Claims

1. A gene therapy vector comprising an expression cassette, the expression cassette comprising a synthetic liver-specific inducible promoter operably linked to a gene, the synthetic liver-specific inducible promoter comprising a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of constitutive androstane receptor (CAR) and retinoid X receptor (RXR),~~.~~

~~2. The gene therapy vector of claim 1~~ wherein the CRE that is capable of being bound and activated by a CAR-RXR heterodimer ~~of~~ comprises or consists of ~~SEQ ID NO: 1 or SEQ ID NO: 2, or~~ a functional variant of a PB-responsive enhancer module (PBREM) sequence ~~SEQ ID NO: 1 or SEQ ID NO: 2~~,~~.~~

~~3. The gene therapy vector of claim 1 or 2 wherein the functional variant of SEQ ID NO: 1 or SEQ ID NO: 2 comprises an NR1 motif from PBREM, preferably the sequence TGTACTTTCCTGACCN (SEQ ID NO: 20) or a sequence that varies at no more than 2, preferably no more than 1, nucleotide position from the sequence TGTACTTTCCTGACCN (SEQ ID NO: 20).~~

~~4. The gene therapy vector of any preceding claim wherein the functional variant of SEQ ID NO: 1 or SEQ ID NO: 2~~which comprises one of the following sequences:

- [TGTACTTTCCTGACCN-S-]$_n$ (SEQ ID NO: 29);
- [CTGTACTTTCCTGACCN-S-]$_n$ (SEQ ID NO: 30); and
- [NCTGTACTTTCCTGACCNTG-S-]$_n$ (SEQ ID NO: 31),

wherein S is an optional spacer and n is from 1 to 5, optionally from 2 to 4, and preferably 3.

2~~5~~. The gene therapy vector of ~~any preceding~~ claim 1 wherein the functional variant of a PBREM sequence~~SEQ ID NO: 1 or SEQ ID NO: 2~~ comprises one of the following sequences:

- TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN (SEQ ID NO: 32);
- TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN-S-TGTACTTTCCTGACCN (SEQ ID NO: 33);
- CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN (SEQ ID NO: 34);
- CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN-S-CTGTACTTTCCTGACCN (SEQ ID NO: 35); and
- NCTGTACTTTCCTGACCNTG-S-NCTGTACTTTCCTGACCNTG (SEQ ID NO: 36); and
- NCTGTACTTTCCTGACCNTG-S-NCTGTACTTTCCTGACCNTG-S-NCTGTACTTTCCTGACCNTG (SEQ ID NO: 37),

wherein S is an optional spacer.

3~~6~~. The gene therapy vector of ~~any preceding~~ claim 1 or claim 2 wherein the functional variant of a PBREM sequence~~SEQ ID NO: 1 or SEQ ID NO: 2~~ comprises one of the following sequences:

- TCTGTACTTTCCTGACCTTG-S-TCTGTACTTTCCTGACCTTG-S-TCTGTACTTTCCTGACCTTG (SEQ ID NO: 38); or
- ACTGTACTTTCCTGACCCTG-S-ACTGTACTTTCCTGACCCTG-S-ACTGTACTTTCCTGACCCTG (SEQ ID NO: 39),

wherein S is an optional spacer.

~~7. The gene therapy vector of any preceding claim wherein the functional variant of SEQ ID NO: 1 or SEQ ID NO: 2 comprises a sequence that is at least 60% identical to either SEQ ID NO: 1 or SEQ ID NO: 2, preferably at least 65%, 75%, 80%, 85%, 90%, 95% or 99 % identical to either SEQ ID NO: 1 or SEQ ID NO: 2.~~

~~8. The gene therapy vector of any preceding claim wherein the functional variant of SEQ ID NO: 1 or SEQ ID NO: 2 comprises a sequence that is at least 90% identical, preferably at least 95% identical, and more preferably perfectly identical to SEQ ID NO: 1 or SEQ ID NO: 2 in the region spanning nucleotides 3 to 18 (preferably the region spanning nucleotides 1 to 20), and at least 70%, 80%, 85%, 90%, 95% or 99% identical across the remainder of SEQ ID NO: 1 or SEQ ID NO: 2.~~

4~~9~~. The gene therapy vector of any preceding claim wherein the CRE that is capable of being bound and activated by a CAR-RXR heterodimer~~functional variant of SEQ ID NO: 1 or SEQ ID NO: 2~~ comprises the following sequence:

NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN (SEQ ID NO: 41)~~, or a sequence at least 90%, preferably 95%, more preferably 99% identical thereto~~.

5~~10~~. The gene therapy vector of any preceding claim comprising a plurality of CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR.

6~~11~~. The gene therapy vector of any preceding claim comprising 2 or 3 CREs that are each capable of being bound and activated by a heterodimer of CAR and RXR.

7~~12~~. The gene therapy vector of any preceding claim comprising a synthetic liver-specific inducible promoter comprising a cis-regulatory module (CRM) comprising or consisting of one of the following sequences:

- NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN-S-NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN (SEQ ID NO: 42); and
- NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN-S-NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN-S-NCTGTACTTTCCTGACCNTGNNNNNGTGNCANCATNNACTTNCCTGANNCN (SEQ ID NO: 43), wherein S is an optional spacer.

8~~13~~. The gene therapy vector of any preceding claim comprising a synthetic liver-specific inducible promoter comprising a CRM comprising or consisting of one of the following sequences:

- TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-S-TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC (SEQ ID NO: 44);

- TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-S-TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC-S-TCTGTACTTTCCTGACCTTGGCACAGTGCCACCATCAACTTGCCTGACACC (SEQ ID NO: 45);
- ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-S-ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-S-ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA (SEQ ID NO: 46); and
- ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA-S-ACTGTACTTTCCTGACCCTGAAGAGGTGGCAGCATGGACTTTCCTGAACCA (SEQ ID NO: 47),

wherein S is an optional spacer.

9~~14~~. The gene therapy vector of any preceding claim which comprises ~~substantially~~ no nucleic acid sequences that would lead to constitutive expression or expression in non-liver cells.

10~~15~~. The gene therapy vector of any preceding claim which comprises a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR operably linked to a minimal promoter or proximal promoter.

11~~16~~. The gene therapy vector of claim 10~~15~~ wherein the minimal promoter is HSV thymidine kinase minimal promoter (MinTK), CMV minimal promoter (CMVmp) or SV40 minimal promoter (SV40mp).

12~~17~~. The gene therapy vector of any preceding claim which comprises a sequence according any one of SEQ ID NOs: 7 to 18 and 59 to 71, or functional variant of any one thereof.

13~~18~~. The gene therapy vector of any preceding claim which comprises a sequence according to SEQ ID NO: 7, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70 or SEQ ID NO: 71, or functional variant of any one thereof.

14~~19~~. The gene therapy vector of any preceding claim wherein the synthetic liver-specific expression cassette comprises sequences providing or coding for one or more of, and preferably all of, a ribosomal binding site, a start codon, a stop codon, a transcription termination sequence, a nucleic acid encoding a posttranscriptional regulatory element and/or a polyA element.

15~~20~~. The gene therapy vector of any preceding claim wherein the gene encodes a protein or RNA.

16~~21~~. The gene therapy vector of any preceding claim wherein the gene encodes a therapeutic expression product, preferably a therapeutic protein suitable for use in treating a disease or condition associated with aberrant gene expression in the liver.

17~~22~~. The gene therapy vector of any preceding claim wherein the gene encodes a site-specific nuclease, such as a meganuclease, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), or the clustered regularly interspaced short palindromic repeats system (CRISPR-Cas).

18~~23~~. The gene therapy vector of any preceding claim which is a viral vector, such as a retroviral, lentiviral, adenoviral, or adeno-associated viral (AAV) vector.

19~~24~~. The gene therapy vector of any preceding claim which is a plasmid.

20~~25~~. An expression cassette comprising a synthetic liver-specific inducible promoter operably linked to a gene, the synthetic liver-specific inducible promoter comprising a CRE that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the synthetic liver-specific inducible promoter is in accordance with any one of claims 1 to 19.

21~~26~~. The expression cassette of claim 20~~25~~ wherein the gene is not a reporter gene.

22~~27~~. The expression cassette of claim 20~~25~~ or 21~~26~~ wherein the gene encodes a therapeutic expression product.

23~~28~~. A synthetic liver-specific inducible promoter comprising a sequence according any one of SEQ ID NOs: 7 to 18 and 59 to 71, or functional variant of any one thereof, wherein the synthetic liver-specific inducible promoter is in accordance with any one of claims 1 to 19.

24~~29~~. A recombinant virion comprising a gene therapy vector according to any one of claims 1 to 21~~24~~, an expression cassette according to any one of claims 10~~25~~ to 22~~27~~, or a promoter according to claim 23~~28~~.

25~~30~~. A pharmaceutical composition comprising a gene therapy vector according to any one of claims 1 to 19~~24~~, an expression cassette according to any one of claims 20~~25~~ to 22~~27~~, a promoter according to claim 23~~28~~, or a virion according to claim 24~~29~~.

26~~31~~. A cell comprising a gene therapy vector according to any one of claims 1 to 19~~24~~, an expression cassette according to any one of claims 20~~25~~ to 22~~27~~, a promoter according to claim 23~~28~~, or a virion according to claim 24~~29~~.

27~~32~~. A cell according to claim 26~~31~~ which is a liver cell, optionally a liver cell in cell culture or a liver cell that is present in the liver of a subject.

2833. A gene therapy vector according to any one of claims 1 to 1924, an expression cassette according to any one of claims 2025 to 2227, a promoter according to claim 2328, a virion according to claim 2429, or pharmaceutical composition according claim 2530 for use in the treatment of a disease.

29. The gene therapy vector, expression cassette, promoter, virion, or pharmaceutical composition for use according to claim 28, wherein the disease to be treated is haemophilia (including haemophilia A or B), familial hypercholesterolemia, ornithine transcarbamylase deficiency, phenylketonuria, ornithine transcarbamylase deficiency, glycogen storage disease, α1-antitrypsin deficiency, hereditary hemochromatosis, tyrosinemia type 1, argininosuccinic aciduria, hepatitis virus infection, non-viral hepatitis, liver cancer, genetic cholestasis, Wilson's disease, and various other liver diseases (such as non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease (ARLD), or lysosomal storage disorders.

3034. A method of producing an expression product *ex vivo*, preferably a therapeutic expression product, in cells, preferably liver cells, the method comprising:

- providing cells comprising an expression cassette comprising a synthetic liver-specific inducible promoter operably linked to a gene, the synthetic liver-specific inducible promoter comprising a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the synthetic liver-specific inducible promoter is in accordance with any one of claims 1 to 19; and
- administering to said cells an inducer which is able to induce expression of the expression product from the gene operably linked to the inducible promoter in said expression cassette.

3135. The method of claim 3034 comprising maintaining said liver cells under suitable conditions for expression of the expression product from the gene.

3236. The method of claim 3034 or 3135 wherein the inducer comprises one or more agents selected from the following list:

phenobarbital (PB); a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin;1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP); 6-(4Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO); Acetaminophen; Buprenorphine; Phenytoin; Carbamazepine; Valproic Acid; Artemisinin and derivatives; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; Thiazolidin-4-one; Estradiol; Estrone ~~and analogues~~; 17α-ethinyl- 3;17ß-estradiol (EE2); Dehydroepiandrost erone (DHEA); 5ß-pregnane-3,20-dione; Diethylstilbestrol; Gingko biloba extract; Galangin; chrysin; baicalein; Diallyl sulphide; Ellagic acid; Resveratrol; Squalestatin-1; Bilobalide; Triclocarban; Triclosan; Dichlorodiphenyltrichloroethane (DDT); Dieldrin; Methoxychlor; Metofluthrin; Permethrin; Pyrethrins; Sulfoxaflor; Diethylhexyl phthalate (DEHP); Cyproconazole; Fluconazole; Propiconazole; FL81; Tri-p-methylphenyl phosphate (TMPP); UM104; and UM145.

33~~37~~. The method of any one of claims 30~~34~~ to 32~~36~~ wherein the inducer comprises one or more agents selected from the following list:

phenobarbital (PB); a flavonoid compound, e.g. flavone, chrysin, baicalein, or galangin; Acetaminophen; Buprenorphine; Phenobarbital; Phenytoin; Carbamazepine; Valproic Acid; Artemisinin and derivatives; Chlorpromazine; Efavirenz; Nevirapine; Rilpivirine; Etravirine; Diazepam; Cyclophosphamid; Ifosfamide; Cerivastatin; Simvastatin; lovastatin; substituted sulphonamides; and Thiazolidin-4-one.

34~~38~~. The method of any one of claims 30~~34~~ to 33~~37~~ comprising ceasing to administer the inducer.

35~~39~~. The method of any one of claims 30~~34~~ to 34~~38~~ comprising varying the concentration of the inducer administered to the cells over time.

36~~40~~. The method of any one of claims 30~~34~~ to 35~~39~~ comprising the step of introducing the synthetic liver-specific expression cassette into the cell.

37~~41~~. Use of a gene therapy vector according to any one of claims 1 to 19~~24~~, an expression cassette according to any one of claims 20~~25~~ to 22~~27~~, a promoter according to claim 23~~28~~, a virion according to claim 24~~29~~ for the manufacture of a pharmaceutical composition for treatment of any condition or disease associated with an aberrant gene expression in the liver.

38. The use of the gene therapy vector, expression cassette, promoter, or virion for the manufacture of a pharmaceutical composition according to claim 37, wherein the condition or disease is haemophilia (including haemophilia A or B), familial hypercholesterolemia, ornithine transcarbamylase deficiency, phenylketonuria, ornithine transcarbamylase deficiency, glycogen storage disease, α1-antitrypsin deficiency, hereditary hemochromatosis, tyrosinemia type 1, argininosuccinic aciduria, hepatitis virus infection, non-viral hepatitis, liver cancer, genetic cholestasis, Wilson's disease, and various other liver diseases (such as non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease (ARLD), or lysosomal storage disorders.

39~~42~~. A method for producing an expression product, the method comprising the steps of:

a) providing a population of eukaryotic cells, preferably animal cells, more preferably mammalian cells, and more preferably liver cells, comprising an expression cassette comprising a synthetic liver-specific inducible promoter operably linked to a gene, the synthetic liver-specific inducible promoter comprising a cis-regulatory element (CRE) that is capable of being bound and activated by a heterodimer of CAR and RXR, wherein the synthetic liver-specific inducible promoter is in accordance with any one of claims 1 to 19;
b) culturing said population of cells; and
c) administering to said population of cells an inducer which is able to induce expression of the expression product from the gene operably linked to the inducible promoter in said expression cassette; and
d) recovering the expression product.