr Asn Arg His Glu Val Arg Lys Met Thr Leu Asp Arg
            405             410             415
```

```
Ser Glu Tyr Thr Ser Leu Ile Pro Asn Leu Arg Asn Val Val Ala Leu
            420             425             430
```

```
Asp Thr Glu Val Ala Ser Asn Arg Ile Tyr Trp Ser Asp Leu Ser Gln
            435             440             445
```

```
Arg Met Ile Cys Ser Thr Gln Leu Asp Arg Ala His Gly Val Ser Ser
    450             455             460
```

```
Tyr Asp Thr Val Ile Ser Arg Asp Ile Gln Ala Pro Asp Gly Leu Ala
465             470             475             480
```

```
Val Asp Trp Ile His Ser Asn Ile Tyr Trp Thr Asp Ser Val Leu Gly
            485             490             495
```

Thr Val Ser Val Ala Asp Thr Lys Gly Val Lys Arg Lys Thr Leu Phe
            500   505   510

Arg Glu Asn Gly Ser Lys Pro Arg Ala Ile Val Val Asp Pro Val His
            515   520   525

Gly Phe Met Tyr Trp Thr Asp Trp Gly Thr Pro Ala Lys Ile Lys Lys
    530   535   540

Gly Gly Leu Asn Gly Val Asp Ile Tyr Ser Leu Val Thr Glu Asn Ile
545   550   555   560

Gln Trp Pro Asn Gly Ile Thr Leu Asp Leu Leu Ser Gly Arg Leu Tyr
            565   570   575

Trp Val Asp Ser Lys Leu His Ser Ile Ser Ser Ile Asp Val Asn Gly
            580   585   590

Gly Asn Arg Lys Thr Ile Leu Glu Asp Glu Lys Arg Leu Ala His Pro
            595   600   605

Phe Ser Leu Ala Val Phe Glu Asp Lys Val Phe Trp Thr Asp Ile Ile
    610   615   620

Asn Glu Ala Ile Phe Ser Ala Asn Arg Leu Thr Gly Ser Asp Val Asn
625   630   635   640

Leu Leu Ala Glu Asn Leu Leu Ser Pro Glu Asp Met Val Leu Phe His
            645   650   655

Asn Leu Thr Gln Pro Arg Gly Val Asn Trp Cys Glu Arg Thr Thr Leu
            660   665   670

Ser Asn Gly Gly Cys Gln Tyr Leu Cys Leu Pro Ala Pro Gln Ile Asn
            675   680   685

Pro His Ser Pro Lys Phe Thr Cys Ala Cys Pro Asp Gly Met Leu Leu
    690   695   700

Ala Arg Asp Met Arg Ser Cys Leu Thr Glu Ala Glu Ala Ala Val Ala
705   710   715   720

Thr Gln Glu Thr Ser Thr Val Arg Leu Lys Val Ser Ser Thr Ala Val
                725   730   735

Arg Thr Gln His Thr Thr Thr Arg Pro Val Pro Asp Thr Ser Arg Leu
            740   745   750

Pro Gly Ala Thr Pro Gly Leu Thr Thr Val Glu Ile Val Thr Met Ser
        755   760   765

His Gln Ala Leu Gly Asp Val Ala Gly Arg Gly Asn Glu Lys Lys Pro
    770   775   780

Ser Ser Val Arg Ala Leu Ser Ile Val Leu Pro Ile Val Leu Leu Val
785   790   795   800

Phe Leu Cys Leu Gly Val Phe Leu Leu Trp Lys Asn Trp Arg Leu Lys
            805   810   815

Asn Ile Asn Ser Ile Asn Phe Asp Asn Pro Val Tyr Gln Lys Thr Thr
            820   825   830

Glu Asp Glu Val His Ile Cys His Asn Gln Asp Gly Tyr Ser Tyr Pro
        835   840   845

Ser Arg Gln Met Val Ser Leu Glu Asp Asp Val Ala
    850   855   860