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(54) **METHODS FOR THE TREATMENT AND PREVENTION OF LIVER DISEASE**

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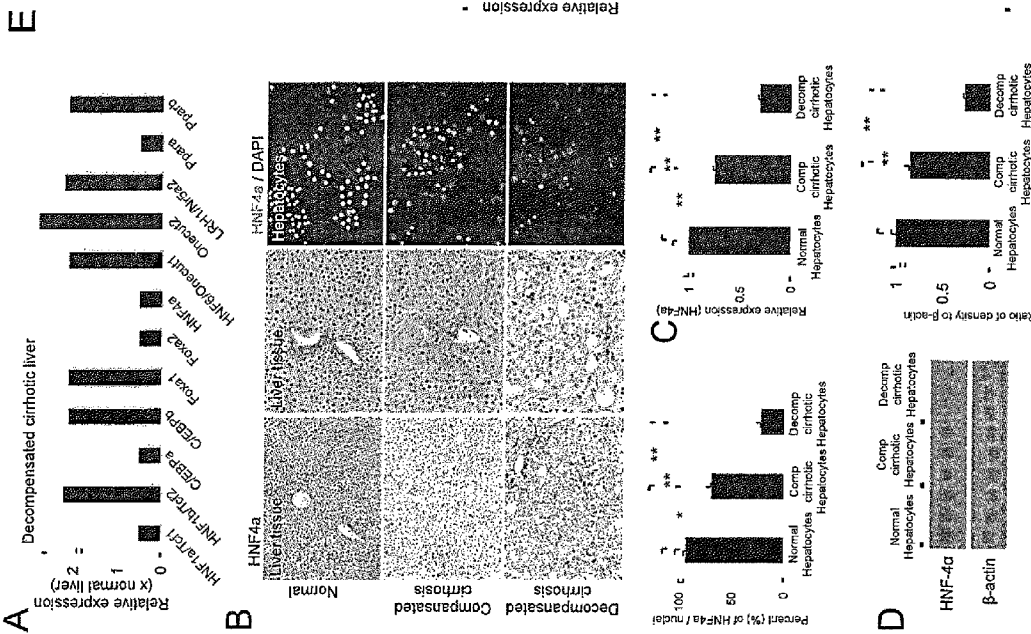
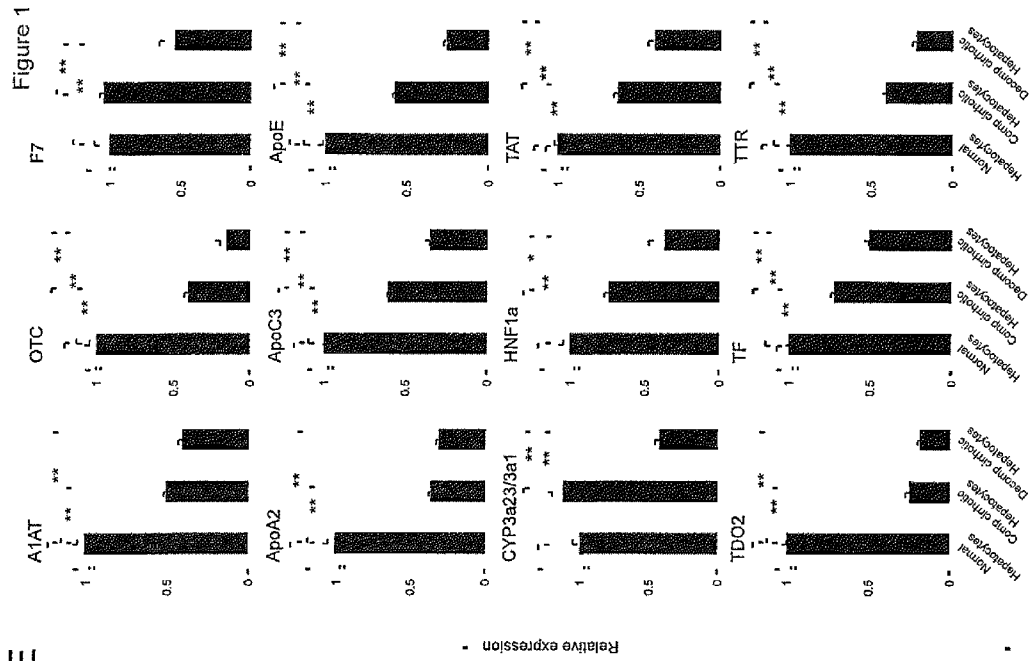
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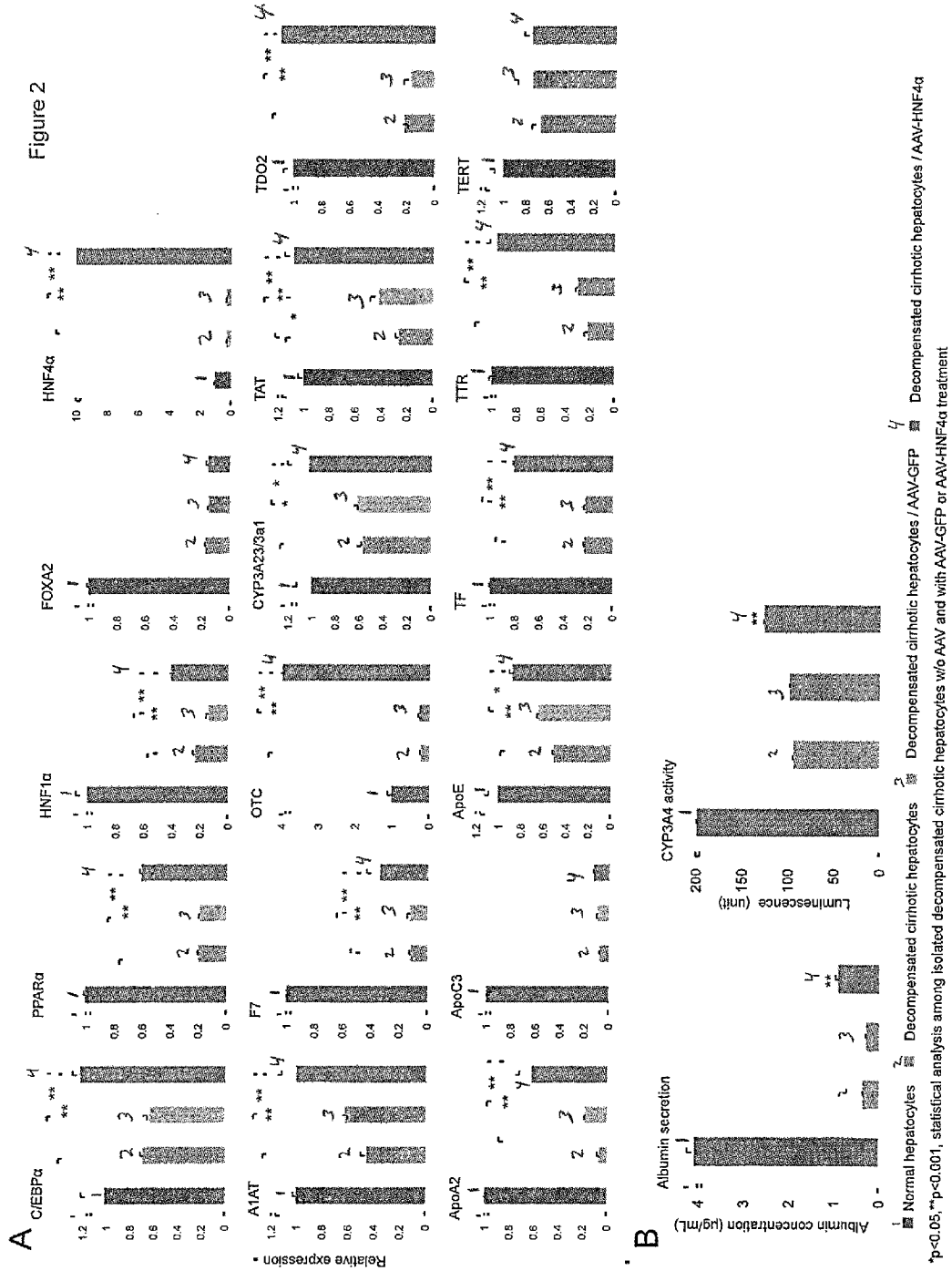
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CPC ..... **C12N 15/113** (2013.01)  
USPC ..... **514/44 R**

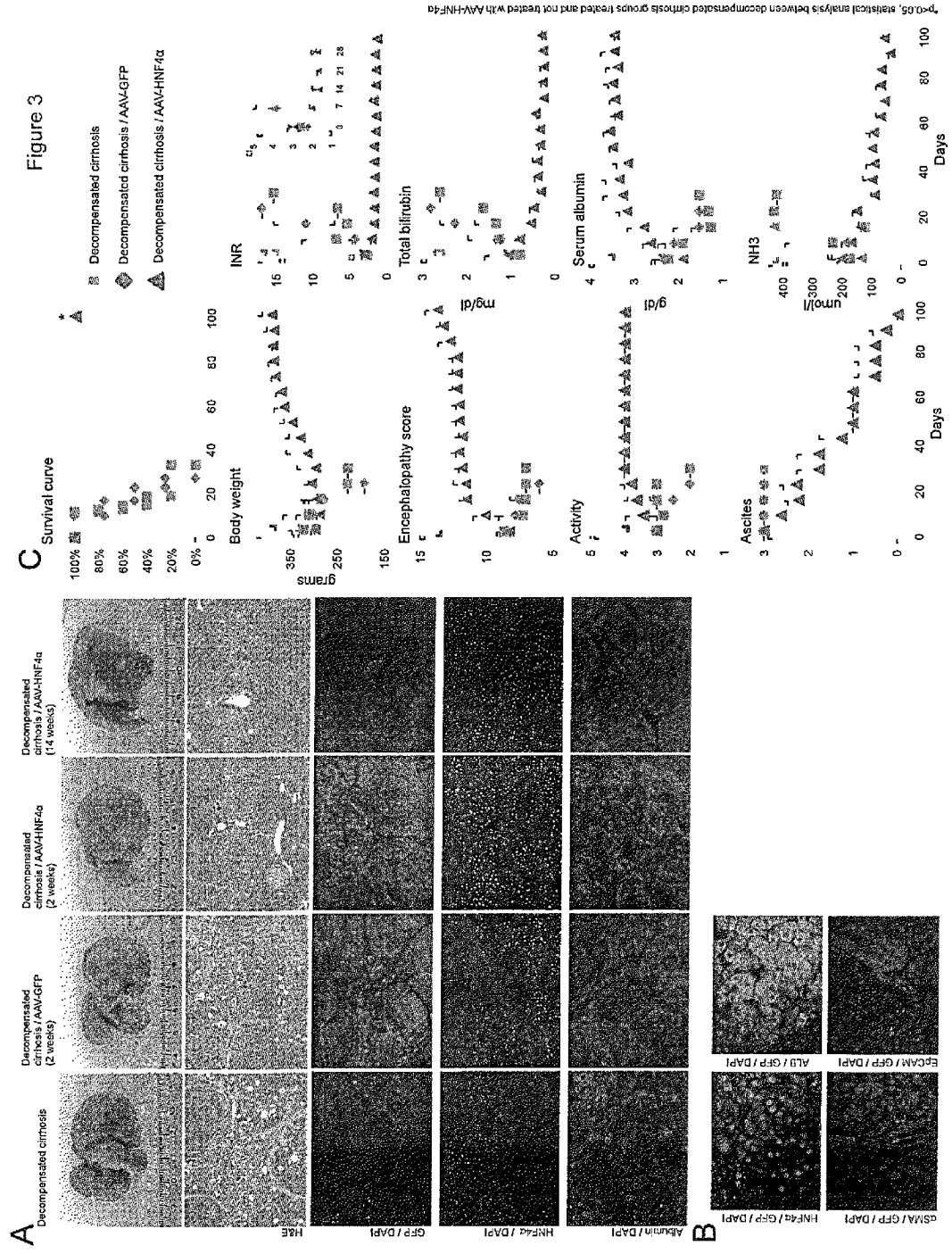
(57) **ABSTRACT**

The presently disclosed invention is directed to the discovery that hepatocyte nuclear factor 4 alpha (HNF4 $\alpha$ ; also known as NR2A1), a transcription factor, reverses hepatocyte dysfunction in an animal model of cirrhosis, resulting in improvement in hepatic function, treatment of cirrhosis, and prolonged survival.



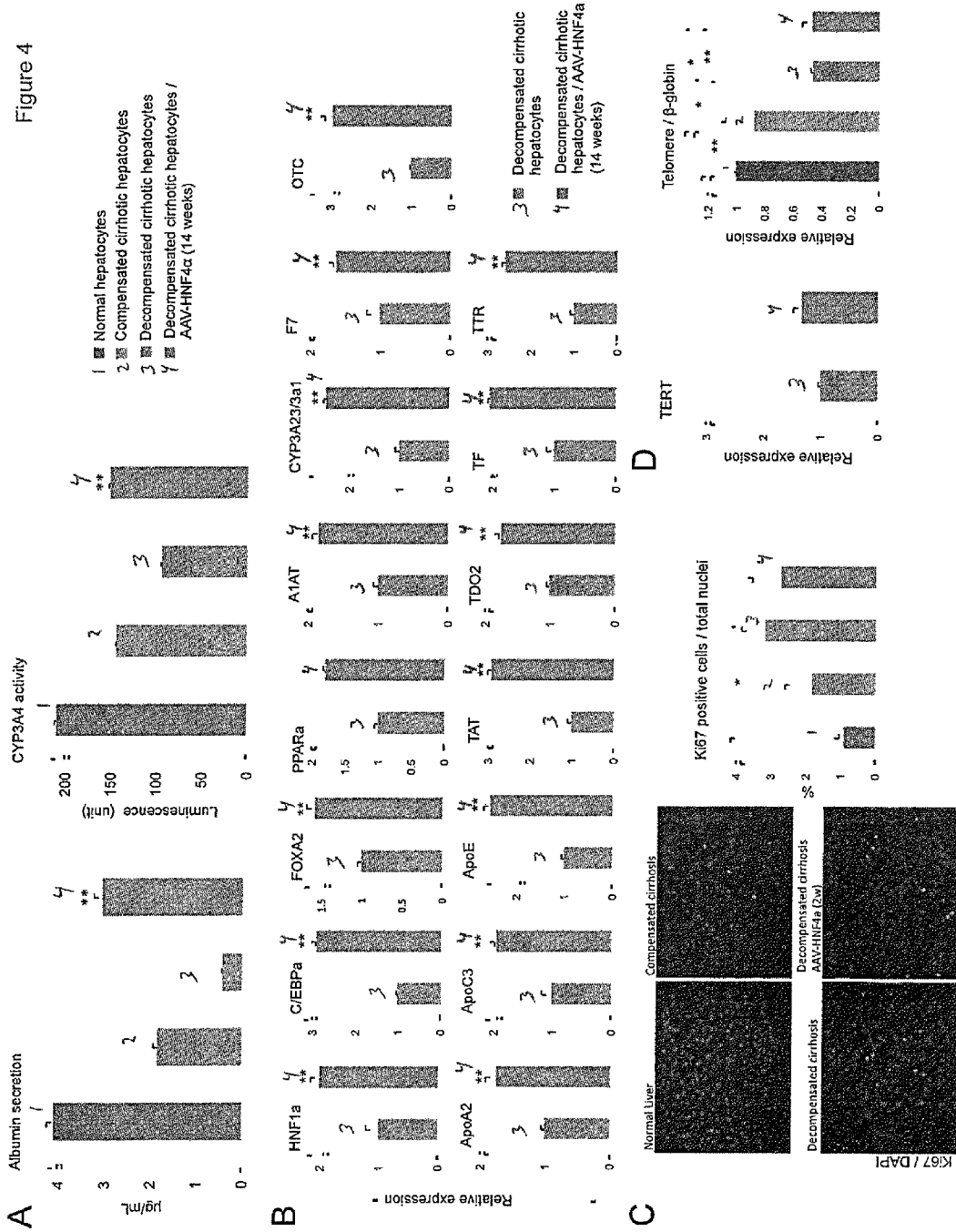
\*p<0.05, \*\*p<0.001, statistical analysis among isolated normal, compensated and decompensated cirrhotic hepatocytes





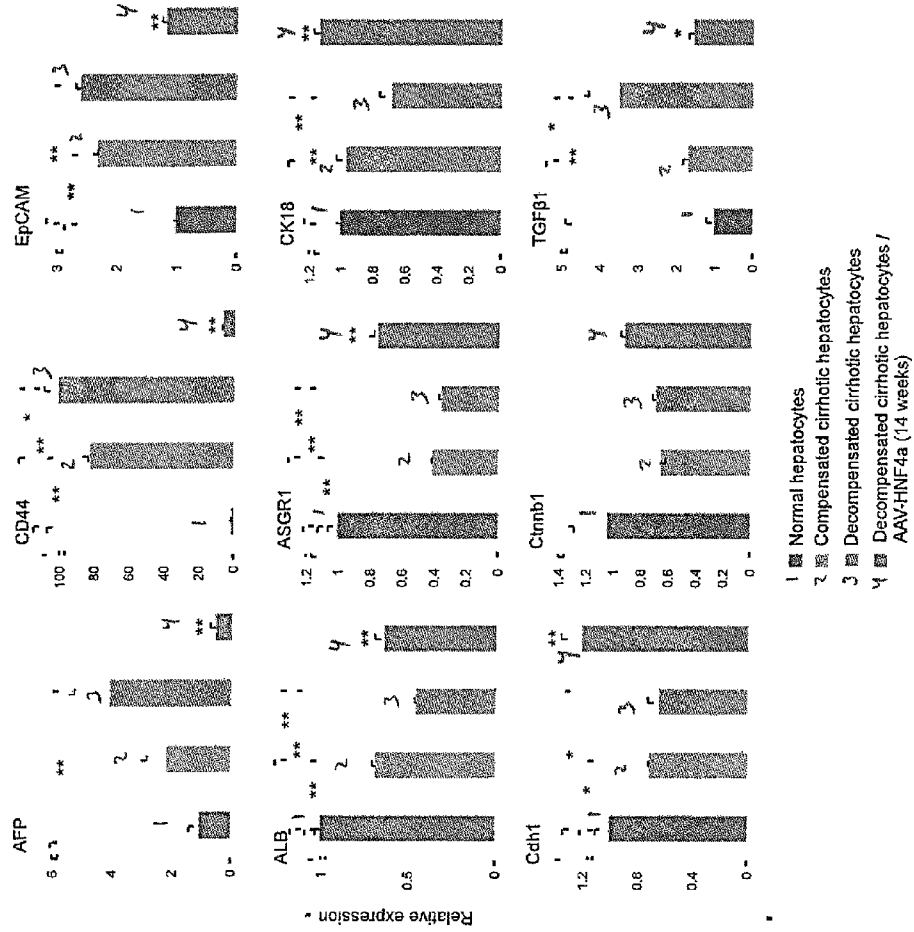
\*p < 0.05, statistical analysis between decompensated cirrhosis groups treated and not treated with AAV-HNF4α

Figure 4



\*p<0.05, \*\*p<0.001, statistical analysis between isolated hepatocytes from decompensated cirrhotic w/o AAV and with AAV-HNF4a treatment

Figure 5



\*p<0.05, \*\*p<0.001, statistical analysis between isolated hepatocytes from decompensated cirrhotic w/o AAV and with AAV-HNF4a treatment

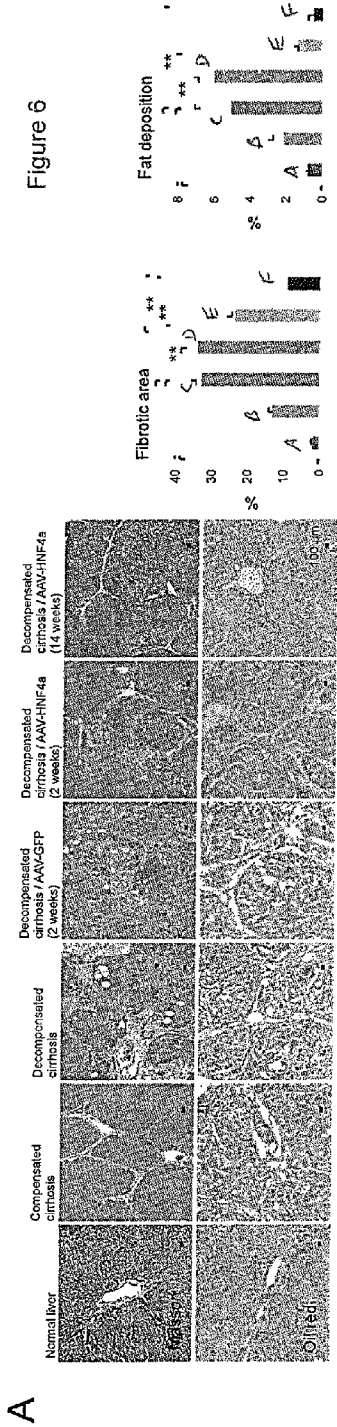
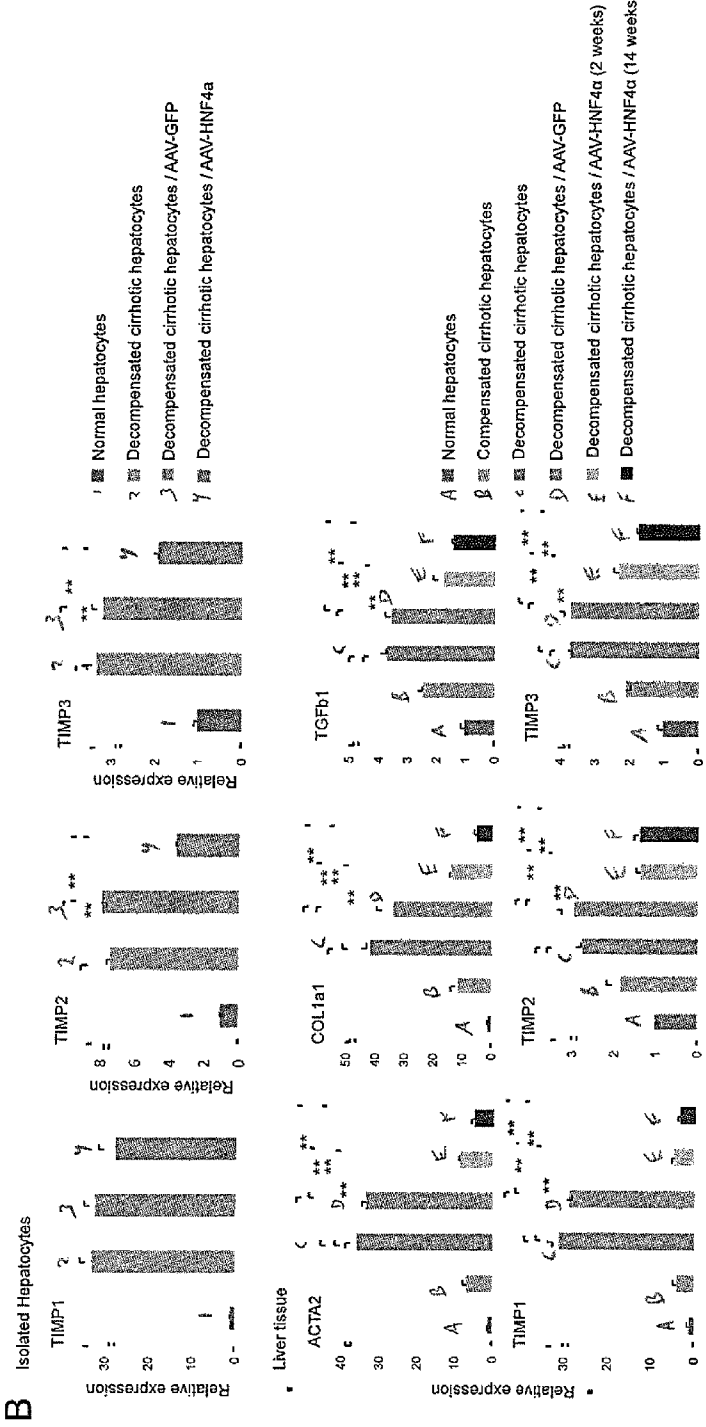
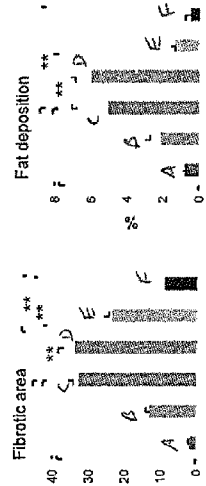


Figure 6



\*p<0.05, \*\*p<0.001, statistical analysis among decompensated cirrhosis w/o AAV and with AAV-GFP or AAV-HNF4a treatment

Figure 7A

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GI:385298686 (SEQ ID NO:5)

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GI:385298687 (SEQ ID NO:6)

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Figure 7B

GI:31077207 (SEQ ID NO:7)

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GI:31077205 (SEQ ID NO:8)

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GI:31077209 (SEQ ID NO:9)

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GI:71725339 (SEQ ID NO:10)

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GI:71725341 (SEQ ID NO:11)

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## METHODS FOR THE TREATMENT AND PREVENTION OF LIVER DISEASE

### CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Application Ser. No. 61/763,744 filed Feb. 12, 2013, the contents of which are incorporated by reference in its entirety.

### GRANT INFORMATION

**[0002]** This invention was made with government support under DK048794 awarded by the National Institutes of Health. The government has certain rights in the invention.

### BACKGROUND

**[0003]** Cirrhosis of the liver, a disease that is difficult to manage, is responsible for 1.2% of all U.S. deaths (1). Cirrhosis is most commonly caused by alcoholism, hepatitis B and hepatitis C, and fatty liver disease, but has many other possible causes such as non-alcoholic steatohepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, hereditary hemochromatosis, Wilson's disease, or alpha 1-antitrypsin deficiency.

**[0004]** Late stages of cirrhosis are characterized by portal hypertension and hepatic encephalopathy, terminal extrahepatic processes that result from fibrosis and vascular remodeling of the cirrhotic liver (2,3). These pathologies are superimposed on liver failure, which results from the inability of hepatocytes to adequately synthesize coagulation factors, conjugate and secrete bilirubin, and regulate metabolism (4-7). Generally, liver damage from cirrhosis cannot be reversed. Therefore, aggressive management can extend life, but the only definitive therapy for end-stage cirrhosis is orthotopic liver transplantation (8).

**[0005]** The cause of organ failure in cirrhosis is poorly understood, but impaired hepatocytes have both intrinsic damage and reside in an abnormal microenvironment (2-8). Studies have shown that somatic cells can be reprogrammed into pluripotent stem cells and fibroblasts or hepatocytes can be reprogrammed into other mature cell lineages following forced expression of selected transcription factors (9,10), although these methods have not been proven in vivo for the treatment of cirrhosis. Accordingly, there is a need for new methods to treat and/or manage cirrhosis and chronic liver disease.

### SUMMARY

**[0006]** The presently disclosed invention is directed to the discovery that hepatocyte nuclear factor 4 alpha (HNF4 $\alpha$ ; also known as NR2A1), a transcription factor, reverses hepatocyte dysfunction in an animal model of cirrhosis, resulting in improvement in hepatic function, treatment of cirrhosis, and prolonged survival.

**[0007]** Accordingly, in one aspect, the present invention provides a method of treating or reducing or preventing hepatic failure or cirrhosis in a subject, e.g., a human subject, comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an HNF4 $\alpha$  agonist to the subject. In one embodiment, the agonist is a small molecule. In another embodiment, the agonist is an HNF4 $\alpha$  nucleic acid molecule. For example, the HNF4 $\alpha$  nucleic acid molecule can be a therapeutic vector comprising a nucleic acid molecule encoding HNF4 $\alpha$ . In one embodiment, a hepa-

toocyte from the subject can be transduced with the therapeutic vector in vitro and reintroduced into the subject, or transduced in vivo. In another embodiment, the agonist is an HNF4 $\alpha$  protein or functional fragment thereof or a peptidomimetic thereof.

**[0008]** In another embodiment, the present invention provides a method of treating or preventing hepatic failure or cirrhosis in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an agonist to a hepatic network factor regulated by HNF4 $\alpha$ , such as, for example, HNF1 $\alpha$ , FOXA2, or CEBP $\alpha$ , alone or in combination with an HNF4 $\alpha$  agonist.

**[0009]** In some embodiments, the agonist is administered alone or in combination with one or more other agent or procedure used for the treatment or prevention of hepatic failure or cirrhosis or symptoms thereof, e.g., interferon therapy, diuretic drugs, transjugular intrahepatic portosystemic shunt (TIPS), paracentesis, antibiotics, drugs for the prevention of variceal bleeding, lactulose, changes to diet, and/or abstinence from alcohol. In one embodiment, the HNF4 $\alpha$  agonist is administered in a subject as a bridge to liver transplantation.

### BRIEF DESCRIPTION OF THE FIGURES

**[0010]** FIGS. 1A-E. Characterization of the hepatocyte transcription factor network in decompensated cirrhosis. (A) Expression changes in the hepatocyte transcription factor network in decompensated cirrhosis (microarray data). (B) Immunohistochemistry; magnification,  $\times 100$  and (cytopins)  $\times 200$  (C) qPCR and (D) western blot for HNF4 $\alpha$  expression in liver tissue and isolated hepatocytes from compensated and decompensated cirrhotic livers. Normal age-matched livers or hepatocytes were used as controls.  $\beta$ -actin was used as the PCR control. (E) Expression of liver-specific genes and genes affected downstream of HNF4 $\alpha$ .

**[0011]** FIGS. 2A-B. Effect of HNF4 $\alpha$  re-expression in isolated hepatocytes from decompensated cirrhotic livers. (A) qRT-PCR analysis of hepatocyte transcription factor network and hepatocyte-specific genes. (B) Albumin synthesis and cytochrome P450 (CYP3A4) activity. Studies were carried out on culture day 2 and compare hepatocytes from normal liver and decompensated cirrhotic livers, the latter also treated with AAV-HNF4 $\alpha$ -GFP or AAV-GFP.

**[0012]** FIGS. 3A-C. Effect of HNF4 $\alpha$  re-expression in rats with decompensated cirrhosis and liver failure. (A) Gross and microscopic (hematoxylin & eosin and fluorescence staining) assessment of decompensated cirrhotic livers two and four-weeks after intervention. Cirrhotic rats with continued severe liver failure, four weeks after their last dose of CCL4, were given a recombinant AAV expressing either HNF4 $\alpha$  and GFP or GFP only; microscopic magnification  $\times 100$ . (B) Fluorescence staining for GFP and co-staining for hepatocyte (albumin) or non-parenchymal liver cell ( $\alpha$ -SMA and EpCAM) markers; magnification  $\times 200$ . (C) Survival and clinical parameters of liver failure in control, AAV-GFP and AAV-HNF4 $\alpha$ -GFP-treated animals with decompensated cirrhosis.

**[0013]** FIGS. 4A-D. Characterization of hepatocytes recovered from treated decompensated cirrhotic rats after HNF4 $\alpha$  re-expression. (A) Albumin synthesis and Cytochrome P450 (CYP3A4) activity in decompensated cirrhotic hepatocytes recovered 14 weeks after AAV-HNF4 $\alpha$ /GFP treatment. Hepatocytes recovered from normal livers and compensated cirrhotic livers were used as controls. (B) qRT-

PCR analysis of hepatocyte transcription factor network and hepatocyte-specific gene expression in hepatocytes recovered from decompensated cirrhotic livers and decompensated cirrhotic livers 14 weeks after AAV-HNF4 $\alpha$ /GFP treatment. (C) Fluorescence staining for Ki67, a proliferation marker, and its quantification in AAV-HNF4 $\alpha$ -GFP-treated decompensated cirrhotic hepatocytes; magnification  $\times 200$  (D) qRT-PCR analysis for TERT expression and telomere length by genomic DNA analysis.

**[0014]** FIG. 5. Effect of HNF4 $\alpha$  re-expression on expression of hepatic progenitor and liver-specific genes in hepatocytes from decompensated cirrhotic livers. qPCR analyses of hepatic progenitor markers (AFP, CD44 and EpCAM), mature hepatic specific genes (Albumin, ASGPR1, CK18), epithelial cell related genes (Cdh1 and Ctnnb1) and the protein TGF $\beta$ 1. Each value represents the mean $\pm$ SD. Statistical analysis was performed among three groups (normal, compensated and decompensated cirrhotic hepatocytes) and between un-treated decompensated cirrhotic hepatocytes and decompensated cirrhotic hepatocytes 14 weeks after in vivo HNF4 $\alpha$  re-expression (\*p<0.05, \*\*P<0.001).

**[0015]** FIGS. 6A-B. Effect of HNF4 $\alpha$  re-expression on histology and fibrosis-related genes in rats with decompensated cirrhosis and liver failure. (A) Masson and oil red stained photomicrographs with quantification of fibrosis and fat deposition in decompensated cirrhotic rat livers two and fourteen weeks after AAV-HNF4 $\alpha$ /GFP therapy. Normal control, compensated cirrhotic and un-treated or AAV-GFP treated decompensated cirrhotic livers were used as controls. Fibrosis and fat deposition decreased significantly two and fourteen weeks after HNF4 $\alpha$  re-expression. Magnification  $\times 100$  (B) qPCR analysis of fibrosis-related genes (TIMP1, TIMP2, TIMP3, ACTA2, COL1a1, and TGF $\beta$ 1) from isolated hepatocytes and tissue recovered from decompensated cirrhotic livers. HNF4 $\alpha$  re-expression induced down regulation of TIMP2 and TIMP3. Each value represents the mean $\pm$ SD. Statistical analysis was performed among four groups (control decompensated cirrhotic hepatocytes, and decompensated cirrhotic hepatocytes 2 after AAV-GFP treatment, or 2 and 14 weeks after AAV-HNF4 $\alpha$ /GFP treatment (\*p<0.05, \*\*P<0.001).

**[0016]** FIGS. 7A-B. (A) Exemplary human HNF4 $\alpha$  nucleic acid sequences, as set forth in SEQ ID NOs:1-6, include HNF4 $\alpha$  transcript variants 1-6, respectively, set forth as Genbank accession numbers GI:385298690, GI:385298689, GI:385298691, GI:385298688, GI:385298686, and GI:385298687, respectively. (B) Exemplary human HNF4 $\alpha$  amino acid sequences, as set forth in SEQ ID NOs:7-12, include HNF4 $\alpha$  transcript variants a-f, respectively, set forth as Genbank accession numbers GI:31077207, GI:31077205, GI:31077209, GI:71725339, GI:71725341, and GI:71725336, respectively.

#### DETAILED DESCRIPTION

**[0017]** The present disclosure describes the discovery that the forced expression of hepatocyte nuclear factor 4 alpha (HNF4 $\alpha$ ; also known as NR2A1), a transcription factor, reverses hepatocyte dysfunction in cirrhosis in vivo. In particular, the present inventors have found that transduction of hepatocytes in cirrhotic animals with irreversible decompensated function produced a profound and immediate improvement in hepatic function and prolonged survival.

**[0018]** Furthermore, the present inventors confirm the role of HNF4 $\alpha$  as an important regulator of the hepatocyte tran-

scription factor network and a master gene for liver function that is down-regulated in advanced cirrhosis. Down-regulation of HNF4 $\alpha$  has a profound effect on the end-stage cirrhotic hepatocyte in vitro, as replenishment of this single factor revitalizes hepatocyte function.

**[0019]** As described herein, using an animal model of cirrhosis and progressive liver failure (11), the inventors have shown that end-stage cirrhotic hepatocytes, previously considered to be senescent and irreversibly dysfunctional, can quickly revert to normal function following transduction with the transcription factor HNF4 $\alpha$ , even though surrounded by an abnormal extracellular matrix. HNF4 $\alpha$  reexpression immediately corrected the phenotype of cultured cirrhotic hepatocytes and reversed terminal end-stage cirrhosis and liver failure in vivo in this animal model. Normalization of function took place in two weeks while portal hypertension, evidenced by the presence of ascites, regressed when histological reversal of cirrhosis was more complete. It was found that HNF4 $\alpha$  acted by phenotypically correcting diseased hepatocytes, not by stimulating their replacement.

**[0020]** Accordingly, the invention provides methods for treating liver failure and cirrhosis (and/or improving liver function in a subject having a cirrhotic liver), comprising administering a therapeutically effective amount of an HNF4 $\alpha$  agonist, e.g., a HNF4 $\alpha$  nucleic acid, protein or functional fragment thereof, peptidomimetic, small molecule, or other drug candidate, to a subject, e.g., a mammal. In one embodiment, the HNF4 $\alpha$  agonist is a therapeutic vector comprising a nucleic acid molecule encoding HNF4 $\alpha$  protein or a functional fragment thereof. In another embodiment, the HNF4 $\alpha$  agonist is a small molecule agonist.

**[0021]** In another embodiment, the present invention provides a method of treating or preventing hepatic failure or cirrhosis in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an agonist to a hepatic network factor regulated by HNF4 $\alpha$ , such as, for example, HNF1 $\alpha$ , FOXA2, or CEBP $\alpha$  (Locker J., *Transcriptional Control of Hepatocyte Differentiation*. In: Monda S P, editor, *Molecular Pathology of Liver Disease*. London, Academic Press; 2001; Kyrimizi, et al. 2006, *Genes Dev.* 20(16):2293-305; Odom et al. 2006 *Mol. Sys. Biol.* 2:2006), alone or in combination with an HNF4 $\alpha$  agonist.

**[0022]** An "individual" or "subject" herein is a vertebrate, such as a human. Mammals include, but are not limited to, humans, primates, farm animals, sport animals, rodents and pets.

**[0023]** An "effective amount" of a substance as that term is used herein is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. In the context of administering a composition that improves liver function, improves liver histology (e.g., fibrosis), or treats liver failure or cirrhosis, an effective amount of an HNF4 $\alpha$  agonist is an amount sufficient to achieve such a modulation as compared to the liver function, histology, or level of cirrhosis when there is no an HNF4 $\alpha$  agonist administered. An effective amount can be administered in one or more administrations.

**[0024]** As used herein, and as well-understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this subject matter, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more

symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, prevention of disease, delay or slowing of disease progression, and/or amelioration or palliation of the disease state. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

#### Agonists

**[0025]** The term "HNF4 $\alpha$  therapeutic" refers to various forms of HNF4 $\alpha$  nucleic acids, polypeptides, peptidomimetics, or small molecules, which can increase expression of liver-specific genes, increase CYP activity, increase liver function, improves liver histology (e.g., fibrosis), treat or prevent cirrhosis, or prolong survival in a subject suffering from liver failure. An HNF4 $\alpha$  therapeutic that mimics, potentiates, or increases the activity of a wild-type HNF4 $\alpha$  polypeptide is a "HNF4 $\alpha$  agonist." An HNF4 $\alpha$  therapeutic includes a gene therapy vector comprising an HNF4 $\alpha$  nucleic acid sequence.

**[0026]** The term "agonist," as used herein, is meant to refer to an agent that mimics or upregulates (e.g., increases, potentiates or supplements) HNF4 $\alpha$  expression or bioactivity, or expression or bioactivity of a hepatic network factor regulated by HNF4 $\alpha$ , such as, for example, HNF1 $\alpha$ , FOXA2, or CEBP $\alpha$ .

**[0027]** An HNF4 $\alpha$  agonist can be a small molecule, a wild-type HNF4 $\alpha$  nucleic acid, protein, derivative, or functional fragment thereof having at least one bioactivity of the wild-type HNF4 $\alpha$  and the ability to increase expression of liver-specific genes, increase CYP activity, increase liver function, improve liver histology (e.g., fibrosis), treat or prevent cirrhosis, or prolong survival in a subject suffering from liver failure. In one embodiment, an HNF4 $\alpha$  agonist is an agent that upregulates expression (which includes forced reexpression) of an HNF4 $\alpha$  gene. An agonist can also be a compound which increases the interaction of a HNF4 $\alpha$  polypeptide with another molecule. Accordingly, a HNF4 $\alpha$  agonist includes a nucleic acid (e.g., using adenoviral expression), micro RNA, e.g., miR-122, which has been shown to be regulated by HNF4 $\alpha$ , (Li, et al, 2011 *J. Hepatol.* 55(3):602-11), a peptidomimetic, protein, or functional fragment thereof, peptide, or small molecule (or other drug candidate) that increases the expression or activity of HNF4 $\alpha$ .

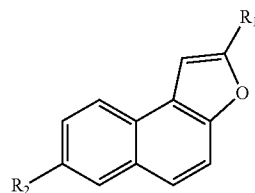
**[0028]** Accordingly, an agonist against a hepatic network factor regulated by HNF4 $\alpha$ , such as, for example, HNF1 $\alpha$ , FOXA2, or CEBP $\alpha$  includes a nucleic acid (e.g., using adenoviral expression), micro RNA, a peptidomimetic, protein, or functional fragment thereof, peptide, or small molecule (or other drug candidate) that increases the expression or activity of a hepatic network factor regulated by HNF4 $\alpha$ .

#### **[0029]** Small Molecule Agonists

**[0030]** Small molecule agonists can be used in the methods of the invention to increase expression or activity of HNF4 $\alpha$  or a hepatic network factor regulated by HNF4 $\alpha$ , such as, for example, HNF1 $\alpha$ , FOXA2, or CEBP $\alpha$ . In one embodiment, exemplary small molecules that can be used in the methods of the invention are described in Le Guével R et al. *Bioorg. Med Chem.*, 2009 Oct. 1; 17(19):7021-30. The small molecules identified therein are synthetic compounds bearing a methoxy group branched on a nitronaphthofuran backbone. The nitro group and a complete naphthofuran backbone were required for full activity of the small molecules tested. Furthermore, adding a hydroxy group at position 7 of the minimal backbone led to an active compound. These compounds were found to

be highly toxic in a human HepG2C3A hepatoma cell assay, except when methylated on the furan ring. One such compound was able to modulate HNF4 $\alpha$ -driven transcription in transfected HepG2C3A cell. Therefore, HNF4 $\alpha$  activity can be modulated (e.g., increased) by small molecules.

**[0031]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula I:

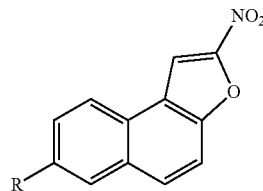


wherein R<sub>1</sub> is selected from the group consisting of NO<sub>2</sub>, COOEt, COOH, CONH<sub>2</sub>, and H; and wherein R<sub>2</sub> is selected from the group consisting of OCH<sub>3</sub>, H, OH, OCH<sub>2</sub>COOEt, OCH<sub>2</sub>COOH, and OMe; wherein Me is methyl and Et is ethyl.

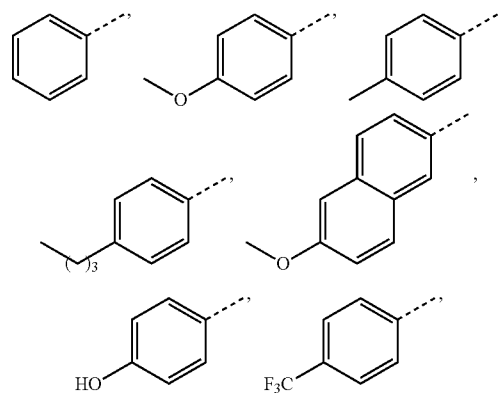
**[0032]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula I wherein R<sub>1</sub> is NO<sub>2</sub> and R<sub>2</sub> is OH.

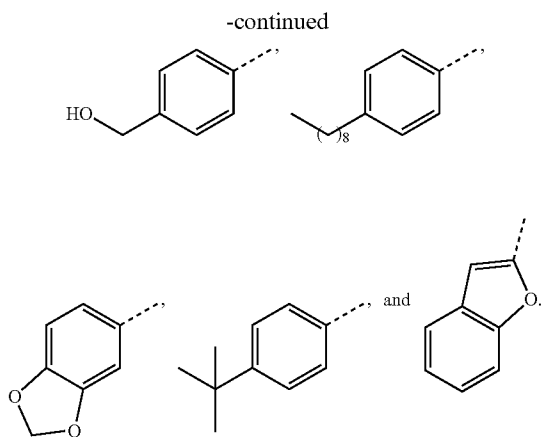
**[0033]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula I wherein R<sub>1</sub> is CONH<sub>2</sub> and R<sub>2</sub> is H.

**[0034]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula II:

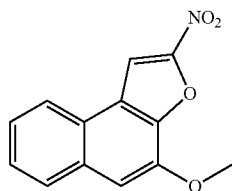


wherein R is selected from the group consisting of:

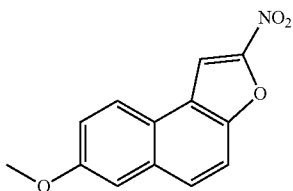




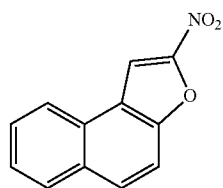
**[0035]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula III:



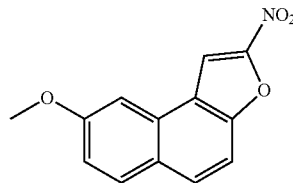
**[0036]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula IV:



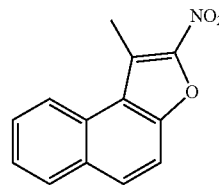
**[0037]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula V:



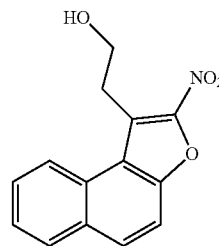
**[0038]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula VI:



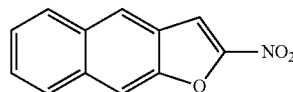
**[0039]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula VII:



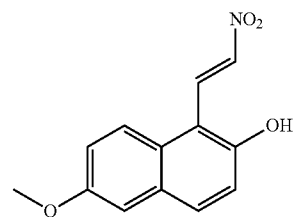
**[0040]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula VIII:



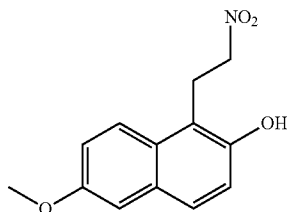
**[0041]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula IX:



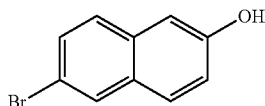
**[0042]** In certain non-limiting embodiments, the small molecule comprises the structure of Formula X:



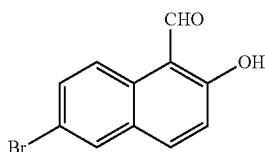
[0043] In certain non-limiting embodiments, the small molecule comprises the structure of Formula XI:



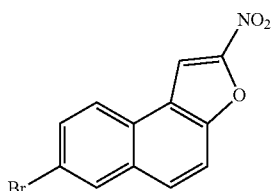
[0044] In certain non-limiting embodiments, the small molecule comprises the structure of Formula XII:



[0045] In certain non-limiting embodiments, the small molecule comprises the structure of Formula XIII:



[0046] In certain non-limiting embodiments, the small molecule comprises the structure of Formula XIV:



[0047] HNF4 $\alpha$  Nucleic Acid Agonists

[0048] The term “HNF4 $\alpha$  nucleic acid” or “HNF4 $\alpha$ ” refers to a nucleic acid encoding an HNF4 $\alpha$  protein, such as, but not limited to, nucleic acids having SEQ ID NOS: 1-6 (Genbank accession numbers GI:385298690, GI:385298689, GI:385298691, GI:385298688, GI:385298686, and GI:385298687, respectively; FIG. 7A), fragments thereof, complement thereof, and derivatives thereof.

[0049] The invention further provides for the use of variant HNF4 $\alpha$  polynucleotides, and fragments thereof, that differ from the nucleotide sequence shown in SEQ ID NOS: 1-6 due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequences shown in SEQ ID NOS: 1-6.

[0050] The invention also provides for the use of HNF4 $\alpha$  nucleic acid molecules encoding the variant polypeptides described above. Such polynucleotides may be naturally occurring, such as allelic variants (same locus), homologs

(different locus), and orthologs (different organism), or may be constructed by recombinant DNA methods or by chemical synthesis. Such non-naturally occurring variants may be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. Accordingly, as discussed herein, the variants can contain nucleotide substitutions, deletions, inversions and insertions.

[0051] Typically, variants have a substantial identity with a nucleic acid molecules of SEQ ID NOS:1-6 and the complements thereof. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions.

[0052] Orthologs, homologs, and allelic variants can be identified using methods well known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is typically at least about 60-65%, 65-70%, 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more homologous to the nucleotide sequence shown in SEQ ID NOS:1-6 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NOS:1-6 or a fragment of the sequence.

[0053] In one embodiment, nucleic acids comprising sequences encoding HNF4 $\alpha$  protein are administered to treat or prevent liver failure or cirrhosis, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0054] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below. For a review of gene therapy in the liver, see Domvri, et al., *Current Gene Therapy* 12(6): 463-483(21) (2012) and Atta, *World J Gastroenterol.* 2010 Aug. 28; 16(32): 4019-4030. For general reviews of the methods of gene therapy, see Kron and Kreppel, *Curr Gene Ther* 12(5):362-73 (2012); Yi et al. *Curr Gene Ther* 11(3):218-28 (2011); Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

[0055] In a preferred aspect, the compound comprises nucleic acid sequences encoding an HNF4 $\alpha$  polypeptide or functional fragment thereof, said nucleic acid sequences being part of expression vectors that express the HNF4 $\alpha$  polypeptide or functional fragments thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the HNF4 $\alpha$  coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. Because of their universal activity, viral promoters were components of many first-generation vectors. However, many of the viral promoters, such as the cytomegalovirus (CMV) promoter, are attenuated or completely shut-off in organs such as the liver. In comparison to viral or housekeeping promoters, tissue- or liver-specific promoters direct

higher levels of expression in vivo. (Acta, World J Gastroenterol. 2010 Aug. 28; 16(32): 4019-4030).

**[0056]** Delivery of nucleic acid into a subject or hepatocyte may be either direct, in which case the subject or hepatocyte is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, hepatocytes are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

**[0057]** The nucleic acid may be directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, *J Biol. Chem.* (1987); 262:4429-4432). The nucleic acid-ligand complexes can also be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In addition, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA (1989); 86:8932-8935; Zijlstra et al., *Nature* (1989); 342:435-438).

**[0058]** The liver is an attractive target for gene therapy because hepatocytes are readily accessible via the blood stream. The endothelium of hepatic sinusoids displays fenestrations that are 100 nm wide and that allow macromolecules such as viral particles to cross the endothelium and reach hepatocytes. Moreover, the hepatic blood flow represents one-fifth of the cardiac output. Thus, any particle injected into the blood circulation can quickly reach the liver. For this reason, the vascular route is commonly used. (Atta, World J Gastroenterol. 2010 Aug. 28; 16(32): 4019-4030).

**[0059]** A method for intravascular regional hydrodynamic delivery of vectors has been developed. The method entails the use of an occlusion balloon catheter into the inferior vena cava and retro dynamically injecting the plasmid in saline solution towards the liver and through the hepatic vein. This retrodynamic hepatic vein gene delivery method has been performed in pigs, and led to liver transgene expression. (Crespo A, *Gene Ther.* 2005; 12:927-935; Eastman S J, *Hum Gene Ther.* 2002; 13:2065-2077; Brunetti-Pierrri N, *Mol Ther.* 2007; 15:732-740; Dariel A, *J Pediatr Surg.* 2009; 44:517-522).

**[0060]** Retrograde administration of adenoviruses into the common bile duct has been shown to induce efficient transgene expression in the liver without causing severe adverse effects, thus supporting the feasibility of adenovirus-mediated gene transfer into the liver in clinical settings by means of endoscopic retrograde cholangiography. (Kuriyama S, *Int J Mol Med.* 2005; 16:503-508; Kuriyama S, *Oncol Rep.*

2005; 13:825-830; Peeters M J, *Hum Gene Ther.* 1996; 7:1693-1699). Repeat administration of adenoviruses into the common bile duct is successful in re-expressing the transgene in the liver. (Tominaga K, *Gut.* 2004; 53:1167-1173; Tsujinoue H, *Int J Oncol.* 2001; 18:575-580).

**[0061]** Another method for delivery of gene products into liver cells is also described in U.S. Patent Application Publication No. 20100010068. This method involves limiting blood flow to the liver during infusion of the vector into the liver.

**[0062]** In a specific embodiment, a viral vector that contains nucleic acid encoding an HNF4 $\alpha$  polypeptide or a functional fragment thereof may be used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* (1993); 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* (1994); 6:291-302. Other references illustrating the use of retroviral vectors in gene therapy are: Anson, *Genet Vaccines Ther* 13; 2(1):9 (2004); Clowes et al., *J. Clin. Invest.* (1994); 93:644-651; Kiem et al., *Blood* (1994); 83:1467-1473; Salmons and Gunzberg, *Human Gene Therapy* (1993); 4:129-141; and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* (1993); 3:110-114.

**[0063]** Adenoviruses are especially attractive vehicles for delivering genes. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems include the liver. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kron and Kreppel, *Curr Gene Ther* 12(5): 362-73 (2012) and Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252: 431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In one embodiment, adenovirus vectors are used.

**[0064]** Adeno-associated virus (AAV) may also be used (Zhong et al. *J Genet Syndr Gene Ther* January 10; S1. pii: 008; High, K A, *Blood*, 120(23):4482-7 (2012); Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Pat. No. 5,436,146). In a preferred embodiment, AAV vectors are used. Vectors that can be used in gene therapy are discussed below in detail below.

**[0065]** Another approach to gene therapy involves transferring a gene to a hepatocyte in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the hepatocytes. The cells are then placed under selection to isolate those hepatocytes that have taken up and are expressing the transferred gene. Those hepatocytes are then delivered to a patient.

**[0066]** In one embodiment, a genetic construct expressing HNF4 $\alpha$  can be introduced into the subject's hepatocytes which can then be propagated ex vivo in, for example, a tolerized non-human animal with a chimeric liver as described in, for example, U.S. Pat. No. 6,525,242. Alterna-

tively, the hepatocytes may be used to colonize the liver of a tolerized animal prior to or contemporaneous with the introduction of the desired transgene via a gene therapy vector. The cells can then be harvested from the chimeric animal and reintroduced into the subject. Hepatocytes used for colonization may be enriched for cells containing the desired construct, for example, by selection by culture conditions, antibody/FACS methods, etc. which eliminate cells lacking the construct.

**[0067]** The nucleic acid can be introduced into a hepatocyte prior to administration in vivo of the resulting recombinant hepatocyte. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92 m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

**[0068]** The resulting recombinant hepatocytes can be delivered to a patient by various methods known in the art. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

**[0069]** Recombinant cells can also be used in gene therapy, where nucleic acid sequences encoding an HNF4 $\alpha$  protein or functional fragment thereof, are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. For example, stem or progenitor cells can be used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used (see e.g. PCT Publication WO 94/08598; Porada and Porada, *J. Genet Syndr Gene Ther.*, May 25; S1. p11:011 (2012); Stemple and Anderson, *Cell* 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.* 61:771 (1986)).

**[0070]** HNF4 $\alpha$  Polypeptide Agonists

**[0071]** The terms "HNF4 $\alpha$  polypeptide," "HNF4 $\alpha$  protein" and "HNF4 $\alpha$ " are intended to encompass polypeptides comprising the amino acid sequence of SEQ ID NOS:7-12 (Genbank accession numbers GI:31077207, GI:31077205, GI:31077209, GI:71725339, GI:71725341, and GI:71725336, respectively; FIG. 7B), fragments thereof (e.g., functional fragments thereof), and variants thereof, and include agonist polypeptides.

**[0072]** In one embodiment, the present invention provides for the use of an isolated or purified HNF4 $\alpha$  polypeptide and variants and fragments thereof. The invention also encompasses the use of sequence variants. Variants include a substantially homologous protein encoded by the same genetic locus in an organism, i.e., an allelic variant. Variants also encompass proteins derived from other genetic loci in an organism, but having substantial homology to the HNF4 $\alpha$  protein of SEQ ID NOS: 7-12. Variants also include proteins

substantially homologous to the HNF4 $\alpha$  protein but derived from another organism, i.e., an ortholog. Variants also include proteins that are substantially homologous to the HNF4 $\alpha$  protein that are produced by chemical synthesis. Variants also include proteins that are substantially homologous to the HNF4 $\alpha$  protein that are produced by recombinant methods.

**[0073]** As used herein, two polypeptides (or regions thereof) are substantially homologous when the amino acid sequences are at least about 60-65%, 65-70%, 70-75%, typically at least about 80-85%, and most typically at least about 90-95%, or 95-99% or more homologous. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence hybridizing to the nucleic acid sequence, or portion thereof, of the sequence shown in SEQ ID NOS:-7-12 under stringent conditions.

**[0074]** The HNF4 $\alpha$  proteins used in the methods of the invention also include HNF4 $\alpha$  polypeptides having additions, deletions or substitutions of amino acid residues (variants) which do not substantially alter the biological activity of the protein. Those individual sites or regions of HNF4 $\alpha$  which may be altered without affecting biological activity may be determined by examination of the structure of the HNF4 $\alpha$  binding domains, for example. Alternatively, one may empirically determine those regions which would tolerate amino acid substitutions by alanine scanning mutagenesis (Cunningham et al. *Science* 244, 1081-1085 (1989)). In this method, selected amino acid residues are individually substituted with a neutral amino acid (e.g., alanine) in order to determine the effects on biological activity.

**[0075]** It is generally recognized that conservative amino acid changes are least likely to perturb the structure and/or function of a polypeptide. Accordingly, the invention encompasses one or more conservative amino acid changes within a HNF4 $\alpha$  protein. Conservative amino acid changes generally involve substitution of one amino acid with another that is similar in structure and/or function (e.g., amino acids with side chains similar in size, charge and shape). Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residue within a HNF4 $\alpha$  protein can be replaced with other amino acid residues from the same side chain family and the altered protein can be tested for retained function using the functional assays described herein. Modifications can be introduced into an antibody of this disclosure by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. If such substitutions result in a retention in biological activity, then more substantial changes may be introduced and/or other additions/deletions may be made and the resulting products screened. In one embodiment, deletions or additions can be from 5-10 residues, alternatively from 2-5 amino acid residues, or from 1-2 residues.

## Vectors

**[0076]** The terms “vector” and “expression vector” mean the vehicle by which a DNA or RNA sequence (e.g., a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g., transcription and translation) of the introduced sequence. Vectors include plasmids, phages, viruses, etc.; they are discussed in greater detail below. A “therapeutic vector” as used herein refers to a vector which is acceptable for administration to an animal, and particularly to a human.

**[0077]** Vectors typically comprise the DNA of a transmissible agent, into which foreign DNA is inserted. A common way to insert one segment of DNA into another segment of DNA involves the use of enzymes called restriction enzymes that cleave DNA at specific sites (specific groups of nucleotides) called restriction sites. Generally, foreign DNA is inserted at one or more restriction sites of the vector DNA, and then is carried by the vector into a host cell along with the transmissible vector DNA. A segment or sequence of DNA having inserted or added DNA, such as an expression vector, can also be called a “DNA construct.” A common type of vector is a “plasmid”, which generally is a self-contained molecule of double-stranded DNA, usually of bacterial origin, that can readily accept additional (foreign) DNA and which can readily introduced into a suitable host cell. A plasmid vector often contains coding DNA and promoter DNA and has one or more restriction sites suitable for inserting foreign DNA. Coding DNA is a DNA sequence that encodes a particular amino acid sequence for a particular protein or enzyme. Promoter DNA is a DNA sequence which initiates, regulates, or otherwise mediates or controls the expression of the coding DNA. Promoter DNA and coding DNA may be from the same gene or from different genes, and may be from the same or different organisms. A large number of vectors, including plasmid and fungal vectors, have been described for replication and/or expression in a variety of eukaryotic and prokaryotic hosts. Non-limiting examples include pKK plasmids (Clonotech), pUC plasmids, pET plasmids (Novagen, Inc., Madison, Wis.), pRSET plasmids (Invitrogen, San Diego, Calif.), pCDNA3 plasmids (Invitrogen), pREP plasmids (Invitrogen), or pMAL plasmids (New England Biolabs, Beverly, Mass.), and many appropriate host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. Recombinant cloning vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes.

**[0078]** Suitable vectors include viruses, such as adenoviruses, adeno-associated virus (AAV), vaccinia, herpesviruses, baculoviruses and retroviruses, parvovirus, lentivirus, bacteriophages, cosmids, plasmids, fungal vectors, naked DNA, DNA lipid complexes, and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

**[0079]** Lentiviral vectors have been reported to deliver genes to primary liver cells efficiently and permanently, integrating into the genome of non-dividing cells such as primary liver cells (Lewis and Emerman “Passage through mitosis is required for oncoretroviruses but not the immunodeficiency virus” *J. Virol.* 1994, 68:510-6). Lentiviral vectors are

described in, for example, Choi et al (2001, *Stem Cells* 2001; 19(3):236-46) or in U.S. Pat. No. 6,218,186.

**[0080]** Viral vectors, especially adenoviral vectors can be complexed with a cationic amphiphile, such as a cationic lipid, polyL-lysine (PLL), and diethylaminoethyl-dextran (DELAE-dextran), which provide increased efficiency of viral infection of target cells (See, e.g., PCT/US97/21496 filed Nov. 20, 1997, incorporated herein by reference). AAV vectors, such as those disclosed in Zhong et al., *J. Genet Syndr Gene Therapy* 2012 Jan. 10; S1. pii: 008, U.S. Pat. Nos. 5,139,941, 5,252,479 and 5,753,500 and PCT publication WO 97/09441, the disclosures of which are incorporated herein, are also useful since these vectors integrate into host chromosomes, with a minimal need for repeat administration of vector. For a review of viral vectors in gene therapy, see McConnell et al., 2004, *Hum Gene Ther.* 15(11):1022-33; McCarty et al., 2004, *Annu Rev Genet.* 38:819-45; Mah et al., 2002, *Clin. Pharmacokinet.* 41(12):901-11; Scott et al., 2002, *Neuromuscul. Disord.* 12(Suppl 1):S23-9.

## Pharmaceutical Compositions

**[0081]** The present invention further provides pharmaceutical compositions which comprise an HNF4 $\alpha$  agonist, e.g., all or portions of HNF4 $\alpha$  polynucleotide sequences, HNF4 $\alpha$  polypeptides or functional fragments thereof, small molecule, or other HNF4 $\alpha$  agonists, alone or in combination with at least one other agent, such as a stabilizing compound, and may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, citrate, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal, parabens, benzylalcohol, benzylalcohol, antioxidants such as ascorbic acid or sodium metabisulfite, and other components such as lysine or glycine. Selection of a particular composition will depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of components suitable for pharmaceutical compositions is found in Remington's *Pharmaceutical Sciences*, 18th ed. A. R. Gennaro, ed. Mack, Easton, Pa. (1980).

**[0082]** The methods of the present invention find use in treating hepatic failure or cirrhosis. Peptides can be administered to the patient intravenously in a pharmaceutically acceptable carrier such as physiological saline. Standard methods for intracellular delivery of peptides can be used (e.g., delivery via liposome). Such methods are well known to those of ordinary skill in the art. The formulations of this invention are useful for parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal. Therapeutic administration of a polypeptide intracellularly can also be accomplished using gene therapy. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

**[0083]** In other embodiments, the pharmaceutical compositions of the present invention can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral or nasal ingestion by a patient to be treated.



**[0084]** Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. For example, a therapeutically effective amount of an HNF4 $\alpha$  agonist is that amount that increases expression of liver-specific genes, increases CYP activity, increases liver function, improves liver histology (e.g., fibrosis), treats or prevents cirrhosis, or prolongs survival in a subject suffering from liver failure. The amount will vary from one individual to another and will depend upon a number of factors, including the overall physical condition of the patient, severity and the underlying cause of liver failure and/or cirrhosis.

**[0085]** A target liver function can be determined by a liver function test, which typically measures, for example, albumin, total bilirubin, direct bilirubin, and/or INR Prothombin Time. The clinical signs of protein dysfunction, manifested by hepatic encephalopathy (which can be measured by an elevated arterial ammonia level), and muscle wasting are manifestations of hepatocyte failure, which can be made worse by portal hypertension. Therefore, improvement in liver function can also be assessed by the presence or absence of fibrosis in the liver, ascites, muscle wasting, ammonia levels, neurologic function (hepatic encephalopathy), etc. in the subject. Liver biopsy, such as a needle biopsy, can be used to assess the degree of fibrosis and cirrhosis of the liver. It is understood that such targets will vary from one individual to another such that physician discretion may be appropriate in determining an actual target liver function for any given patient. Nonetheless, determining a target liver function is well within the level of skill in the art.

**[0086]** The formulations of the invention can be administered for prophylactic and/or therapeutic treatments. For example, in alternative embodiments, pharmaceutical compositions of the invention are administered in an amount sufficient to treat, prevent and/or ameliorate liver failure and/or cirrhosis. As is well known in the medical arts, dosages for any one patient depends upon many factors, including stage of the disease or condition, the severity of the disease or condition, the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and interaction with other drugs being concurrently administered.

**[0087]** Accordingly, in some embodiments of the present invention, HNF4 $\alpha$  nucleotide and HNF4 $\alpha$  amino acid sequences or other HNF4 $\alpha$  agonists can be administered to a patient alone, or in combination with one or more other nucleotide sequences, drugs, lifestyle changes, etc. used in the treatment or prevention of liver failure and/or cirrhosis or symptoms thereof (for example, interferon therapy, diuretic drugs, transjugular intrahepatic portosystemic shunt (TIPS), paracentesis, antibiotics, drugs for the prevention of variceal bleeding, lactulose, changes to diet, and/or abstinence from alcohol) or in pharmaceutical compositions where it is mixed with excipient(s) or other pharmaceutically acceptable carriers. In one embodiment, the HNF4 $\alpha$  agonist is administered in a subject as a bridge to liver transplantation.

**[0088]** In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert. In another embodiment of the present invention, HNF4 $\alpha$  polynucleotide sequences or HNF4 $\alpha$  amino acid sequences or other HNF4 $\alpha$  agonists may be administered alone to individuals subject to or suffering from liver failure and/or cirrhosis. The dosage regimen also takes into consid-

eration pharmacokinetics parameters well known in the art, i.e., the active agents' rate of absorption, bioavailability, metabolism, clearance, and the like (see, e.g., Hidalgo-Aragones (1996) *J. Steroid Biochem. Mol. Biol.* 58:611-617; Groning (1996) *Pharmazie* 51:337-341; Fotherby (1996) *Contraception* 54:59-69; Johnson (1995) *J. Pharm. Sci.* 84:1144-1146; Rohatagi (1995) *Pharmazie* 50:610-613; Brophy (1983) *Eur. J. Clin. Pharmacol.* 24:103-108; the latest Remington's, supra). The state of the art allows the clinician to determine the dosage regimen for each individual patient, active agent and disease or condition treated. Guidelines provided for similar compositions used as pharmaceuticals can be used as guidance to determine the dosage regimen, i.e., dose schedule and dosage levels, administered practicing the methods of the invention are correct and appropriate.

**[0089]** Single or multiple administrations of formulations can be given depending on the dosage and frequency as required and tolerated by the patient. The formulations should provide a sufficient quantity of active agent to effectively treat, prevent or ameliorate or liver failure and/or cirrhosis or symptoms thereof as described herein. For example, an exemplary pharmaceutical formulation for oral administration can be in a daily amount of between about 0.1 to 0.5 to about 20, 50, 100 or 1000 or more  $\mu\text{g}$  per kilogram of body weight per day of protein. In an alternative embodiment, dosages are from about 1 mg to about 4 mg per kg of body weight per patient per day of protein are used. For example, in one embodiment a therapeutically effective amount of a polypeptide of this invention is a dosage of between about 0.025 to 0.5 milligram per 1 kilogram of body weight of the patient; or, a therapeutically effective amount is a dosage of between about 0.025 to 0.2 milligram, or 0.05 to 0.1 milligram, or 0.075 to 0.5 milligram, or 0.2 to 0.4 milligram, of the compound per 1 kilogram of body weight of the patient. In another embodiment, a single dose is sufficient to achieve the desired results.

**[0090]** In one embodiment, the HNF4 $\alpha$  agonists of the invention are administered once, twice, or three times per week for all indications except the surgery indication, by intravenous (IV) or subcutaneous (SC) injection to reach a suggested target liver function range. Once the target liver function has been achieved, a maintenance dosing schedule is established which will vary depending upon the patient.

**[0091]** Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (LD50, the dose lethal to 50% of the population; and ED50, the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

**[0092]** The following Example is offered to more fully illustrate the invention, but is not to be construed as limiting the scope thereof.

## Example 1

Upregulation of HNF4 $\alpha$  to Treat Hepatic Failure

**[0093]** This Example illustrates that end-stage cirrhotic hepatocytes, previously considered to be senescent and irreversibly dysfunctional, can revert to normal function following transduction with nucleic acid encoding the transcription factor HNF4 $\alpha$ , even though surrounded by an abnormal extracellular matrix. As described herein, HNF4 $\alpha$  reexpression immediately corrected the phenotype of cultured cirrhotic hepatocytes and reversed terminal end-stage cirrhosis and liver failure *in vivo*.

**[0094]** More than a decade ago, the inventors developed a unique model in rats, using chronic administration of CCl<sub>4</sub>, to produce a syndrome of cirrhosis and progressive liver failure that greatly resembles human disease (11). While the latter has different etiologies that include HBV, HCV, alcohol, or NASH/metabolic syndrome, the CCl<sub>4</sub>-injured rat reproduces the most important feature of advanced cirrhosis, the irreversibly decompensated hepatocyte.

**[0095]** Previously, the inventors analyzed the transcriptome of hepatocytes recovered from advanced cirrhotic livers (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE22977>; Liu L, Yannam G R, Nishikawa T, Yamamoto T et al. *Hepatology* 2012 May; 55(5):1529-39, the contents of which are expressly incorporated herein by reference) and also transplanted them. Microarrays showed marked decreases in the expression of HNF4 $\alpha$ , Foxa2, C/EBP $\alpha$ , and HNF1 $\alpha$ , molecules that are part of the network of hepatocyte-enriched transcription factors, sequentially established during development, that regulate the mature hepatocyte phenotype, controlling expression of proteins of coagulation, biliary metabolism, and lipid metabolism (12, 13). Transplantation of hepatocytes from end-stage cirrhotic rats into non-cirrhotic host livers eventually restored their regenerative capacity (14), but the delay in restoration suggested that the process was not the simple expansion of engrafted cells that occurs with normal regeneration.

**[0096]** Since deficient transcription factors could explain hepatocyte impairment, the inventors investigated the therapeutic effects of forced re-expression. HNF4 $\alpha$  was chosen for this therapy because it is the central regulator of the hepatocyte transcription factor network, has no other hepatocyte homolog, and showed the greatest reduction in the decompensated hepatocyte (FIG. 1A).

**[0097]** As described herein, cirrhosis was induced in Lewis rats by treatment with phenobarbital and carbon tetrachloride (CCl<sub>4</sub>). Animals treated for 14 weeks had normal liver function (compensated cirrhosis), whereas 26-28 weeks of CCl<sub>4</sub> produced decompensated liver function with elevated bilirubin, decreased albumin, prolonged prothrombin time, and hepatic encephalopathy scores of 8 $\pm$ 0.7 vs. a normal score of 15 (11). All animals were assessed for liver function 4 weeks after receiving their last dose of CCl<sub>4</sub> to guarantee that alterations in liver function were not the consequence of the acute effects of CCl<sub>4</sub> administration.

**[0098]** To confirm the inventors' previous microarray studies (Liu L, Yannam G R, Nishikawa T, Yamamoto T et al. *Hepatology* 2012 May; 55(5):1529-39), a detailed analysis of the expression of HNF4 $\alpha$  and its target genes in isolated hepatocytes and liver tissue was performed. qRT-PCR analysis confirmed severe downregulation of HNF4 $\alpha$  expression, and quantification of HNF4 $\alpha$  in hepatocytes by Western blot and by immunofluorescent staining of cytospin samples gave

similar results (FIG. 1B-D). Immunohistochemical localization of HNF4 $\alpha$  showed expression in nuclei of hepatocytes but not in bile duct or other non-parenchymal cells. Nuclear HNF4 $\alpha$  was present in the majority of hepatocytes from animals with compensated cirrhosis but was severely diminished in hepatocytes in the nodular livers of decompensated cirrhosis, where it selectively localized to the periportal region. Down-regulation of HNF4 $\alpha$  expression has also been reported in human cirrhosis (15, 16). Thus, a significant decrease of HNF4 $\alpha$  in hepatocytes correlates with decompensation in cirrhosis. As HNF4 $\alpha$  affects the expression of many liver-specific target genes involved in glucose, lipid, amino acid, xenobiotic, and drug metabolism (17), the expression of  $\alpha$ 1-antitrypsin; apolipoproteins A2, C3, and E; cytochrome P450 3a23, coagulation factor VII, hepatocyte nuclear factor 1 $\alpha$ , ornithine transcarbamylase, tyrosine aminotransferase, tryptophan 2,3-dioxygenase, transferrin, and transthyretin was evaluated (FIG. 1E). All of these genes were severely down-regulated in advanced cirrhosis in parallel to HNF4 $\alpha$ .

**[0099]** To assess whether forced reexpression of HNF4 $\alpha$  could affect the function of cirrhotic hepatocytes, *in vitro* culture system was first used. Hepatocytes, isolated from animals with cirrhosis and decompensated liver function, were transduced with adeno-associated virus (AAV) vectors to express HNF4 $\alpha$  and GFP or GFP alone. At 48 hours, qRT-PCR analysis showed HNF4 $\alpha$  reexpression restored to nearly normal levels the hepatic transcription factors C/EBP $\alpha$ , HNF1 $\alpha$ , and PPAR $\alpha$ , and the phenotypic targets genes important for liver-specific activity (FIG. 2A). HNF4 $\alpha$  expression also improved secretion of albumin into the culture supernatant—severely impaired in hepatocytes isolated from decompensated cirrhosis (14)—and activity of Cytochrome P450 3A4, a major enzyme of xenobiotic metabolism (FIG. 2B).

**[0100]** In previous studies using this model of decompensated liver failure from cirrhosis, it was shown by the inventors that after withdrawal of CCl<sub>4</sub> and a four-week observation period, 100% of untreated animals die of progressive hepatic failure in 2 to 3 weeks. Intrasplenic hepatocyte transplantation dramatically improved function and survival, but was only effective for a period of weeks to months (11,18) despite transplantation with syngeneic hepatocytes. The therapeutic benefit was moderate, since the end-stage cirrhotic rats still died of progressive hepatic failure and persistent severe cirrhosis. With this background, animals with liver failure and cirrhosis were transduced to re-express HNF4 $\alpha$  in their hepatocytes by intravenous infusion of 3 $\times$ 10<sup>11</sup> AAV-HNF4 $\alpha$ -GFP genomes. Animals sacrificed 2 weeks after infusion demonstrated high transduction efficiency uniformly distributed in most hepatocytes. Moreover, the impaired albumin expression of decompensated cirrhosis was dramatically improved and its expression increased until the time of sacrifice at 100 days following the observation period (FIG. 3A). Administration of the AAV-GFP control vector did not affect liver function. Co-staining for GFP, albumin,  $\alpha$ -smooth muscle, and EpCam indicated transduction of approximately 60-80% of hepatocytes, but not non-parenchymal cells (FIG. 3B). Pathophysiologic testing showed striking and persistent improvement in liver function, ascites, activity, and neurologic function, and survival was prolonged to the end-point of the study at 100 days post observation after CCl<sub>4</sub> withdrawal (FIG. 3C).

**[0101]** Functional analysis of cells isolated from treated animals showed significant improvement of albumin secretion and CYP3A4 activity (FIG. 4A). In addition, there was improvement in expression levels of HNF4 $\alpha$  target genes (FIG. 4B) and decreased expression of the hepatic progenitor cell markers AFP, CD44, and EpCAM (FIG. 5). The healing effects of HNF4 $\alpha$  reexpression did not depend on proliferation, since there was no increase apparent in Ki67 staining (FIG. 4C). HNF4 $\alpha$  did not significantly augment TERT expression and telomere length in the cirrhotic hepatocytes remained critically short (FIGS. 40). Thus, HNF4 $\alpha$  acted by phenotypically correcting diseased hepatocytes, not by stimulating their replacement.

**[0102]** The corrected hepatocytes had a striking extrinsic effect on the extracellular matrix, because the fibrotic remodeling of the liver, the hallmark of cirrhosis, was also corrected. Histologic studies showed improvement in fat deposition and fibrosis as early as 2 weeks following treatment with AAV-HNF4 $\alpha$ -GFP and significant further improvement at 100 days (FIG. 3, FIG. 6A), results that are in sharp contrast to the inventors' previous experience with hepatocyte transplantation (11, 18). Expression of TIMP1-3, natural inhibitors of metalloproteinases that degrade the extracellular matrix, and TGF $\beta$ , a hepatocyte-secreted cytokine that stimulates fibrogenesis was also investigated. TIMP2 and TIMP3 expression was immediately down-regulated in vitro following HNF4 $\alpha$  re-expression, while TGF $\beta$  and TIMPs 1, 2, and 3 were down-regulated within 2 weeks of HNF4 $\alpha$  transduction in cirrhotic rats (FIG. 6B).

**[0103]** These studies show that down-regulation of HNF4 $\alpha$  has a profound effect on the end-stage cirrhotic hepatocyte in vitro, since replenishment of this single factor immediately revitalizes function. Moreover, transduction of hepatocytes in cirrhotic animals with apparently irreversible decompensated function produced a profound and immediate improvement in hepatic function. Normalization of function took place in two weeks while portal hypertension, evidenced by the presence of ascites, regressed when histological reversal of cirrhosis was more complete. This outcome is consistent with the central role of HNF4 $\alpha$  within the hepatocyte transcription factor network (12, 20, 21). Impaired expression of HNF4 $\alpha$  could reflect direct regulation or inhibition of another network factor that activates HNF4 $\alpha$  transcription (22-24) and could result from either cell-extrinsic or cell-intrinsic mechanisms. Toxins, chemical injury, and cytokines generated from inflammation or injured cells can all induce inhibition of critical transcription factors (25). Such extrinsic mechanisms should be corrected immediately by removing the injury in vivo, or by culturing the hepatocytes in vitro. However, neither withdrawing CCl<sub>4</sub> nor primary cell culture effectively reversed the hepatocyte dysfunction. Since down regulation of network factors HNF1 $\alpha$ , FOXA2, CEBP $\alpha$ , and PPAR $\alpha$  was also clear in these studies, it is likely that HNF4 $\alpha$ , or another network gene, is the critical target of an inhibitory pathway. These data indicate the target is HNF4 $\alpha$ , since its reexpression restores the other three factors. Research on hepatocyte injury has highlighted three candidate pathways that could mediate this inhibition—TNF $\alpha$ -NF $\kappa$ B, IL6-Stat3, and TGF $\beta$ -SMAD—and the inventors' preliminary research showed NF $\kappa$ B and TGF $\beta$  signatures by microarray and direct transcript analysis (26). Thus, it is likely that cytokine/injury effects alter expression of the hepatocyte transcription factor network by extrinsic mechanisms, with the result that network factors establish a new steady-state equilibrium in the

dysfunctional hepatocyte that can no longer compensate to restore normal gene expression. This has important therapeutic implications, because it may require only transient therapy with HNF4 $\alpha$  to restore the transcription factor network once the injury has been moderated.

**[0104]** Further studies (see Example 3 below) will determine the efficacy of this intervention when there may be ongoing hepatocellular insults, as is the case with clinical cirrhosis, typically associated with hepatitis or alcohol abuse.

**[0105]** These studies indicate that in addition to regeneration mediated by expansion of mature hepatocytes or differentiation and expansion of induced progenitors, normalized function can be accomplished by transcriptional reprogramming with reversal of de-differentiation but not senescence. The results also indicate that HNF4 $\alpha$  therapy could be effective in treating advanced liver cirrhosis with impaired hepatic function as a bridge to organ transplantation or possibly even as destination therapy.

#### Material and Methods

**[0106]** Animals: Lewis rats were obtained from Charles River Laboratory (Cambridge, Mass.) and were maintained in isolation cages in the Department of Laboratory and Animal Resources at the University of Pittsburgh. Animals were housed in temperature- and light-dark cycle-controlled rooms. All procedures performed on animals were approved by the University of Pittsburgh Animal Care and Use Committees, and thus within the guidelines for humane care of laboratory animals.

**[0107]** Induction of liver cirrhosis: Liver cirrhosis was induced as described beginning in four-week-old inbred male Lewis rats, weighing 100 to 130 g, using Phenobarbital (Sigma Chem. Co. St. Louis, Mo.) and carbon tetrachloride (CCl<sub>4</sub>, Sigma) (27). Rats were given Phenobarbital (0.5 g/L) added to the drinking water. Starting two weeks later, CCl<sub>4</sub> (diluted 1:9 in the olive oil) was administered by gavage on a full stomach twice a week. Following an initial dose of 0.2 ml/kg each subsequent dose was adjusted weekly on the basis of changes in body weight. If the body weight increased or remained unchanged CCl<sub>4</sub> was continued at 0.2 ml/kg twice weekly. When body weight decreased by 1-5 g the dose of CCl<sub>4</sub> was reduced to 0.15 ml/kg, and if body weight decreased by 6-10 g the CCl<sub>4</sub> was reduced to 0.1 ml/kg. In rats that lost more than 10 g of body weight, CCl<sub>4</sub> was withheld until reassessment one week later. All animals were monitored by body weight, activity, amount of ascites, and by hepatic encephalopathy (HE) score, which constituted a coma scale based on spontaneous levels of flexion, grasping, righting, placement, corneal, and head-shaking reflexes. Normal activity score was 5; maximum ascites score was 3; and a HE score of 15 indicated normal behavior (28). Whole blood was obtained at different time points and analyzed for bilirubin and albumin using a microfluidic metabolic assay system (Piccolo-Abaxis, Union City, Calif.). Ammonia (NH<sub>3</sub>) and INR in serum were measured in the clinical laboratory at the Children's Hospital of Pittsburgh.

**[0108]** When rats received a minimum of 2.6 ml CCl<sub>4</sub> and developed persistent ascites, an activity score of <4 and an HE score of <10, laboratory tests were performed weekly to estimate liver function. Phenobarbital and CCl<sub>4</sub> were discontinued when (1) plasma total bilirubin levels exceeded 0.5 mg/dl (normal <0.2 mg/dl); (2) the INR exceeded 1.7; (3) plasma ammonia concentrations were above 90 mmol/l (normal <70 mmol/l); (4) ascites was found to be persistent by

clinical examination, and (5) the hepatic encephalopathy (HE) score was persistently <10. If four weeks after complete cessation of phenobarbital and carbon tetrachloride treatment, the laboratory measures, encephalopathy score and ascites did not improve, rats were considered to have chronic liver failure from decompensated cirrhosis. All animals receiving CCl<sub>4</sub> were observed for four weeks after receiving their last dose of CCl<sub>4</sub> to eliminate the acute effects of toxin exposure before hepatocytes were recovered for analysis and transplantation.

**[0109]** Animals required 2.8±0.2 mL CCl<sub>4</sub> over 26 to 28 weeks to generate cirrhosis that produced irreversible liver failure, and these animals died approximately 2-4 weeks after the four-week observation period with progressive worsening of liver function if they received no treatment. Animals with cirrhosis without liver failure received 13 to 14 weeks of CCl<sub>4</sub>, and a total dose of 1.3±0.1 mL of CCl<sub>4</sub>. Laboratory tests and ascites resolved quickly in all of these animals after the four week observation period after discontinuing carbon tetrachloride.

**[0110]** Isolation of hepatocytes: Hepatocytes were isolated from donor rats by in situ collagenase perfusion as originally described by Berry and Friend and later modified by Seglen (29, 30). Briefly, a 20 G cannula (Becton, Dickinson Infusion Therapy Systems Inc., Sandy, Utah) was inserted into the portal vein. Perfusion using 0.5 mM EGTA in Leffert's buffer was started via the portal vein for 10 minutes, and the inferior vena cava (IVC) was cut for drainage. The liver was then perfused with collagenase (Liberase, Roche, Germany) at 21 units/200 mL in Leffert's solution for 10 minutes. The perfusion time varied from 10 to 20 minutes based on the consistency of liver tissue in response to collagenase digestion. After perfusion, the liver was excised and chopped into small millimeter sized pieces using a scalpel in a 10 cm sterile tissue culture dish. Cells were then collected in Leffert's solution

containing 2.5 mM CaCl<sub>2</sub> and filtered through a 200 micron nylon mesh to remove aggregated cells and residual tissue, centrifuged at 50 g for 3 minutes, and washed three times with chilled Dulbecco's Modified Eagle's Medium (DMEM). Viability was assessed by Trypan blue exclusion and by plating efficiency at 24 hours. Cell viability, as determined by trypan blue exclusion and plating efficiency, was required to be 80% or greater to be acceptable for in vitro analysis. For cytospin samples, 5×10<sup>4</sup> hepatocytes were centrifuged at 50 g for 5 minutes for attachment to slides. After air-drying, the cells were fixed with 4% paraformaldehyde for 15 minutes and preserved at -80° C.

**[0111]** Hepatocyte culture: Five hundred thousand hepatocytes isolated from control and cirrhotic livers were seeded into individual wells of collagen-coated 6-well plates (Becton Dickinson Labware, NJ) and cultured at 37° in 5% CO<sub>2</sub> in F-12 medium (DMEM supplemented with 5% FBS, 2 mM Glutamine, 100 U/mL Penicillin, 100 ug/mL Streptomycin, 100 nM dexamethasone, 0.872 uM insulin and 5 ng/mL epithelial growth factor). Tissue culture medium was changed after overnight culture following the isolation and freshly exchanged everyday. The supernatant was collected 24 hours later to determine albumin secretion.

**[0112]** Total RNA extraction and quantitative real-time PCR (QPCR): RNA was extracted from isolated rat hepatocytes or liver tissue using the RNeasy Mini Kit (QIAGEN, Valencia Calif.) according to the manufacturer's instructions. RNA quantity and integrity were evaluated using a NANO DROP 1000 spectrometer (Thermo Fisher Scientific Inc). cDNA was reverse transcribed from 1 ug total RNA using SuperScript III reverse transcriptase (Invitrogen). Each gene expression was measured using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, Calif., USA), using a ABI 7500 real time PCR System. The sequences of the primers used for this Example are listed in Table 1.

TABLE 1

Primer sequences		
Primer Name	Primer sequence	Length of PCR product
HNF4a-F	5'-ATGGACATGGCTGACTACAGTGCT-3' (SEQ ID NO: 13)	204 bp
HNF4a-R	5'-ACAGCTTGAGGCTCCGTAGTGTTT-3' (SEQ ID NO: 14)	
A1AT-F	5'-TCTAGAGGGCCTGGAGTTCA-3' (SEQ ID NO: 15)	99 bp
A1AT-R	5'-TCACTGTCTGGCCTCTTGAG-3' (SEQ ID NO: 16)	
ACTA2-F	5'-TTCAATGTCCCTGCCATGTA-3' (SEQ ID NO: 17)	94 bp
ACTA2-R	5'-CATCTCCAGAGTCCACCACA-3' (SEQ ID NO: 18)	
ACTB-F	5'-TTGCTGACAGGATGCAGAAG-3' (SEQ ID NO: 19)	122 bp
ACTB-R	5'-CAGTGAGGCCAGGATAGAGC-3' (SEQ ID NO: 20)	
AFP-F	5'-GCCCAGCATAACGAAGAAAACA-3' (SEQ ID NO: 21)	176 bp
AFP-R	5'-TCTCTTTGTCTGGAAGATTTCCT-3' (SEQ ID NO: 22)	
ALB-F	5'-TCTGCACACTCCAGACAAG-3' (SEQ ID NO: 23)	114 bp
ALB-R	5'-AGTCACCCATCACCCTCTTC-3' (SEQ ID NO: 24)	
ApoA2-F	5'-GGCAAGGATTTGATGGAGAA-3' (SEQ ID NO: 25)	108 bp

TABLE 1-continued

Primer sequences		
Primer Name	Primer sequence	Length of PCR product
ApoA2-R	5'-CCCAGTCTCTGGACAAAGG-3' (SEQ ID NO: 26)	
ApoC3-F	5'-ACATGGAACAAGCCTCCAAG-3' (SEQ ID NO: 27)	75 bp
ApoC3-R	5'-TGGCCACCACAGCTATATCA-3' (SEQ ID NO: 28)	
ApoE-F	5'-TGAACCGCTTCTGGGATTAC-3' (SEQ ID NO: 29)	85 bp
ApoE-R	5'-TGTGTGACTTGGGAGCTCTG-3' (SEQ ID NO: 30)	
ASGR1-F	5'-GGAGGATCTGAGGGAAGACC-3' (SEQ ID NO: 31)	125 bp
ASGR1-R	5'-GGCAGCAGATCCTTTCAGAG-3' (SEQ ID NO: 32)	
CD44-F	5'-TTTGGTGGCACACAGCTTG-3' (SEQ ID NO: 33)	104 bp
CD44-R	5'-ATGGAATACACCTGCGTAACGG-3' (SEQ ID NO: 34)	
Cdh1-F	5'-GAAGGCTAAGCACAAACAGC-3' (SEQ ID NO: 35)	99 bp
Cdh1-R	5'-AAGCACTTGACCCTGGTACG-3' (SEQ ID NO: 36)	
C/EBPa-F	5'-GCCAAGAAGTCGGTGGATAA-3' (SEQ ID NO: 37)	125 bp
C/EBPa-R	5'-AACACCTTCTGCTGCGTCTC-3' (SEQ ID NO: 38)	
CK18-F	5'-GCTCAGATCTTTGCGAATTC-3' (SEQ ID NO: 39)	204 bp
CK18-R	5'-CGCTTCGATTTCTGTCTCC-3' (SEQ ID NO: 40)	
COL1a1-F	5'-TGGTCTCAAGTTTCCAAG-3' (SEQ ID NO: 41)	123 bp
COL1a1-R	5'-TTACCAGCTTCCCCATCATC-3' (SEQ ID NO: 42)	
Ctnnb1-F	5'-TGCAGAAAATGGTTGCTTTG-3' (SEQ ID NO: 43)	98 bp
Ctnnb1-R	5'-GCTTTCCTGATTGCCGTAAG-3' (SEQ ID NO: 44)	
CYP3a23/3a1-F	5'-ATGGAGATCACAGCCAGTC-3' (SEQ ID NO: 45)	130 bp
CYP3a23/3a1-R	5'-CGATCTCCTCCTGAGTTTC-3' (SEQ ID NO: 46)	
EpCAM-F	5'-TGAGAATGGTGAATGCCAGT-3' (SEQ ID NO: 47)	101 bp
EpCAM-R	5'-GAGTCATCTCCGCTTCATC-3' (SEQ ID NO: 48)	
F7-F	5'-TAACCAGGAGGAAGCACAC-3' (SEQ ID NO: 49)	102 bp
F7-R	5'-CTTCATTGCACTCCCTCTCC-3' (SEQ ID NO: 50)	
Foxa2-F	5'-CCATCCGTCTCTCTCTCC-3' (SEQ ID NO: 51)	112 bp
Foxa2-R	5'-TCGAACATGTTGCCAGAGTC-3' (SEQ ID NO: 52)	
HNF1a-F	5'-GACGTCTCCAGGTCTCAACC-3' (SEQ ID NO: 53)	119 bp
HNF1a-R	5'-CACCCGTGTTAGTGAACGTG-3' (SEQ ID NO: 54)	
OTC-F	5'-CTCACCCCTCAGCTGGATAGG-3' (SEQ ID NO: 55)	101 bp
OTC-R	5'-CCCTTTGGAGTAGCTGCTTG-3' (SEQ ID NO: 56)	
Ppara-F	5'-AATGCAATCCGTTTTGGAAG-3' (SEQ ID NO: 57)	116 bp
Ppara-R	5'-GCCAGAGATTTGAGGTCTGC-3' (SEQ ID NO: 58)	
TAT-F	5'-CATCGTGGACAACATGAAGG-3' (SEQ ID NO: 59)	101 bp
TAT-R	5'-CAGGGTCTGTAGGCAGGTTTC-3' (SEQ ID NO: 60)	
TD02-F	5'-TTGAAGGGTCTGGAAGAGGA-3' (SEQ ID NO: 61)	119 bp

TABLE 1-continued

Primer sequences		
Primer Name	Primer sequence	Length of PCR product
TDO2-R	5'-TCATCGAACAAGCAGAGCAG-3' (SEQ ID NO: 62)	
TERT-F	5'-GCATCTGACCCGAGTCTCTC-3' (SEQ ID NO: 63)	105 bp
TERT-R	5'-GAATGGCCTGAGCTTTTCAG-3' (SEQ ID NO: 64)	
TF-F	5'-AATGGAGATGGCAAAGAGGA-3' (SEQ ID NO: 65)	100 bp
TF-R	5'-GAGAGCCGAACAGTTGGAAG-3' (SEQ ID NO: 66)	
TGFb1-F	5'-GGACTCTCCACCTGCAAGAC-3' (SEQ ID NO: 67)	100 bp
TGFb1-R	5'-GACTGGCGAGCCTTAGTTTG-3' (SEQ ID NO: 68)	
TIMP1-F	5'-GGTTCCTGGCATAATCTGA-3' (SEQ ID NO: 69)	99 bp
TIMP1-R	5'-ATGGCTGAACAGGGAAACAC-3' (SEQ ID NO: 70)	
TIMP2-F	5'-TGGACGTTGGAGGAAAGAAG-3' (SEQ ID NO: 71)	97 bp
TIMP2-R	5'-TCCCAGGGCACATAAAGTC-3' (SEQ ID NO: 72)	
TIMP3-F	5'-TGTGCAACTTTGTGGAGAGG-3' (SEQ ID NO: 73)	84 bp
TIMP3-R	5'-AATTGCAACCCAGGTGGTAG-3' (SEQ ID NO: 74)	
TTR-F	5'-TCGTACTGGAAGGCTCTTGG-3' (SEQ ID NO: 75)	120 bp
TTR-R	5'-GTAGGAGTACGGGCTGAGCA-3' (SEQ ID NO: 76)	

\* These primers were used for quantitative RT-PCR

**[0113]** The PCR reaction was programmed as follows: initial denaturing at 95° C. for 10 min followed by 95° C. for 15 sec, 60° C. for 1 min, cycled 40 times. The median cycle threshold value and the relative cycle threshold method were used for analysis. All cycle threshold values were normalized to the expression of the housekeeping gene ACTB. All reactions were performed with four biological replicates and three technical replicates with reference dye normalization. All values were normalized to control normal hepatocytes. A p value of less than 0.05 was used for the significance cutoff point for all genes tested. ANOVA (Tukey-Kramer multiple comparison test) was used for statistical comparison within experimental groups. Each value represents the mean±SD.

**[0114]** DNA extraction and telomere length measurement by QPCR: Genomic DNA was extracted from isolated rat hepatocytes using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia Calif.) according to the manufacturer's instructions. The samples were used for examination of telomere length in QPCR using the modified Cawthon's method (31). Briefly, telomere and single copy gene ( $\beta$ -globin) PCRs were performed in separate 96-well plates using the same DNA sample. The telomere/single copy gene (T/S) ratio was calculated as the index of telomere length in each sample. Triplicate PCR reactions for each sample were performed with Power SYBR Green PCR Master Mix, DNA and primer pairs using the ABI 7500 real time PCR System. Primers for telomeres and  $\beta$ -globin were added to a final concentrations of 250 nM. The primer sequences were tel-1, 5'-GGTTTTTGAGGGTGAGGGTGAGGGT-GAGGGTGAGGGT-3' (SEQ ID NO:77); tel-2, 5'-TC-

CCGACTATCCCTATCCCTATCCCTATCCCTATCCCTATCCCTA-3 (SEQ ID NO:78);  $\beta$ -globin-F, 5'-CAGCAAGTGGGAAGGTGTAATCC-3' (SEQ ID NO:79);  $\beta$ -globin-R, 5'-CCCATTCTATCATCAACGGGTA-CAA-3' (SEQ ID NO:80). PCR was performed at 95° C. for 10 min, followed by 40 cycles at 95° C. for 15 sec, and 54° C. for 2 min for the telomere reaction or 40 cycles at 95° C. for 15 sec, 60° C. for 60 sec for the  $\beta$ -globin reaction. Standard curves for both telomere length and the single copy gene were generated from five concentrations (42, 25.2, 15.1, 9.1, 5.4 ng/aliquot) of a reference DNA sample serially diluted 1.68 fold with PCR grade water.

**[0115]** Western blot analysis: Isolated hepatocyte fractions were lysed in RIPA buffer (Sigma Aldrich) with 1% protease inhibitor cocktail (Calbiochem) on ice. The supernatant was collected as cellular protein after centrifugation at 8000 g for 15 min. and the supernatant was preserved at -80° C. Forty micrograms of the cellular protein was electrophoresed in a 4-12% bis-tris gel and then transferred to a nitrocellulose membrane (Invitrogen). The immunological detection of HNF4 $\alpha$  and beta-actin was performed as previously described. Antibodies for HNF4 $\alpha$  and beta-actin were purchased from Abcam Inc. (Cambridge, Mass.) and Cell Signaling Technology, Inc. (Beverly, Mass.) respectively. After incubation with a horseradish peroxidase-conjugated secondary antibody for 1 h, immune detection of each band was performed with SuperSignal West Pico Chemiluminescent Substrate (PIERCE, Rockford, Ill.). The density of each band was measured using Image J software.

**[0116]** Histology: Five-micrometer-thick sections were prepared from paraffin embedded liver tissue fixed in 4% paraformaldehyde (PFA) and two slides each were used for hematoxylin and eosin (H & E) and Masson trichrome staining according to the manufacturer's protocols from Sigma. Oil red O staining was performed to detect cellular deposition of triglycerides using 5 micron sections from OTC compound embedded frozen liver tissues fixed in 2% PFA. Briefly, after fixation in 10% formaldehyde for 15 minutes at room temperature, sections were washed twice with deionized water for 1 minute, and then rinsed in 60% isopropanol for 1 minute. Subsequently, the sections were immersed in the oil red O working solution for 30 min at 37° C. After washing three times with deionized water, sections were counterstained using Gill's haematoxylin for 60 seconds to visualize nuclei. Sections were then rinsed with water for 10 min and mounted using crystal mount (Biomedica corp., Foster city, CA). Images were taken at low magnification (100×) using an Olympus Provis light microscope. To quantify detection of fibrotic areas in the Masson trichrome stained sections or fat deposition on oil red O stained sections, the signal intensity was measured over five different fields using ImageJ software and the average value was used as the semi-quantitative measurement of fibrosis or fat deposition within the liver.

**[0117]** Immunohistochemistry: Sections, five-microns in thickness, were heated in a microwave oven in citrate sodium buffer, pH6.0 (DAKO, CA) for 5-10 minutes at 95° C., and cooled at room temperature for 20 minutes. After blocking with PBS and 2% FCS for 15 minutes, samples were incubated with one or a combination of diluted primary antibodies (1:100) for 1 hour at room temperature. After washing three times in PBS, samples were incubated with diluted secondary antibodies conjugated with fluorochromogen (1:250) for 60 minutes at room temperature for immunofluorescence staining. Samples were then finally mounted in VECTASHIELD (Vector Laboratories, Inc., Burlingame, Calif.) containing DAPI solution. Immunohistochemistry was also performed using the same method described above, except for the blocking of endogenous peroxydase by incubation in methanol and 0.3% hydroxyperoxydase for 20 minutes. The VECTASTAIN ABC kit for mouse IgG (Vector Laboratories, Inc) was used to detect the immunocomplex signal and the samples were counterstained using Gill's haematoxylin for 60 seconds. The stained samples were examined using Olympus Provis light microscope. Mouse anti-HNF4 $\alpha$  monoclonal antibody, rabbit anti-GFP polyclonal antibody, rabbit anti-Epcam polyclonal antibody, rabbit Ki67 polyclonal antibody, mouse anti-alpha smooth muscle actin monoclonal antibody were purchased from Abcam Inc., mouse anti-GFP monoclonal antibody from Cell Signaling Technology, Inc. and sheep anti-rat albumin polyclonal antibody from Bethyl Laboratories, Inc. (Montgomery, Tex.) as primary antibodies in this study. Alexa Fluor 488-conjugated goat anti-rabbit IgG and anti-mouse IgG antibodies and Alexa Fluor 594-conjugated goat anti-rabbit IgG, anti-mouse IgG and donkey anti-sheep IgG antibody were purchased from Invitrogen as secondary antibodies. For calculation of percent positive cells at least four low power digital images (100×) per sample were analyzed using an Olympus Provis light microscope using ImageJ software.

**[0118]** AAV cloning and virus preparation: Two AAV vectors were used for these studies; one capable of expressing GFP from an IRES promoter, pAAV-GFP, and the other capable of expressing HNF4 $\alpha$  under control of a CMV pro-

moter and GFP under control of the IRES promoter, pAAV-HNF4 $\alpha$ /GFP. Cells were transduced in vitro at an MOI of 2000, and the transduction efficiency in vitro was >90%.

**[0119]** A 1.5 kb EcoRI fragment of rat-HNF4 $\alpha$  2 was cloned into the bicistronic plasmid pAAV-IRES-GFP (Cell Biolabs, Inc; San Diego, Calif.), which expresses HNF4 $\alpha$  under control of the CMV promoter and GFP under control of the IRES promoter. AAV vector preparation was performed using the helper-virus-free CaPO<sub>4</sub> triple transfection method. Briefly, HEK293 cells were grown in DMEM with 10% FCS to 75% confluence in 12 150 cm<sup>2</sup> flasks. For each flask, 15  $\mu$ g of each plasmid [pAAV-CMV-HNF4 $\alpha$ /IRES-GFP, pladenos (32), pAAV-DJ (33)] was added to 3 ml of 300 mM CaCl<sub>2</sub>. This mixture was then added to 3 ml of 2X-HEPES-buffered saline (50 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid], 280 mM NaCl, 1.5 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.1), vortexed and let stand for 5 minutes, then added to the flask and incubated at 37° C. for 4-6 hrs. Media was changed to DMEM with 2% FCS and cultured for 48-72 hrs. For harvest, EDTA was added to the flask to a final concentration of 10 mM and incubated for 3-5 min. Cells were dislodged by gentle shaking and cells and media were harvested and centrifuged at 100×g. The cell pellet was suspended in DMEM and subjected to 3 freeze-thaw cycles in dry-ice/ethanol and 37° C. baths. Cell debris was removed by centrifugation at 10,000×g for 10 min. Supernatant was collected and virus was purified and concentrated using the ViraBind™ AAV purification kit (Cell Biolabs, Inc; San Diego, Calif.) per manufacturer's instructions. Viral titer in genome copies (gc)/ml was determined by dot blot of serial dilutions of virus using linearized pAAV-CMV-HNF4 $\alpha$ /IRES-GFP as a standard and a HNF4 $\alpha$  cDNA fragment as probe.

**[0120]** Transduction to express HNF4 $\alpha$ : For in vitro studies, freshly isolated hepatocytes were plated at a density of 0.5 million cells per well in collagen-coated 6-well plates. After incubation in DMEM/F12 for 2 hours at 37° C. and 5% CO<sub>2</sub>, the culture medium was changed to one containing each AAV vector at an MOI of 2000. Cells were incubated for 24 hours at 37° C. and 5% CO<sub>2</sub>, and then washed twice with PBS. Two ml of fresh culture medium was added into each well, starting 24 hours after AAV transduction (Day 0), and repeated daily up to day 7. Supernatant samples were collected and preserved at -80° C. After viral transduction, cell samples were also collected from each well on day 2 for extraction of genomic DNA, total RNA and whole cellular protein for analysis.

**[0121]** For AAV transduction in vivo, 3.0×10<sup>11</sup> viral genomes of AAV-DJ-HNF4 $\alpha$ -IRES-GFP (n=5) and AAVDJ-IRES-GFP (n=4) were injected into the tail vein. Five animals with decompensated cirrhosis were also monitored that received no AAV infection as a control group. All animals were monitored and scored for clinical changes and by laboratory tests every week until expiration or until the end of the observation period at 14 weeks after viral infection. Untreated animals and animals treated with the control AAV-GFP vector developed progressively worsening liver function and died with a mean survival of 19 days. Treated rats showed improvement in clinical parameters, INR, total bilirubin, serum albumin level, and ammonia. All were nearly at normal levels within 2 weeks of treatment, and sustained those levels for at least 100 days. One animal treated with AAVDJ-HNF4 $\alpha$ -IRES-GFP was sacrificed 2 weeks after viral injection to estimate early transduction efficiency. Survival assessment was statistically performed by log-rank test among three

groups (\* $p < 0.05$ ). Value represent mean $\pm$ SD. Specimens were collected following euthanasia and samples were preserved unfixed at  $-80^{\circ}\text{C}$ ., embedded in paraffin after 4% PFA fixation, and embedded in OTC compound after 2% PFA fixation. Vector transduction efficiency was estimated by immune detection for GFP.

**[0122]** Albumin measurement: Albumin levels from hepatocyte culture supernatants were measured by ELISA according to manufacture's instructions (Bethyl Laboratories, TX). Five hundred thousand freshly isolated viable hepatocytes were seeded into each well of a collagen-coated 6 well plate containing 2 ml DMEM/F12 and incubated overnight at  $37^{\circ}\text{C}$ . in 5%  $\text{CO}_2$ . The culture medium was replaced with 2 ml of new every day. Collected samples were stored at  $-80^{\circ}\text{C}$ . prior to analyses. For the ELISA, high binding 96 well plates (Corning, N.Y.) were coated with 100  $\mu\text{L}$  of an anti-rat albumin affinity purified antibody (1:100) (Bethyl laboratories) in 0.05M carbonate-bicarbonate, pH 9.6 for 1 hr at  $25^{\circ}\text{C}$ . Plates were washed three times with 50 mM Tris, 0.14M NaCl, and 0.05% (v/v) Tween 20 pH 8.0. Nonspecific binding was blocked by adding 1% BSA for 1 h at  $25^{\circ}\text{C}$ . After washing three times, 200  $\mu\text{L}$  of each sample (in duplicate) and standards were added and incubated for 1 h at  $25^{\circ}\text{C}$ . Samples were diluted as needed up to 1:150000 in 50 mM Tris, 0.14M NaCl, 1% BSA, and 0.05% (v/v) Tween 20 pH 8.0). Wells were then washed five times, and 100  $\mu\text{L}$  of an anti-albumin HRP conjugate, diluted 1:10000 in the above buffer was added. Plates were incubated for 1 h at  $25^{\circ}\text{C}$ . After washing, 100  $\mu\text{L}$  of substrate ABTS (KPL, MD) was added in each well. After 30 min, the reaction was stopped by adding 1% SDS to each well. Absorbance was measured at 405 nm using a Benchmark microplate reader (Biorad, CA). The absence of cross reactivity with fetal bovine serum (present in F-12 medium) was verified using samples of culture medium used as blanks. Lewis rat serum was used as a positive control.

**[0123]** CYP3A4 activity assay: Isolated cells were cultured in DMEM/F12 at density of 0.5 million cells per well in collagen-coated 6-well plates, as described above. CYP3A4 activity was measured on day 2 of culturing, when expression following HNF4 $\alpha$  vector transduction had peaked, using the P450-Glo™ CYP3A4 assay kit (Promega Corporation, Madison, Wis.), according to manufacture's instructions. Briefly, the culture medium was exchanged with one ml of fresh media (without Phenol red) containing 3  $\mu\text{M}$  Luciferin-IPA. After incubation for 30 minutes at  $37^{\circ}\text{C}$ ., 200  $\mu\text{L}$  of culture supernatant was collected from each well and transferred into luminometer tubes. Then 200  $\mu\text{L}$  of Luciferin detection reagent was added into each tube. The mixture was incubated for 20 minutes at room temperature, protected from light, and the luminescence was immediately detected using a TD-20/20 luminometer (Turner BioSystems, Inc., Sunnyvale, Calif.).

**[0124]** Statistical analysis: Differences among group results were deemed significant when the P-value was less than 0.05. Statistical analyses were performed using the Tukey-Kramer multiple comparisons procedure and Student's t-test using SPSS v16.0 software (SPSS Inc., Chicago, Ill., USA). Survival in vivo was evaluated by log-rank test. Each value represents the mean $\pm$ SD. Statistical analysis was performed among three groups (normal, compensated and decompensated cirrhotic hepatocytes) and between un-treated decompensated cirrhotic hepatocytes and decompensated cirrhotic hepatocytes 14 weeks after in vivo HNF4 $\alpha$  re-expression (\* $p < 0.05$ , \*\* $P < 0.001$ ).

### Example 2

#### Effect of Transient Expression of Exogenous HNF4 $\alpha$ in Cirrhotic Rats

**[0125]** Short-term HNF4 $\alpha$  re-expression may reactivate the hepatocyte transcription factor network. Alternatively, the therapeutic effects could require continuous expression of exogenous HNF4 $\alpha$ . Determination of which scenario is accurate will profoundly affect clinical therapeutic strategies. Therefore, a recombinant AAV vector is constructed that expresses HNF4 $\alpha$  from an inducible promoter controlled by Tet-ON/OFF system.

**[0126]** The bicistronic vector ("Tet-ON:AAV-CMV-rTA/TRE-HNF4 $\alpha$ ", modeled after Zheng et al. (2012 *BMC Cancer*, 12:153) will express the rTA (Tet repressor) under the control of a CMV promoter and the human HNF4 $\alpha$  cDNA under the control of the TRE (tet regulated promoter). The CMV-rTA is 2200 bp and the TRE-HNF4 $\alpha$  is 2100 bp, a total within the 5 kb capacity of AAV. Tet ON:AAV-CMV rTA/TRE-GFP is generated as a control virus. Transduction efficiency and efficacy of the conditional constructs is confirmed by immunochemistry and in vitro studies.

**[0127]** An alternative approach will be to simultaneously administer separate viruses containing the rTA and TRE-HNF4 or TRE-GFP. IV injection on 2-consecutive days can also be done to achieve maximal target cell transduction with no increased immune response. Tetracycline inducible systems have been widely used to administer regulated gene expression, but expression can be leaky. Therefore, a newer third generation Tetracycline-inducible system, the Tet-ON-3 G (Clontech), will be utilized, which has been engineered to have reduced basal expression and enhanced sensitivity to doxycycline.

**[0128]** HNF4 $\alpha$  expression is induced by administration of doxycycline (1 mg/ml in drinking water) and animals will be assessed weekly for improved hepatic function (INR, total bilirubin, serum NH3, albumin, and encephalopathy score). Exogenous HNF4 $\alpha$  expression is halted by withdrawal of doxycycline at one, 2, 4, 6, and 8 weeks after correction of hepatic function and assessed for sustained normalization of hepatic function and survival. If liver function does not deteriorate 2 weeks after withdrawal of exogenous HNF4 $\alpha$  expression (i.e., animals are improving), liver specimens are collected and HNF4 $\alpha$  expression is determined by immunohistochemistry, Western blot, northern blot, and qPCR. Hepatocytes are also isolated from treated cirrhotic livers and examined following cytospin for gene expression by IHC (HNF4 $\alpha$ , albumin, etc.). In addition, RNA is collected from hepatocytes for qPCR analysis to assess HNF4 $\alpha$  expression and associated changes in liver specific gene expression. Exogenous and endogenous HNF4 $\alpha$  expression is distinguished by qRT-PCR, since the mRNAs differ in their 3'-untranslated regions.

### Example 3

#### Effect of Ongoing CCl $_4$ Treatment on Liver Function and Gene Expression in Cirrhotic Rats with Liver Failure Treated with AAV-HNF4/GFP

**[0129]** Human cirrhosis patients have continuing injury, whether from alcohol, HCV, or other chronic insults. Therefore, how HNF4 $\alpha$  therapy modulates continuing injury with CCl $_4$  is studied. Rats with end-stage cirrhosis whose liver



function has been corrected following administration with AAV-HNF4 $\alpha$  receive ongoing treatment with CCl<sub>4</sub>, starting 2 weeks after AAV treatment. Animals are assessed weekly by blood tests for maintenance of improved hepatic function following AAV-HNF4 $\alpha$  treatment. CCl<sub>4</sub> treatment is discontinued if the hepatic encephalopathy score falls below 9 (Kobayashi, et al. 2000, *Hepatology* 31(4):851; Bures, et al. Innate and motivated behavior. Techniques and Basic Experiments for the Study of Brain and Behavior. New York: Elsevier; 1976, p. 37-45)). If hepatic function deteriorates but does not correct within 2 weeks of stopping treatment with CCl<sub>4</sub>, animals are sacrificed and liver specimens and isolated hepatocytes are examined for HNF4 $\alpha$  and liver-specific gene expression.

#### Example 4

##### To Determine the Mechanism of HNF4 $\alpha$ Downregulation in Human End-Stage Cirrhosis and to Examine Whether Exogenous HNF4 $\alpha$ can Correct for Loss of Function

**[0130]** To extend the rat observations to human liver disease, control and cirrhotic livers are examined for function and changes in gene expression and chromatin. There are a wide variety of fibrotic liver diseases, with some differences from the rat CCl<sub>4</sub> model. Nevertheless, the focus on liver failure via transcriptionally regulated hepatocyte decompensation is distinctive, and there is a striking similarity between the rat and human diseases. It has been found that HNF4 $\alpha$  expression is a dramatically diminished in cirrhotic livers with decompensated hepatic function (Berasain, C. et al. 2003 *Hepatology* 38(1):148-57). Thus, the extent to which networks altered in rodent studies are similarly altered in human hepatocytes will be determined. Since diminished HNF4 $\alpha$  expression has been identified, further examination focuses on the extent to which providing exogenous HNF4 $\alpha$  to human hepatocytes will correct hepatic function toward normal.

Characterization of Transplanted Hepatocytes Derived from Cirrhotic Livers.

**[0131]** In the animal studies described herein, the yield of hepatocytes recovered by collagenase digestion of cirrhotic livers was significantly lower than from age-matched controls, but hepatocyte viability and plating efficiency were not statistically different among groups. However, hepatocytes from the livers of rats with decompensated cirrhosis functioned less well in vitro than those from all other donor groups. Preliminary studies of human disease were performed, using explanted diseased livers recovered during liver transplantation at Children's Hospital of Pittsburgh. Cirrhotic livers were classified according to severity based on Child-Pugh Classification of severity of liver disease (determined based on the criteria set forth in Table 2, below). Cells were isolated from cirrhotic livers, from the livers of patients with liver-based metabolic disorders, and from pediatric age-matched controls. There was no difference in viability or plating efficiency within groups. However, albumin secretion and urea synthesis was significantly diminished in hepatocytes from Child-B livers. A decrease in inducible CYP 1A1/1A2 activity in hepatocytes from human Child-B but not Child-A cirrhotic hepatocytes was observed. As cirrhosis became more advanced, human hepatocytes showed an increase in mRNA expression for progenitor cell genes, most markedly for CD44 and EpCAM.

TABLE 2

Criteria for Child-Pugh Classification			
Scores for each of five clinical and biochemical measurements are combined for a Grade, Grade A = 5-6 Grade B = 7-9 Grade C = 10-15			
Clinical and Biochemical	Points Scored for Increasing Abnormality		
	1	2	3
Measurements			
Hepatic encephalopathy (grade)*	None	1 and 2	3 and 4
Ascites	Absent	Mild	Moderate
Total bilirubin (mg/dl)	<2.0	2.0-3.0	>3.0
Serum albumin (g/dl)	>3.5	2.8-3.5	<2.8
Prothrombin time (sec. prolonged) or Prothrombin time INR**	<4 or <1.7	4-6 or 1.7-2.3	>6 or >2.3

\*According to grading of Trey, Burns, and Saunders (1996).

\*\*Lucey MR, et al. Minimal Criteria for Placement of Adults on the Liver Transplant Waiting List *Liver Transplantation and Surgery*, Vol. 3, No 6 (November), 1997: pp 628-637.

HNF4 $\alpha$  Expression is Downregulated in Human Livers with Advanced Cirrhosis.

**[0132]** As in rat cirrhosis, HNF4 $\alpha$  downregulation occurs in human livers with advanced cirrhosis (Berasain, C. et al. 2003 *Hepatology* 38(0):148-57). This finding was confirmed by qPCR of cirrhotic and control hepatocytes recovered at Children's Hospital of Pittsburgh. By qRT-PCR, levels of HNF4 $\alpha$  were significantly lower than that found in control normal livers.

Research Design:

**[0133]** Characterization of Human Livers and Hepatocytes Derived from these Livers.

**[0134]** Normal livers from nonheart-beating donors, and livers representing different stages of fibrosis and cirrhosis are recovered for analysis. Serum bilirubin, albumin, INR level, extent of ascites, and encephalopathy are recorded for each liver where hepatocytes are recovered. Tissue biopsies assess the extent of regenerative hyperplasia and cirrhosis. Extent of collagen deposition is determined by Masson-Trichrome and Sirius red-stained sections using ImageJ software. The yield of hepatocytes from cirrhotic and control livers recovered by collagenase digestion, including their viability and plating efficiency, us recorded. Four groups are established based on severity of donor liver disease. Group 1: normal control livers; Group 2: livers with significant liver injury but associated with no, or minimal, change in hepatic function (Child-Pugh A); Group 3: livers with modest loss of function (Child-Pugh B); Group 4: livers with severe loss of hepatic function (Child-Pugh C).

Baseline Functional Analysis and Gene Expression Profile of Hepatocytes Recovered from Human Livers.

**[0135]** As with hepatocytes derived from our rat model, measure human hepatocyte function is measured [albumin secretion, urea synthesis, qPCR for expression of liver-specific genes including HNF4 $\alpha$ , and baseline and induced cytochrome P450 activity; evidence of replicative senescence [qRT-PCR for NF-kB, telomerase expression, functional telomerase activity, and telomere length (Southern blot analysis)].

**[0136]** Differences among groups are analyzed statistically using ANOVA. It has been calculated that at least 10 samples representing each experimental group is required for comparison.

Human-Rat Comparison of the Dynamic Genetic Changes Associated with Progressive Cirrhosis and Liver Failure.

**[0137]** RNA-Seq analysis is carried out and expression levels of selected liver specific genes are also examined by quantitative PCR. As in the rodent studies, transcriptome analysis provides a fundamental resource for elucidating the mechanisms by which cells derived from normal, compensated, and decompensated cirrhotic livers develop hepatic dysfunction. The cluster analysis of the rat studies revealed signatures of NF $\kappa$ B stimulation and HNF4 $\alpha$  inhibition (see above) (Liu, L. et al. 2012 *Hepatology* 55(5):1529-39). It is hypothesized that similar patterns of expression will discriminate normal vs. compensated vs. decompensated cirrhosis in human livers. Hepatocytes, recovered as outlined above, and donor liver segments are processed for mRNA and subsequent analysis by RNA-Seq. transcriptomic dynamics of normal liver cells is defined, followed by a similar analysis of cells derived from compensated cirrhotic livers, culminating in an analysis of the transcriptomic dynamics of hepatocytes from decompensated cirrhotic livers.

Transduction of Hepatocytes from Cirrhotic Human Livers Associated with Decompensated Function to Express HNF-4a.

**[0138]** Adult human hepatocytes derived from end-stage cirrhotic livers are transduced to express HNF4 $\alpha$  by AAV. MOI is optimized to attain >80% transduction of human hepatocytes without injury. An AAV vector has been generated containing the gene encoding human HNF4 $\alpha$ . However, a new vector expressing both HNF4 $\alpha$  and GFP is constructed to allow easy assessment of transduction efficiency. Controls receive a GFP-expressing vector.  $5 \times 10^5$  human hepatocytes are cultured in each well of a 6-well plate. Following transduction, liver-specific gene expression is assessed in hepatocytes from normal and decompensated Child's A, B, and C livers. Following transduction, supernatants are collected daily from hepatocytes for measurement of albumin and urea secretion. Furthermore, mRNA is recovered from hepatocytes and qRT-PCR is performed to assess AAT, ApoA, ApoC, Cyp3A4, FVII, HNF1 $\alpha$ , TAT, Tdo2, TF, TTR, and endogenous and exogenous HNF4 $\alpha$  expression. All values are normalized to control normal hepatocytes.

Transplantation of HNF4 $\alpha$  Treated Human Hepatocytes from Cirrhotic Livers into Immune Deficient Mice FAH k/o Mice.

**[0139]** Control human hepatocytes and AAV-HNF4 $\alpha$  transduced hepatocytes are transplanted into immune-deficient FAH K/O (FRG or FRG-NOD) mice that provide a selective repopulation advantage to donor cells. FRG/N mice (6-12 week old), maintained on 2-(2-nitro-4-trifluoro-methyl-benzoyl)-1,3 cyclohexanedione (NTBC)-containing drinking water at 16 mg/L, receive  $5 \times 10^9$  pfu Ad-uPA i.p. before transplantation (Lieber, A. et al. 1995 *Hum Gene Ther.* 6(8):1029-37; Azuma, H. et al. 2007 *Nature Biotechnology* 25(8):903-10). 106 viable human hepatocytes are then transplanted into the spleen for engraftment in the liver. NTBC is gradually decreased (1.6 mg/l, day 0-2; 0.8 mg/l, day 3-4; 0.4 mg/l, day 5-6) and completely withdrawn one week after transplantation. Two weeks after stopping NTBC, animals are placed back on the drug for 5 d and then taken off again. NTBC may need to be repeatedly restarted for 5 days each time the body weight decreases by 20 percent in order to allow expansion of

transplanted hepatocytes. Human serum albumin level is used to non-invasively assess the extent to which engrafted donor hepatocytes function and expand in the non-cirrhotic FRG/N liver environment over time. 4-8 weeks after transplantation, when human serum albumin approaching 0.5 mg/ml indicates engraftment by human hepatocytes approximating 5-10% repopulation, hepatocytes are isolated by in situ collagenase perfusion (Berry, M N. et al. 1969 *PMCID:PMCID2107801*; Seglen P A, 1976 *Method Cell Biol.* 13:29-83). Isolated cells are placed in fresh media containing homogentisic acid (HGA), the intermediate metabolite in the pathway between 4-hydroxyphenylpyruvate dioxygenase and FAH, for 24 hrs. Donor cells that express FAH survive this treatment, whereas FAH-deficient cells do not survive (Kubo S. et al. 1998 *PNA-SUSA* 95(16):9552-7).

**[0140]** As predicted, is was found that FAH-deficient hepatocytes die in a dose-dependent fashion. As with hepatocytes derived from control and cirrhotic rats, human hepatocyte function: albumin secretion, urea synthesis, and qPCR is measured for expression of liver-specific genes including HNF4 $\alpha$ , baseline and induced cytochrome P450 activity (CYP3A4, 2C9, 1A1, 1A2), and evidence of replicative senescence (telomerase expression, functional telomerase activity, and telomere length) (Kitada T, et al. 1995 *Biochem Biophys Res Commun* 211(1):33-9; Rudolph L K et al. Telomeres and telomerases in experimental liver cirrhosis. Chisari F V, et al. editors. In: *The Liver: Biology and Pathobiology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1000-10; Wiemann S U, et al. 2002, *FASEB J.* 16(9): 935-42).

**[0141]** Differences between experimental and controls are analyzed statistically using ANOVA. At least 10 samples representing each group are required for comparison.

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## SEQUENCE LISTING

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cagactcgtt cttctgggaa ccctgcccac tcccaggacc aagattggcc tgaggctgca	3180
ctaaaattca cttagggctg agcatcctgt ttgctgataa atattaagga gaattcatga	3240
ctcttgacag cttttctctc ttcactcccc aagtcaaggg gaggggtggc aggggtctgt	3300
ttcctggaag tcaggctcat ctggcctgtt ggcatggggg tgggacagtg tgcacagtgt	3360
gggggacagg gagggctaag caggcctggg tttgagggct gctccggaga ccgtcactcc	3420
aggtgcattc tggaaagcatt agaccccagg atggagcgac cagcatgtca tccatgtgga	3480

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atcttggtgg ctttgaggac attctggaaa atgccactga ccagtgtgaa caaaagggat 3540
gtgttatggg gctggagggtg tgattaggtg ggagggaaac tgttgaccg actcctgccc 3600
cctgtcaaac actgaccct ctgagtggtt ggaggcagtg cccagtgcc cagaaatccc 3660
accattagtg attgtttttt atgagaaaga ggcgtggaga agtattgggg caatgtgtca 3720
gggaggaatc accacatccc tacggcagtc ccagccaagc cccaatccc agcggagact 3780
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tcttgggaga aaggttgaga taagaatgtc ccatggagtg ccgtgggcaa cagtggcct 4020
tcatgggaac aatctgttg agcaggggt cagttctctg ctgggaatct accccttct 4080
ggaggagaaa cccattccc ctttaataact ttattgtaat gtgagaaaca caaaacaaag 4140
tttacttttt tgactctaag ctgacatgat attagaaat ctctcctct cttttttttt 4200
ttttttttt tttttggcta cttgagttgt ggtcctaaaa cataaatct gatggacaaa 4260
cagaggggtg ctggggggac aagcgtgggc acaatttccc caccaagaca ccctgatcct 4320
caggcgggtc tcaggagctt ctaaaaatcc gcctggctct cctgagagtg gacagaggag 4380
aggagaggtt cagaaatgaa cgctcttcta tttctgtca ttaccaagcc aattactttt 4440
gccaaaatth tctgtgatct gccctgatta agatgaattg tgaatttac atcaagcaat 4500
tatcaaaagc ggctgggtcc catcagaacg acccacatct ttctgtgggt gtgaatgtca 4560
ttaggtcttg cgtgacccc tgagccccc tcaactgccgc ctgatggggc aaagaaacaa 4620
aaaaattht ttactcttct gtgttttaac aaaagtttat aaaacaaat aatggcgcga 4680
tatgttttct aaaaaaaaa aaaaaaa 4707

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&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 4737

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 2

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ggtttgaag gaaggcagag agggcactgg gaggaggcag tgggagggcg gagggcgggg 60
gccttcgggg tgggcgcccc gggtagggca ggtggccgcg gcgtggaggc agggagaatg 120
cgactctcca aaacctcgt cgacatggac atggccgact acagtgtctc actggacca 180
gcctacacca ccctggaatt tgagaatgtg caggtgttga cgatgggcaa tgacacgtcc 240
ccatcagaag gcaccaacct caacgcgccc aacagcctgg gtgtcagcgc cctgtgtgcc 300
atctgcgggg accgggccac gggcaaacac tacggtgcct cgagctgtga cgctgcaag 360
ggcttcttcc ggaggagcgt gcggaagaac cacatgtact cctgcagatt tagccggcag 420
tgctggttg acaaagacaa gaggaaccag tgccgctact gcaggctcaa gaaatgcttc 480
cgggctggca tgaagaagga agccgtccag aatgagcggg accggatcag cactcgaag 540
tcaagctatg aggacagcag cctgcctccc atcaatgcgc tcctgcaggc ggaggtcctg 600
tcccacaga tcacctcccc cgtctccggg atcaacggcg acattcgggc gaagaagatt 660
gccagcatcg cagatgtgtg tgagtccatg aaggagcagc tgctggttct cgttgagtg 720
gccaagtaca tccagcttct ctgcgagctc cccctggacg accaggtggc cctgctcaga 780

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gcccattgctg gcgagcacct gctgctcgga gccaccaaga gatccatggt gttcaaggac	840
gtgctgctcc taggcaatga ctacattgtc cctcggcact gcccgagct ggcggagatg	900
agccgggtgt ccatacgcat ccttgacgag ctggtgctgc ccttcagga gctgcagatc	960
gatgacaatg agtatgccta cctcaaagcc atcatcttct ttgaccaga tgccaagggg	1020
ctgagcgatc cagggagat caagcggctg cgttcccagg tgcaggtgag cttggaggac	1080
tacatcaacg accgccagta tgactcgcgt ggccgcttg gagagctgct gctgctgctg	1140
cccaccttgc agagcatcac ctggcagatg atcgagcaga tccagttcat caagetcttc	1200
ggcatggcca agattgacaa cctgttgca g gatgctgc tgggagggc cccagcgat	1260
gcaccccatg cccaccacc cctgcacct cacctgatgc agaacatat gggaaaccaac	1320
gtcatcgctt ccaacacaat gcccaactcac ctgagcaacg gacagatgtg tgagtggccc	1380
cgacccaggg gacagcgagc caccctgag accccacagc cctcaccgcc aggtggctca	1440
gggtctgagc cctataagct cctgcggga gccgtcgcca caatgctca gccctctct	1500
gccatcccc agccgacct caccaagcag gaagtattct agcaagcgc tggggcttgg	1560
gggtccact ggctcccc agccccctaa gagagcact ggtgatcag tggtcacggc	1620
aaaggaagac gtgatgccag gaccagtccc agagcaggaa tgggaaggat gaagggcccg	1680
agaacatggc ctaagggcca catcccactg ccaccctga cgcctgctc tggataaaa	1740
gactttgact tggggagacc tctactgct tggacaactt ttctcatgtt gaagccactg	1800
ccttcacctt caccttcate catgtccaac ccccgacttc atcccaaagg acagccgct	1860
ggagatgact tgaggcctta cttaaaccga gctcccttct tccctagcct ggtgcttctc	1920
ctctcctagc cctgtcatg gtgtccagac agagccctgt gaggtgggt ccaattgttg	1980
cacttggggc accttctcc tctctctgct gctgccccca cctctgctgc ctccctctgc	2040
tgtaacctg ctgagccatc ccgtcttctc caacaccacc tctccagagg ccaaggaggc	2100
cttgaaacg attccccag tcatctggg aacatgtgt aagcactgac tgggaccagg	2160
caccaggcag ggtctagaag gctgtggtga gggaaagcgc ctttctctc caaccaaac	2220
tcatcctct tcttcaggga cttgggtggg tacttgggtg aggatccctg aaggccttca	2280
accgagaaa acaaacccag gttggcgact gcaacaggaa cttggagtgg agaggaaaag	2340
catcagaaag aggcagacca tccaccaggc ctttgagaaa gggtagaatt ctggctggta	2400
gagcaggtga gatgggacat tccaaagaac agcctgagcc aaggcctagt ggtagtaaga	2460
atctagcaag aattgaggaa gaatgggtgt ggagaggat gatgaagaga gagaggcct	2520
gctggagagc atagggtctg gaacaccagg ctgaggtcct gatcagcttc aaggagtatg	2580
cagggagctg ggcttcaga aatgaacac agcagttctg cagaggacgg gaggtggaa	2640
gctgggaggt caggtgggtt ggatgatata atcggggtga gagtaatgag gcttggggct	2700
ggagaggaca agatgggtaa accctcacat cagagtgaca tccaggagga ataagctccc	2760
agggcctgct tcaagetctt ccttactccc aggcactgct ttaaggcctc tgacatgat	2820
catctcattt aatcctcctt tctctcctat taacctagag attgtttttg ttttttattc	2880
tctctctccc tccccgcct caccgcccc actcctcct aacctagaga ttgttacaga	2940
agtgaaatt gcgttctaag aggtgaagtg attttttttc tgaactcac acaactagga	3000
agtggctgag tcaggacttg aaccaggctc tccctggatc agaacaggag ctcttaacta	3060

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cagtggtga atagcttctc caaaggetcc ctgtgttctc accgtgatca agttgagggg 3120
cttcggctc ccttctacag cctcagaaac cagactcggt cttctgggaa cctgccccac 3180
teccaggacc aagattggcc tgaggctgca ctaaaattca cttagggtcg agcatcctgt 3240
ttgctgataa atattaagga gaattcatga ctcttgacag cttttctctc ttcactcccc 3300
aagtcaaggg gaggggtgga aggggtctgt ttcttggaag tcaggctcat ctggcctgtt 3360
ggcatggggg tgggacagtg tgcacagtgt gggggcaggg gagggctaag caggcctggg 3420
ttgagggct gctccggaga ccgtcactcc aggtgcattc tggaagcatt agaccccagg 3480
atggagcgac cagcatgtca tccatgtgga atcttggtgg ctttgaggac attctggaaa 3540
atgccactga ccagtgtgaa caaaagggat gtgttatggg gctggagggtg tgattaggta 3600
ggagggaaac tgttgaccg actcctgccc cctgctcaac actgaccctc ctgagtgggt 3660
ggaggcagt cccagtgcc cagaaatccc accattagtg attgtttttt atgagaaaga 3720
ggcgtggaga agtattgggg caatgtgtca gggaggaatc accacatccc tacggcagtc 3780
ccagccaagc cccaatccc agcggagact gtgcctgct cagagctccc aagccttccc 3840
ccaccacctc actcaagtgc cctgaaatc cctgccagac ggctcagcct ggtctgcggt 3900
aaggcagga ggctggaacc atttctgggc attgtgtgca ttcccactgt gttcctccac 3960
ctcctcctc cagcgttgct cagacctctg tcttgggaga aaggtgaga taagaatgct 4020
ccatggagt cctggggcaa cagtggcctc tcatgggaac aatctgttg agcagggggt 4080
cagttctctg ctgggaatct acccctttct ggaggagaaa ccattccac ctttaataact 4140
ttattgtaat gtgagaaaca caaaacaaag tttacttttt tgactctaag ctgacatgat 4200
attagaaat ctctcgtct cttttttttt tttttttttt tttttggcta cttgagttgt 4260
ggctcctaaa cataaaatct gatggacaaa cagagggttg ctggggggac aagcgtgggc 4320
acaatttccc caccaagaca ccctgatctt caggcgggtc tcaggagctt ctaaaaatcc 4380
gcatggctct cctgagagt gacagaggag aggagagggt cagaaatgaa cgctcttcta 4440
tttcttgta ttaccaagcc aattactttt gccaaatttt tctgtgatct gccctgatta 4500
agatgaattg tgaatttac atcaagcaat tatcaaagcg ggctgggtcc catcagaacg 4560
accacatct ttctgtgggt gtgaatgtca ttaggtcttg cgctgacccc tgagcccca 4620
tcaactgccg ctgatggggc aaagaacaa aaaacatttc ttactcttct gtgttttaac 4680
aaaagtatat aaaacaaat aatggcgca tatgttttct aaaaaaaaa aaaaaaa 4737

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&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 1628

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 3

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ggtttgaaag gaaggcagag agggcactgg gaggagcag tgggagggcg gagggcgggg 60
gccttcgggg tgggcgcccc gggtagggca ggtggccgcy gcgtggagge agggagaatg 120
cgactctcca aaacctcgt cgacatggac atggccgact acagtgtctc actggacca 180
gcctacacca ccctggaatt tgagaatgtg caggtgttga cgatgggcaa tgacacgtcc 240
ccatcagaag gcaccaacct caacgcgccc aacagcctgg gtgtcagcgc cctgtgtgcc 300
atctgctggg accgggccac gggcaaacac tacggtgcct cgagctgtga cggctgcaag 360

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ggcttcttcc ggaggagcgt gcggaagaac cacatgtact cctgcagatt tagccggcag 420
tgcgtggtgg acaaagacaa gaggaaccag tgccgctact gcaggctcaa gaaatgcttc 480
cgggctggca tgaagaagga agccgtccag aatgagcggg accggatcag cactcgaagg 540
tcaagctatg aggacagcag cctgcctccc atcaatgcgc tcctgcaggc ggaggctctg 600
tcccacaga tcacctcccc cgtctccggg atcaacggcg acattcgggc gaagaagatt 660
gccagcatcg cagatgtgtg tgagtccatg aaggagcagc tgctggttct cgttgagtgg 720
gccaagtaca tcccagcttt ctgcgagctc cccctggacg accaggtggc cctgctcaga 780
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agccgggtgt ccatacgcct ccttgacgag ctggtgctgc cctccagga gctgcagatc 960
gatgacaatg agtatgccta cctcaaagcc atcatcttct ttgaccaga tgccaagggg 1020
ctgagcgate cagggagat caagcggctg cgttcccagg tgcaggtgag cttggaggac 1080
tacetcaacg accgccagta tgactcgcgt ggccgctttg gagagctgct gctgctgctg 1140
cccaccttgc agagcatcac ctggcagatg atcgagcaga tccagttcat caagctcttc 1200
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cccctcagct ccttggcttc cccactgtgc cgtttgggc aagttgetta acctgtctgt 1380
gcctcagttt cctcaccaga aaaatgggaa caaggcaatg gtctatttgt tcaggcaccg 1440
agaacctagc acgtgccagt cactgttcta agtgctggca attcagaaa gaacaagatc 1500
tttgcctcgc gggagctgt gtgtgtgtga gtatgtatgg atcgctggat atctgtgat 1560
atgccctgat gtgcgtgcat gtgtatataa agcctcacat tttatgattt tgaataaac 1620
aggttaata 1628

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&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 4558

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 4

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ggccatggte agcgtgaacg cgcctcgg ggctccagtg gagagttctt acgacacgtc 60
cccacagaa ggcaccaacc tcaacgcgcc caacagcctg ggtgtcagcg ccctgtgtgc 120
catctgcggg gaccgggcca cgggcaaaca ctacgggtgcc tcgagctgtg acggctgcaa 180
gggcttcttc cggaggagcg tgccgaagaa ccacatgtac tcctgcagat ttagccggca 240
gtgcgtggtg gacaaagaca agaggaacca gtgccgttac tgcaggctca agaatgctt 300
ccgggctggc atgaagaagg aagccgtcca gaatgagcgg gaccggatca gactcgaag 360
gtcaagctat gaggacagca gcctgccctc catcaatgcg ctctgcagg cggaggctct 420
gtcccagacg atcacctccc ccgtctccgg gatcaacggc gacattcggg cgaagaagat 480
tgccagcadc gcagatgtgt tgagtccat gaaggagcag ctgctggttc tcgttgagtg 540
ggccaagtac atcccagctt tctgcgagct cccctggac gaccaggtgg ccctgctcag 600
agcccatgct ggcgagcacc tgctgctcgg agccaccaag agatccatgg tgttcaagga 660
cgtgctgctc ctaggcaatg actacattgt ccctcggcac tgcccggagc tggcggagat 720

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gagccgggtg tccatagcea tccttgacga gctggtgctg cccttcagag agctgcagat	780
cgatgacaat gagtatgcct acctcaaagc catcatcttc ttgacccag atgccaaggg	840
gctgagcgat ccaggaaga tcaagcggct gcgttcccag gtgcaggtga gcttgaggga	900
ctacatcaac gaccgccagt atgactcgcg tggccgcttt ggagagctgc tgctgctgct	960
gcccaccttg cagagcatca cctggcagat gatcgagcag atccagtcca tcaagctctt	1020
cgcatggcc aagattgaca acctgttgca ggagatgctg ctgggagggg cccccagcga	1080
tgcaccccat gcccaccacc ccctgcacc tcacctgatg caggaacata tgggaaccaa	1140
cgctcatggt gccaacacaa tgcccactca cctcagcaac ggacagatgt gtgagtggcc	1200
ccgaccaggg ggacagcgag ccaccctga gacccacag ccctcaccgc caggtggctc	1260
agggctgag ccctataagc tcctgocggg agccgtcgc acaatcgtca agccctctc	1320
tgccatcccc cagccgacca tcaccaagca ggaagtatc tagcaagccg ctggggcttg	1380
ggggctccac tggtccccc cagcccccta agagagcacc tggtgatcac gtggtcacgg	1440
caaaggaaga cgtgatgcca ggaccagtcc cagagcagga atgggaagga tgaagggccc	1500
gagaacatgg cctaagggcc acatcccact gccacccttg acgcccctg ctggataaca	1560
agacttgac ttggggagac ctctactgcc ttggacaact tttctcatgt tgaagcact	1620
gccttcacct tcacctcat ccattgcca cccccgactt catcccaaag gacagccgcc	1680
tgagatgac ttgagccctt acttaaaccc agctcccttc ttccctagcc tgggtcttct	1740
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gcacttgggg caecttgctc ctctctctgc tgetgcccc acctctgctg cctccctctg	1860
ctgtcacctt gctcagccat ccctcttct ccaacaccac ctctccagag gccaaaggag	1920
ccttggaac gattccccca gtcattctgg gaacatgttg taagcactga ctgggaccag	1980
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ctcatctcc ttcttcaggg acttgggtgg gtacttgggt gaggatccct gaaggccttc	2100
aacccgagaa aacaaaccca ggttggcgac tgcaacagga acttgagtg gagaggaaaa	2160
gcatcagaaa gaggcagacc atccaccag cctttgagaa agggtagaat tctggctggt	2220
agagcaggtg agatgggaca ttccaagaa cagcctgagc caaggcctag tggtagtaag	2280
aatctagcaa gaattgagga agaattggtg gggagagggg tgatgaagag agagagggcc	2340
tgctggagag catagggctt ggaacaccag gctgaggtcc tgatcagctt caaggagtat	2400
gcaggagct gggcttcag aaaatgaaca cagcagttct gcagaggacg ggaggctgga	2460
agctgggag tcaggtgggg ttgatgatat aatgcccgtg agagtaatga ggttggggc	2520
tgagaggac aagatgggta aaccctcaca tcagagtgac atccaggagg aataagctcc	2580
cagggcctgt ctcaagctct tccttactcc caggcactgt ctaaggcat ctgacatgca	2640
tcatctcatt taatctcccc ttctcccta ttaacctaga gattgttttt gttttttatt	2700
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aagtgaaat tgcgttctaa gaggtgaagt gatttttttt ctgaaactca cacaactagg	2820
aagtggctga gtcaggactt gaaccaggt ctccctggat cagaacagga gctcttaact	2880
acagtggctg aatagcttct ccaaaggctc cctgtgttct caccgtgatc aagttgaggg	2940
gcttcggct ccttctaca gcctcagaaa ccagactcgt tctctggga acctgccc	3000

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ctcccaggac caagattggc ctgaggetgc actaaaatc acttagggtc gagcatcctg 3060
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caagtcaagg ggagggtgg caggggtctg tttcctggaa gtcaggctca tctggcctgt 3180
tggeatgggg gtgggacagt gtgcacagtg tgggggcagg ggagggctaa gcaggcctgg 3240
gtttgagggc tgctccggag accgtcactc caggtgcatt ctggaagcat tagaccccag 3300
gatggagcga ccagcatgtc atccatgtgg aatcttggtg gctttgagga cattctggaa 3360
aatgccactg accagtgtga acaaaagga tgtgttatgg ggctggaggt gtgattaggt 3420
aggagggaaa ctggtggacc gactcctgcc cctgctcaa cactgacccc tctgagtgg 3480
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cctcctccct ccagcgttgc tcagacctc gtcttgggag aaagggtgag ataagaatgt 3840
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cacaatttcc ccaccaagac accctgatct tcaggcgggt ctcaggagct tctaaaaatc 4200
cgcatggctc tcctgagagt ggacagagga gaggagaggg tcagaaatga acgctcttct 4260
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gaccacatc tttctgtggg tgtgaaatgc attaggtctt gcgctgaccc ctgagcccc 4440
atcactgcc cctgatgggg caaagaaaca aaaaacattt cttactcttc tgtgttttaa 4500
caaaagttha taaaacaaaa taaatggcgc atatgttttc taaaaaaaaa aaaaaaaaa 4558

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&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 4528

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 5

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ggccatggtc agcgtgaacg cgcctcctcg ggctccagtg gagagtctt acgacacgtc 60
cccatcagaa ggcaccaacc tcaacgcgcc caacagcctg ggtgtcagcg cctgtgtg 120
catctcgggg gaccgggcca cgggcaaaaca ctacgggtgcc tcgagctgtg acggctgcaa 180
gggcttcttc cggaggagcg tgcggaagaa ccacatgtac tcctgcagat ttagccggca 240
gtgctgtggt gacaaagaca agaggaacca gtgccgttac tgcaggctca agaaatgctt 300
ccgggctggc atgaagaagg aagccgtcca gaatgagcgg gaccggatca gactcgaag 360
gtcaagctat gaggacagca gcctgccctc catcaatgcg ctctgcagg cggaggtcct 420
gtcccgacag atcacctccc ccgtctcgg gatcaacggc gacattcggg cgaagaagat 480

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tgccagcacc	gcagatgtgt	gtgagtcac	gaaggagcag	ctgctggttc	tcgttgagtg	540
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agcccatgct	ggcgagcacc	tgctgctcgg	agccaccaag	agatccatgg	tgttcaagga	660
cgtgctgctc	ctaggcaatg	actacattgt	ccctcggcac	tgcccggagc	tggcggagat	720
gagccgggtg	tccatacgc	tccttgacga	gctggtgctg	cccttccagg	agctgcagat	780
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gctgagcgat	ccaggaaga	tcaagcggct	gcgttcccag	gtgcaggtga	gcttgagga	900
ctacatcaac	gaccgccagt	atgactcgcg	tggccgcttt	ggagagctgc	tgctgctgct	960
gcccaccttg	cagagcatca	cctggcagat	gatcgagcag	atccagtcca	tcaagctctt	1020
cggcatggcc	aagattgaca	acctgttgca	ggagatgctg	ctgggagggt	ccccagcga	1080
tgacccccat	gcccaccacc	ccctgcaccc	tcacctgatg	caggaacata	tgggaaccaa	1140
cgctcatcgtt	gccaacacaa	tgcccactca	cctcagcaac	ggacagatgt	ccaccctga	1200
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gtacttgggt	gaggatccct	gaaggccttc	aaccggagaa	aacaaacca	ggttggcgac	2100
tgcaacagga	acttggagtg	gagaggaaaa	gcatacagaaa	gaggcagacc	atccaccagg	2160
ccttttagaa	agggtagaat	tctggctggt	agagcaggtg	agatgggaca	ttccaaagaa	2220
cagcctgagc	caaggcctag	tggtagtaag	aatctagcaa	gaattgagga	agaatggtgt	2280
gggagagggg	tgatgaagag	agagagggcc	tgctggagag	catagggtct	ggaacaccag	2340
gctgagggtc	tgatcagctt	caaggagtat	gcaggggagct	gggcttccag	aaaatgaaca	2400
cagcagttct	gcagaggagc	ggaggctgga	agctgggagg	tcagggtggg	tggatgatat	2460
aatgcgggtg	agagtaatga	ggcctggggc	tggagaggac	aagatgggta	aaccctcaca	2520
tcagagtgac	atccaggagg	aataagctcc	cagggcctgt	ctcaagctct	tccttactcc	2580
caggcactgt	cttaaggcat	ctgacatgca	tcctctcatt	taatcctccc	ttcctcccta	2640
ttaacctaga	gattgttttt	gttttttatt	ctcctcctcc	ctccccccc	tcacccgccc	2700
cactccctcc	taacctagag	attgttacag	aagctgaaat	tgctttctaa	gagggtgaagt	2760

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cctgtgttct caccgtgatc aagttgaggg gcttccggct cccttctaca gectcagaaa 2940
ccagactcgt tcttctggga accctgccca ctcccaggac caagattggc ctgaggctgc 3000
actaaaattc acttagggtc gagcatcctg tttgctgata aatattaagg agaattcatg 3060
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gtttactttt ttgactctaa gctgacatga tattagaaaa tctctcgctc tctttttttt 4020
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tgccaaattt ttctgtgatc tgccctgatt aagatgaatt gtgaaattta catcaagcaa 4320
ttatcaaage gggctgggct ccatcagaac gaccacatc tttctgtggg tgtgaatgtc 4380
attaggtctt gcgctgacct ctgagcccc atcactgccc cctgatgggg caaagaaca 4440
aaaaacattt ctactcttc tgtgttttaa caaaagtta taaaacaaa taaatggcgc 4500
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&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 1449

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 6

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ggccatggtc agcgtgaacg cgcccctcgg ggctccagtg gagagttctt acgacacgtc 60
cccctcagaa ggcaccaacc tcaacgcgcc caacagcctg ggtgtcagcg ccctgtgtgc 120
catctgcggg gaccgggcca cgggcaaaaca ctacggtgcc tcgagctgtg acggctgcaa 180
gggcttcttc cggaggagcg tgcggaagaa ccacatgtac tcctgcagat ttagccggca 240

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gtgcgtggtg gacaaagaca agaggaacca gtgccgctac tgcaggctca agaaatgctt 300
ccgggctggc atgaagaagg aagccgtcca gaatgagcgg gaccggatca gactcgaag 360
gtcaagctat gaggacagca gcttgcctc catcaatgcg ctctgcagg cggaggctct 420
gtcccagacag atcacctccc ccgtctccgg gatcaacggc gacattcggg cgaagaagat 480
tgccagcatc gcagatgtgt gtgagtccat gaaggagcag ctgctggttc tcgttgagt 540
ggccaagtac atcccagctt tctgcgagct cccctggac gaccagggtg ccttgcctcag 600
agcccatgct ggcgagcacc tgctgctcgg agccaccaag agatccatgg tgttaagga 660
cgtgtgctc ctaggcaatg actacattgt ccctcggcac tgcccggagc tggcggagat 720
gagccgggtg tccatagca tccttgacga gctgggtgctg cccttcagg agctgcagat 780
cgatgacaat gagtatgctt acctcaaagc catcatcttc tttgaccag atgccaaggg 840
gctgagcgat ccaggaaga tcaagcggct gcgttcccag gtgcaggatga gcttgaggga 900
ctacatcaac gaccgccagt atgactcggc tggccgcttt ggagagetgc tgetgctget 960
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tatgcccgta tgtgcgtgca tgtgtatata aagcctcaca ttttatgatt ttgaaataa 1440
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&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 464

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 7

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Met Arg Leu Ser Lys Thr Leu Val Asp Met Asp Met Ala Asp Tyr Ser
1           5           10           15
Ala Ala Leu Asp Pro Ala Tyr Thr Thr Leu Glu Phe Glu Asn Val Gln
20           25           30
Val Leu Thr Met Gly Asn Asp Thr Ser Pro Ser Glu Gly Thr Asn Leu
35           40           45
Asn Ala Pro Asn Ser Leu Gly Val Ser Ala Leu Cys Ala Ile Cys Gly
50           55           60
Asp Arg Ala Thr Gly Lys His Tyr Gly Ala Ser Ser Cys Asp Gly Cys
65           70           75           80
Lys Gly Phe Phe Arg Arg Ser Val Arg Lys Asn His Met Tyr Ser Cys
85           90           95
Arg Phe Ser Arg Gln Cys Val Val Asp Lys Asp Lys Arg Asn Gln Cys
100          105          110
Arg Tyr Cys Arg Leu Lys Lys Cys Phe Arg Ala Gly Met Lys Lys Glu
115          120          125
Ala Val Gln Asn Glu Arg Asp Arg Ile Ser Thr Arg Arg Ser Ser Tyr
130          135          140

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Glu Asp Ser Ser Leu Pro Ser Ile Asn Ala Leu Leu Gln Ala Glu Val  
 145 150 155 160  
 Leu Ser Arg Gln Ile Thr Ser Pro Val Ser Gly Ile Asn Gly Asp Ile  
 165 170 175  
 Arg Ala Lys Lys Ile Ala Ser Ile Ala Asp Val Cys Glu Ser Met Lys  
 180 185 190  
 Glu Gln Leu Leu Val Leu Val Glu Trp Ala Lys Tyr Ile Pro Ala Phe  
 195 200 205  
 Cys Glu Leu Pro Leu Asp Asp Gln Val Ala Leu Leu Arg Ala His Ala  
 210 215 220  
 Gly Glu His Leu Leu Leu Gly Ala Thr Lys Arg Ser Met Val Phe Lys  
 225 230 235 240  
 Asp Val Leu Leu Leu Gly Asn Asp Tyr Ile Val Pro Arg His Cys Pro  
 245 250 255  
 Glu Leu Ala Glu Met Ser Arg Val Ser Ile Arg Ile Leu Asp Glu Leu  
 260 265 270  
 Val Leu Pro Phe Gln Glu Leu Gln Ile Asp Asp Asn Glu Tyr Ala Tyr  
 275 280 285  
 Leu Lys Ala Ile Ile Phe Phe Asp Pro Asp Ala Lys Gly Leu Ser Asp  
 290 295 300  
 Pro Gly Lys Ile Lys Arg Leu Arg Ser Gln Val Gln Val Ser Leu Glu  
 305 310 315 320  
 Asp Tyr Ile Asn Asp Arg Gln Tyr Asp Ser Arg Gly Arg Phe Gly Glu  
 325 330 335  
 Leu Leu Leu Leu Leu Pro Thr Leu Gln Ser Ile Thr Trp Gln Met Ile  
 340 345 350  
 Glu Gln Ile Gln Phe Ile Lys Leu Phe Gly Met Ala Lys Ile Asp Asn  
 355 360 365  
 Leu Leu Gln Glu Met Leu Leu Gly Gly Ser Pro Ser Asp Ala Pro His  
 370 375 380  
 Ala His His Pro Leu His Pro His Leu Met Gln Glu His Met Gly Thr  
 385 390 395 400  
 Asn Val Ile Val Ala Asn Thr Met Pro Thr His Leu Ser Asn Gly Gln  
 405 410 415  
 Met Ser Thr Pro Glu Thr Pro Gln Pro Ser Pro Pro Gly Gly Ser Gly  
 420 425 430  
 Ser Glu Pro Tyr Lys Leu Leu Pro Gly Ala Val Ala Thr Ile Val Lys  
 435 440 445  
 Pro Leu Ser Ala Ile Pro Gln Pro Thr Ile Thr Lys Gln Glu Val Ile  
 450 455 460

<210> SEQ ID NO 8  
 <211> LENGTH: 474  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Arg Leu Ser Lys Thr Leu Val Asp Met Asp Met Ala Asp Tyr Ser  
 1 5 10 15  
 Ala Ala Leu Asp Pro Ala Tyr Thr Thr Leu Glu Phe Glu Asn Val Gln  
 20 25 30  
 Val Leu Thr Met Gly Asn Asp Thr Ser Pro Ser Glu Gly Thr Asn Leu

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35			40			45									
Asn	Ala	Pro	Asn	Ser	Leu	Gly	Val	Ser	Ala	Leu	Cys	Ala	Ile	Cys	Gly
50					55						60				
Asp	Arg	Ala	Thr	Gly	Lys	His	Tyr	Gly	Ala	Ser	Ser	Cys	Asp	Gly	Cys
65					70					75				80	
Lys	Gly	Phe	Phe	Arg	Arg	Ser	Val	Arg	Lys	Asn	His	Met	Tyr	Ser	Cys
			85						90					95	
Arg	Phe	Ser	Arg	Gln	Cys	Val	Val	Asp	Lys	Asp	Lys	Arg	Asn	Gln	Cys
			100					105						110	
Arg	Tyr	Cys	Arg	Leu	Lys	Lys	Cys	Phe	Arg	Ala	Gly	Met	Lys	Lys	Glu
		115					120					125			
Ala	Val	Gln	Asn	Glu	Arg	Asp	Arg	Ile	Ser	Thr	Arg	Arg	Ser	Ser	Tyr
		130					135					140			
Glu	Asp	Ser	Ser	Leu	Pro	Ser	Ile	Asn	Ala	Leu	Leu	Gln	Ala	Glu	Val
145					150					155				160	
Leu	Ser	Arg	Gln	Ile	Thr	Ser	Pro	Val	Ser	Gly	Ile	Asn	Gly	Asp	Ile
			165						170					175	
Arg	Ala	Lys	Lys	Ile	Ala	Ser	Ile	Ala	Asp	Val	Cys	Glu	Ser	Met	Lys
			180					185						190	
Glu	Gln	Leu	Leu	Val	Leu	Val	Glu	Trp	Ala	Lys	Tyr	Ile	Pro	Ala	Phe
		195					200					205			
Cys	Glu	Leu	Pro	Leu	Asp	Asp	Gln	Val	Ala	Leu	Leu	Arg	Ala	His	Ala
		210					215				220				
Gly	Glu	His	Leu	Leu	Leu	Gly	Ala	Thr	Lys	Arg	Ser	Met	Val	Phe	Lys
225					230					235				240	
Asp	Val	Leu	Leu	Leu	Gly	Asn	Asp	Tyr	Ile	Val	Pro	Arg	His	Cys	Pro
			245						250					255	
Glu	Leu	Ala	Glu	Met	Ser	Arg	Val	Ser	Ile	Arg	Ile	Leu	Asp	Glu	Leu
			260					265						270	
Val	Leu	Pro	Phe	Gln	Glu	Leu	Gln	Ile	Asp	Asp	Asn	Glu	Tyr	Ala	Tyr
		275					280					285			
Leu	Lys	Ala	Ile	Ile	Phe	Phe	Asp	Pro	Asp	Ala	Lys	Gly	Leu	Ser	Asp
		290					295				300				
Pro	Gly	Lys	Ile	Lys	Arg	Leu	Arg	Ser	Gln	Val	Gln	Val	Ser	Leu	Glu
305					310					315				320	
Asp	Tyr	Ile	Asn	Asp	Arg	Gln	Tyr	Asp	Ser	Arg	Gly	Arg	Phe	Gly	Glu
			325						330					335	
Leu	Leu	Leu	Leu	Leu	Pro	Thr	Leu	Gln	Ser	Ile	Thr	Trp	Gln	Met	Ile
			340					345						350	
Glu	Gln	Ile	Gln	Phe	Ile	Lys	Leu	Phe	Gly	Met	Ala	Lys	Ile	Asp	Asn
		355					360					365			
Leu	Leu	Gln	Glu	Met	Leu	Leu	Gly	Gly	Ser	Pro	Ser	Asp	Ala	Pro	His
		370					375				380				
Ala	His	His	Pro	Leu	His	Pro	His	Leu	Met	Gln	Glu	His	Met	Gly	Thr
385					390					395				400	
Asn	Val	Ile	Val	Ala	Asn	Thr	Met	Pro	Thr	His	Leu	Ser	Asn	Gly	Gln
			405						410					415	
Met	Cys	Glu	Trp	Pro	Arg	Pro	Arg	Gly	Gln	Ala	Ala	Thr	Pro	Glu	Thr
			420					425						430	
Pro	Gln	Pro	Ser	Pro	Pro	Gly	Gly	Ser	Gly	Ser	Glu	Pro	Tyr	Lys	Leu
		435					440					445			

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Leu Pro Gly Ala Val Ala Thr Ile Val Lys Pro Leu Ser Ala Ile Pro  
 450 455 460

Gln Pro Thr Ile Thr Lys Gln Glu Val Ile  
 465 470

<210> SEQ ID NO 9  
 <211> LENGTH: 417  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Arg Leu Ser Lys Thr Leu Val Asp Met Asp Met Ala Asp Tyr Ser  
 1 5 10 15

Ala Ala Leu Asp Pro Ala Tyr Thr Thr Leu Glu Phe Glu Asn Val Gln  
 20 25 30

Val Leu Thr Met Gly Asn Asp Thr Ser Pro Ser Glu Gly Thr Asn Leu  
 35 40 45

Asn Ala Pro Asn Ser Leu Gly Val Ser Ala Leu Cys Ala Ile Cys Gly  
 50 55 60

Asp Arg Ala Thr Gly Lys His Tyr Gly Ala Ser Ser Cys Asp Gly Cys  
 65 70 75 80

Lys Gly Phe Phe Arg Arg Ser Val Arg Lys Asn His Met Tyr Ser Cys  
 85 90 95

Arg Phe Ser Arg Gln Cys Val Val Asp Lys Asp Lys Arg Asn Gln Cys  
 100 105 110

Arg Tyr Cys Arg Leu Lys Lys Cys Phe Arg Ala Gly Met Lys Lys Glu  
 115 120 125

Ala Val Gln Asn Glu Arg Asp Arg Ile Ser Thr Arg Arg Ser Ser Tyr  
 130 135 140

Glu Asp Ser Ser Leu Pro Ser Ile Asn Ala Leu Leu Gln Ala Glu Val  
 145 150 155 160

Leu Ser Arg Gln Ile Thr Ser Pro Val Ser Gly Ile Asn Gly Asp Ile  
 165 170 175

Arg Ala Lys Lys Ile Ala Ser Ile Ala Asp Val Cys Glu Ser Met Lys  
 180 185 190

Glu Gln Leu Leu Val Leu Val Glu Trp Ala Lys Tyr Ile Pro Ala Phe  
 195 200 205

Cys Glu Leu Pro Leu Asp Asp Gln Val Ala Leu Leu Arg Ala His Ala  
 210 215 220

Gly Glu His Leu Leu Leu Gly Ala Thr Lys Arg Ser Met Val Phe Lys  
 225 230 235 240

Asp Val Leu Leu Leu Gly Asn Asp Tyr Ile Val Pro Arg His Cys Pro  
 245 250 255

Glu Leu Ala Glu Met Ser Arg Val Ser Ile Arg Ile Leu Asp Glu Leu  
 260 265 270

Val Leu Pro Phe Gln Glu Leu Gln Ile Asp Asp Asn Glu Tyr Ala Tyr  
 275 280 285

Leu Lys Ala Ile Ile Phe Phe Asp Pro Asp Ala Lys Gly Leu Ser Asp  
 290 295 300

Pro Gly Lys Ile Lys Arg Leu Arg Ser Gln Val Gln Val Ser Leu Glu  
 305 310 315 320

Asp Tyr Ile Asn Asp Arg Gln Tyr Asp Ser Arg Gly Arg Phe Gly Glu





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260					265					270					
Phe	Asp	Pro	Asp	Ala	Lys	Gly	Leu	Ser	Asp	Pro	Gly	Lys	Ile	Lys	Arg
	275						280					285			
Leu	Arg	Ser	Gln	Val	Gln	Val	Ser	Leu	Glu	Asp	Tyr	Ile	Asn	Asp	Arg
	290					295					300				
Gln	Tyr	Asp	Ser	Arg	Gly	Arg	Phe	Gly	Glu	Leu	Leu	Leu	Leu	Leu	Pro
305					310					315					320
Thr	Leu	Gln	Ser	Ile	Thr	Trp	Gln	Met	Ile	Glu	Gln	Ile	Gln	Phe	Ile
			325						330					335	
Lys	Leu	Phe	Gly	Met	Ala	Lys	Ile	Asp	Asn	Leu	Leu	Gln	Glu	Met	Leu
		340						345					350		
Leu	Gly	Gly	Ser	Pro	Ser	Asp	Ala	Pro	His	Ala	His	His	Pro	Leu	His
		355					360						365		
Pro	His	Leu	Met	Gln	Glu	His	Met	Gly	Thr	Asn	Val	Ile	Val	Ala	Asn
	370					375					380				
Thr	Met	Pro	Thr	His	Leu	Ser	Asn	Gly	Gln	Met	Cys	Glu	Trp	Pro	Arg
385					390					395					400
Pro	Arg	Gly	Gln	Ala	Ala	Thr	Pro	Glu	Thr	Pro	Gln	Pro	Ser	Pro	Pro
				405					410					415	
Gly	Gly	Ser	Gly	Ser	Glu	Pro	Tyr	Lys	Leu	Leu	Pro	Gly	Ala	Val	Ala
			420					425					430		
Thr	Ile	Val	Lys	Pro	Leu	Ser	Ala	Ile	Pro	Gln	Pro	Thr	Ile	Thr	Lys
		435					440						445		
Gln	Glu	Val	Ile												
	450														

&lt;210&gt; SEQ ID NO 11

&lt;211&gt; LENGTH: 442

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 11

Met	Val	Ser	Val	Asn	Ala	Pro	Leu	Gly	Ala	Pro	Val	Glu	Ser	Ser	Tyr
1				5					10					15	
Asp	Thr	Ser	Pro	Ser	Glu	Gly	Thr	Asn	Leu	Asn	Ala	Pro	Asn	Ser	Leu
		20						25					30		
Gly	Val	Ser	Ala	Leu	Cys	Ala	Ile	Cys	Gly	Asp	Arg	Ala	Thr	Gly	Lys
		35					40					45			
His	Tyr	Gly	Ala	Ser	Ser	Cys	Asp	Gly	Cys	Lys	Gly	Phe	Phe	Arg	Arg
	50					55					60				
Ser	Val	Arg	Lys	Asn	His	Met	Tyr	Ser	Cys	Arg	Phe	Ser	Arg	Gln	Cys
65					70						75			80	
Val	Val	Asp	Lys	Asp	Lys	Arg	Asn	Gln	Cys	Arg	Tyr	Cys	Arg	Leu	Lys
			85						90					95	
Lys	Cys	Phe	Arg	Ala	Gly	Met	Lys	Lys	Glu	Ala	Val	Gln	Asn	Glu	Arg
		100						105					110		
Asp	Arg	Ile	Ser	Thr	Arg	Arg	Ser	Ser	Tyr	Glu	Asp	Ser	Ser	Leu	Pro
		115					120						125		
Ser	Ile	Asn	Ala	Leu	Leu	Gln	Ala	Glu	Val	Leu	Ser	Arg	Gln	Ile	Thr
	130					135						140			
Ser	Pro	Val	Ser	Gly	Ile	Asn	Gly	Asp	Ile	Arg	Ala	Lys	Lys	Ile	Ala
145					150						155				160

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Ser Ile Ala Asp Val Cys Glu Ser Met Lys Glu Gln Leu Leu Val Leu
      165                               170                               175

Val Glu Trp Ala Lys Tyr Ile Pro Ala Phe Cys Glu Leu Pro Leu Asp
      180                               185                               190

Asp Gln Val Ala Leu Leu Arg Ala His Ala Gly Glu His Leu Leu Leu
      195                               200                               205

Gly Ala Thr Lys Arg Ser Met Val Phe Lys Asp Val Leu Leu Leu Gly
      210                               215                               220

Asn Asp Tyr Ile Val Pro Arg His Cys Pro Glu Leu Ala Glu Met Ser
      225                               230                               235                               240

Arg Val Ser Ile Arg Ile Leu Asp Glu Leu Val Leu Pro Phe Gln Glu
      245                               250                               255

Leu Gln Ile Asp Asp Asn Glu Tyr Ala Tyr Leu Lys Ala Ile Ile Phe
      260                               265                               270

Phe Asp Pro Asp Ala Lys Gly Leu Ser Asp Pro Gly Lys Ile Lys Arg
      275                               280                               285

Leu Arg Ser Gln Val Gln Val Ser Leu Glu Asp Tyr Ile Asn Asp Arg
      290                               295                               300

Gln Tyr Asp Ser Arg Gly Arg Phe Gly Glu Leu Leu Leu Leu Pro
      305                               310                               315                               320

Thr Leu Gln Ser Ile Thr Trp Gln Met Ile Glu Gln Ile Gln Phe Ile
      325                               330                               335

Lys Leu Phe Gly Met Ala Lys Ile Asp Asn Leu Leu Gln Glu Met Leu
      340                               345                               350

Leu Gly Gly Ser Pro Ser Asp Ala Pro His Ala His His Pro Leu His
      355                               360                               365

Pro His Leu Met Gln Glu His Met Gly Thr Asn Val Ile Val Ala Asn
      370                               375                               380

Thr Met Pro Thr His Leu Ser Asn Gly Gln Met Ser Thr Pro Glu Thr
      385                               390                               395                               400

Pro Gln Pro Ser Pro Pro Gly Gly Ser Gly Ser Glu Pro Tyr Lys Leu
      405                               410                               415

Leu Pro Gly Ala Val Ala Thr Ile Val Lys Pro Leu Ser Ala Ile Pro
      420                               425                               430

Gln Pro Thr Ile Thr Lys Gln Glu Val Ile
      435                               440

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&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 395

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 12

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Met Val Ser Val Asn Ala Pro Leu Gly Ala Pro Val Glu Ser Ser Tyr
  1           5           10           15

Asp Thr Ser Pro Ser Glu Gly Thr Asn Leu Asn Ala Pro Asn Ser Leu
  20           25           30

Gly Val Ser Ala Leu Cys Ala Ile Cys Gly Asp Arg Ala Thr Gly Lys
  35           40           45

His Tyr Gly Ala Ser Ser Cys Asp Gly Cys Lys Gly Phe Phe Arg Arg
  50           55           60

Ser Val Arg Lys Asn His Met Tyr Ser Cys Arg Phe Ser Arg Gln Cys
  65           70           75           80

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<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 14

acagcttgag gctccgtagt gttt 24

<210> SEQ ID NO 15  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 15

tctagagggc ctggagtcca 20

<210> SEQ ID NO 16  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 16

tcactgtctg gcctgttgag 20

<210> SEQ ID NO 17  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 17

ttcaatgtcc ctgccatgta 20

<210> SEQ ID NO 18  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 18

catctccaga gtccagcaca 20

<210> SEQ ID NO 19  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 19

ttgctgacag gatgcagaag 20

<210> SEQ ID NO 20  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

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<400> SEQUENCE: 20  
cagtgaggcc aggatagagc 20

<210> SEQ ID NO 21  
<211> LENGTH: 21  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 21  
gcccagcata cgaagaaaac a 21

<210> SEQ ID NO 22  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 22  
tctctttgtc tggaagcatt cct 23

<210> SEQ ID NO 23  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 23  
tctgcacact cccagacaag 20

<210> SEQ ID NO 24  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 24  
agtcacccat caccgtcttc 20

<210> SEQ ID NO 25  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 25  
ggcaaggatt tgatggagaa 20

<210> SEQ ID NO 26  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 26  
cccagttctc tggacaaagg 20

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<210> SEQ ID NO 27  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
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<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 27

acatggaaca agcctccaag 20

<210> SEQ ID NO 28  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 28

tggccaccac agctatatca 20

<210> SEQ ID NO 29  
<211> LENGTH: 20  
<212> TYPE: DNA  
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<220> FEATURE:  
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<400> SEQUENCE: 29

tgaaccgctt ctgggattac 20

<210> SEQ ID NO 30  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 30

tgtgtgactt gggagctctg 20

<210> SEQ ID NO 31  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 31

ggaggatctg agggaagacc 20

<210> SEQ ID NO 32  
<211> LENGTH: 20  
<212> TYPE: DNA  
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<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 32

ggcagcagat cctttcagag 20

<210> SEQ ID NO 33  
<211> LENGTH: 19

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<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 33

tttgggtggca cacagcttg 19

<210> SEQ ID NO 34  
<211> LENGTH: 22  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 34

atggaataca cctgcgtaac gg 22

<210> SEQ ID NO 35  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 35

gaaggcctaa gcacaacagc 20

<210> SEQ ID NO 36  
<211> LENGTH: 20  
<212> TYPE: DNA  
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<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 36

aagcacttga ccttggtagc 20

<210> SEQ ID NO 37  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 37

gccaagaagt cggtggataa 20

<210> SEQ ID NO 38  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 38

aacacettct getgctctc 20

<210> SEQ ID NO 39  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide



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<400> SEQUENCE: 39

gctcagatct ttgcaattc 20

<210> SEQ ID NO 40

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 40

cgcttcgatt tctgtctcc 19

<210> SEQ ID NO 41

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 41

tggtcctcaa ggtttccaag 20

<210> SEQ ID NO 42

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 42

ttaccagctt ccccatcatc 20

<210> SEQ ID NO 43

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 43

tgcagaaaat ggttgctttg 20

<210> SEQ ID NO 44

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 44

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<210> SEQ ID NO 45

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 45

atggagatca cagcccagtc 20

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<210> SEQ ID NO 46  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 46  
cgatctcctc ctgcagtttc 20

<210> SEQ ID NO 47  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 47  
tgagaatggt gaatgccagt 20

<210> SEQ ID NO 48  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 48  
gagtcattctc cgccttcac 20

<210> SEQ ID NO 49  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 49  
taaccagga ggaagcacac 20

<210> SEQ ID NO 50  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 50  
cttcattgca ctccctctcc 20

<210> SEQ ID NO 51  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 51  
ccatccgtea ttctctctcc 20

<210> SEQ ID NO 52  
<211> LENGTH: 20

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<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 52

tcgaacatgt tgccagagtc 20

<210> SEQ ID NO 53  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 53

gacgtctcca ggtctcaacc 20

<210> SEQ ID NO 54  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 54

caccctgtgt agtgaacgtg 20

<210> SEQ ID NO 55  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 55

ctcaccctca gctggatagg 20

<210> SEQ ID NO 56  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 56

ccctttggag tagctgcttg 20

<210> SEQ ID NO 57  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 57

aatgcaatcc gttttggaag 20

<210> SEQ ID NO 58  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

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<400> SEQUENCE: 58

gccagagatt tgaggtctgc 20

<210> SEQ ID NO 59

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 59

catcgtggac aacatgaagg 20

<210> SEQ ID NO 60

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

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<210> SEQ ID NO 61

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 61

ttgaagggtc tggaagagga 20

<210> SEQ ID NO 62

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 62

tcacgaaca agcagagcag 20

<210> SEQ ID NO 63

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 63

gcatctgacc cgagtctctc 20

<210> SEQ ID NO 64

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 64

gaatggcctg agcttttcag 20

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<210> SEQ ID NO 65  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 65

aatggagatg gcaaagagga 20

<210> SEQ ID NO 66  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 66

gagagccgaa cagttggaag 20

<210> SEQ ID NO 67  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 67

ggactctcca cctgcaagac 20

<210> SEQ ID NO 68  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 68

gactggcgag ccttagtttg 20

<210> SEQ ID NO 69  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 69

ggttccttg cataatctga 20

<210> SEQ ID NO 70  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 70

atggctgaac agggaaacac 20

<210> SEQ ID NO 71  
<211> LENGTH: 20

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<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 71

tggacgttg aggaaagaag 20

<210> SEQ ID NO 72  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 72

tcccagggca caataaagtc 20

<210> SEQ ID NO 73  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 73

tgtgcaactt tgtggagagg 20

<210> SEQ ID NO 74  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 74

aattgcaacc caggtggtag 20

<210> SEQ ID NO 75  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 75

tcgtactgga aggctcttgg 20

<210> SEQ ID NO 76  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 76

gtaggagtac gggctgagca 20

<210> SEQ ID NO 77  
<211> LENGTH: 37  
<212> TYPE: DNA  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic oligonucleotide

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<400> SEQUENCE: 77

ggtttttgag ggtgagggtg agggtgaggg tgagggt 37

<210> SEQ ID NO 78

<211> LENGTH: 39

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 78

tcccgactat ccctatccct atccctatcc ctatcccta 39

<210> SEQ ID NO 79

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 79

cagcaagtgg gaaggtgtaa tcc 23

<210> SEQ ID NO 80

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 80

cccattctat catcaacggg taaaa 25

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1. A method of treating or preventing hepatic failure or cirrhosis in a subject, comprising administering a therapeutically effective amount of a pharmaceutical composition comprising an HNF4 $\alpha$  agonist to the subject.

2. The method of claim 1, wherein the agonist is a small molecule.

3. The method of claim 1, wherein the agonist is an HNF4 $\alpha$  nucleic acid molecule.

4. The method of claim 3, wherein the HNF4 $\alpha$  nucleic acid molecule is expressed by a therapeutic vector.

5. The method of claim 4, wherein a hepatocyte from the subject is transduced with the therapeutic vector in vitro and reintroduced into the subject.

6. The method of claim 1, wherein the subject is a human.

7. The method of claim 1, wherein the pharmaceutical composition comprises at least one of a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative, and adjuvant.

8. The method of claim 1, wherein the HNF4 $\alpha$  agonist is administered in conjunction with one or more additional agents for the treatment or prevention of hepatic failure or cirrhosis.

\* \* \* \* \*