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 CAR and 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 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 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

5. The gene therapy vector of any preceding claim wherein the functional variant of 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.

6. The gene therapy vector of any preceding claim wherein the functional variant of 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.

9. The gene therapy vector of any preceding claim wherein the 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.

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.

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.

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.

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.

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.

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.

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

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.

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.

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.

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

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.

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).

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.

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

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.

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

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

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.

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

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

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

32. A cell according to claim 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.

33. A gene therapy vector according to any one of claims 1 to 24, an expression cassette according to any one of claims 25 to 27, a promoter according to claim 28, a virion

according to claim 29, or pharmaceutical composition according claim 30 for use in the treatment of a disease.

34. 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; 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.

35. The method of claim 34 comprising maintaining said liver cells under suitable conditions for expression of the expression product from the gene.

36. The method of claim 34 or 35 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.

37. The method of any one of claims 34 to 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.

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

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

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

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

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;
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.