CLAIMS

1. A method of generating hepatic cells comprising increasing the expression of at least three or more transcription factors, wherein the three or more transcription factors are selected from the group consisting of: FOXA1 or FOXA3; NR1I2 or NR1I3; HHEX; CREB3L3; GATA4; KLF9; ATF5; MLXIPL; FOXA2; AR; ARID3C; CEBPA; CUX2; EPAS1; HLF; HNF1A; HNF4A; HNF4G; KLF15; NCOA2; NR0B2; NR1H4; NR5A2; ONECUT1; ONECUT2; PPARA; PROX1; RORA; RORC; RXRA; SALL1; SMAD1; SREBF1; STAT3; TSHZ2; XBP1; ZBTB16; and variants thereof, in a non-hepatic cell population and culturing the cell population to obtain hepatic cells.

2. The method as defined in claim 1, wherein the three or more transcription factors are selected from the group consisting of: (1) FOXA1, FOXA2 or FOXA3, (2) NR1I2 or NR1I3, and (3) one or more of: HHEX, CREB3L3, GATA4, KLF9, ATF5, MLXIPL, ARID3C, CUX2, HNF4A, HNF4G, NR0B2, ONECUT1, ONECUT2, RXRA, SALL1, SREBF1, and variants thereof.

3. The method as defined in claim 2, wherein the three or more transcription factors are selected from the group consisting of: (1) FOXA1, FOXA2 or FOXA3; (2) NR1I2 or NR1I3; and (3) one or more of: HHEX, CREB3L3, GATA4, KLF9, ATF5, MLXIPL, HNF4A, HNF4G, ONECUT1, RXRA, SREBF1; and variants thereof.

4. The method as defined in claim 3, wherein the three or more transcription factors are selected from the group consisting of: (1) FOXA1, FOXA2 or FOXA3; (2) NR1I2 or NR1I3; and (3) one or more of: HHEX, CREB3L3, GATA4, KLF9, ATF5, MLXIPL, HNF4A, RXRA, SREBF1; and variants thereof.

5. The method as defined in any one of claims 1 to 4, wherein the three transcription factors comprise FOXA1, CREB3L3, NR1I2, or variants thereof.

6. The method as defined in any one of claims 1 to 5, wherein the three transcription factors comprise FOXA1, HHEX, NR1I2, or variants thereof.

7. The method as defined in any one of claims 1 to 6, wherein the method comprises increasing the expression of FOXA1, CREB3L3, NR1I2, HHEX, or variants thereof.

8. The method as defined in any one of claims 1 to 7, wherein the method comprises increasing the expression of at least four or more of the transcription factors, in particular five, six or seven or more of the transcription factors.

9. The method as defined in any one of claims 1 to 8, wherein the method comprises increasing the expression of between three and seven of the transcription factors.

10. The method as defined in any one of claims 1 to 9, wherein the non-hepatic cell population comprises source cells.

11. The method as defined in any one of claims 1 to 10, wherein the non-hepatic cell population comprises pluripotent stem cells, in particular induced pluripotent stem cells.

12. The method as defined in any one of claims 1 to 11, wherein the method comprises generating hepatic cells by cellular reprogramming of pluripotent stem cells or induced pluripotent stem cells.

13. The method as defined in any one of claims 1 to 12, wherein the hepatic cells are human hepatic cells.

14. The method as defined in any one of claims 1 to 13, which additionally comprises monitoring the cell population for at least one characteristic of a hepatic cell.

15. The method as defined in claim 14, wherein the characteristic is selected from one or more of:

(i) expression of one or more hepatic cell markers, such as Glucose-6-phosphatase, Albumin, α1-Antitrypsin (AAT), Fumarylacetoacetase (FAH), Cytokeratin 8 (CK8), Cytokeratin 18 (CK18), Asialoglycoprotein Receptor (ASGR), Alcohol Dehydrogenase 1, Arginase Type I, Cytochrome p450 3A4 (CYP3A4), Cytochrome p450 2C9 (CYP2C9), UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1), Liver-specific Organic Anion Transporter (LST-1), or a combination thereof;

(ii) activity of glucose-6-phosphatase, CYP3A4, CYP2C9, albumin synthesis and secretion, bile production or secretion, urea production, or xenobiotic detoxification; or

(iii) hepatic cell morphological features.

16. The method as defined in claim 14 or claim 15, wherein the characteristic comprises a hepatic cell marker selected from albumin and CYP3A4.

17. The method as defined in any one of claims 1 to 16, wherein the expression of the transcription factors is increased by contacting the cell population with the transcription factors, or one or more agents that activate or increase the expression or amount of the transcription factors.

18. The method as defined in any one of claims 1 to 17, wherein expression of the transcription factors is under controlled transcription.

19. The method as defined in any one of claims 1 to 18, wherein a sequence encoding one or more of the transcription factors is introduced into the cell population using a method comprising:

- targeted insertion of a coding sequence for a transcriptional regulator protein into a first genetic safe harbour site of the cell; and

- targeted insertion of an inducible cassette into a second genetic safe harbour site of the cell, wherein said inducible cassette comprises said transcription factor sequence operably linked to an inducible promoter, and said promoter is regulated by the transcriptional regulator protein.

20. The method as defined in any one of claims 1 to 19, which comprises culturing under suitable conditions for at least 4 days.

21. A method for the production of hepatic cells from a source cell, comprising the steps of:

a) targeted insertion of a gene encoding a transcriptional regulator protein into a first genetic safe harbour site of the source cell; and

b) targeted insertion of at least three or more transcription factors, wherein the three or more transcription factors are selected from the group consisting of: FOXA1 or FOXA3; NR1I2 or NR1I3; HHEX; CREB3L3; GATA4; KLF9; ATF5; MLXIPL; FOXA2; AR; ARID3C; CEBPA; CUX2; EPAS1; HLF; HNF1A; HNF4A; HNF4G; KLF15; NCOA2; NR0B2; NR1H4; NR5A2; ONECUT1; ONECUT2; PPARA; PROX1; RORA; RORC; RXRA; SALL1; SMAD1; SREBF1; STAT3; TSHZ2; XBP1; ZBTB16; and variants thereof, operably linked to an inducible promoter into a second genetic safe harbour site of the source cell, wherein said inducible promoter is regulated by the transcriptional regulator protein; and

c) culturing the source cell(s) comprising the insertions to obtain hepatic cells.

22. Use of at least three or more transcription factors, wherein the three or more transcription factors are selected from the group consisting of: FOXA1 or FOXA3; NR1I2 or NR1I3; HHEX; CREB3L3; GATA4; KLF9; ATF5; MLXIPL; FOXA2; AR; ARID3C; CEBPA; CUX2; EPAS1; HLF; HNF1A; HNF4A; HNF4G; KLF15; NCOA2; NR0B2; NR1H4; NR5A2; ONECUT1; ONECUT2; PPARA; PROX1; RORA; RORC; RXRA; SALL1; SMAD1; SREBF1; STAT3; TSHZ2; XBP1; ZBTB16; and variants thereof, to generate hepatic cells.

23. A cell obtainable by any one of the methods defined in claims 1 to 21.

24. A cell comprising one or more exogenous expression cassettes encoding at least three or more transcription factors, wherein the three or more transcription factors are selected from the group consisting of: FOXA1 or FOXA3; NR1I2 or NR1I3; HHEX; CREB3L3; GATA4; KLF9; ATF5; MLXIPL; FOXA2; AR; ARID3C; CEBPA; CUX2; EPAS1; HLF; HNF1A; HNF4A; HNF4G; KLF15; NCOA2; NR0B2; NR1H4; NR5A2; ONECUT1; ONECUT2; PPARA; PROX1; RORA; RORC; RXRA; SALL1; SMAD1; SREBF1; STAT3; TSHZ2; XBP1; ZBTB16; and variants thereof.

25. The cell as defined in claim 23 or claim 24, wherein the cell is an engineered hepatic cell.

26. The cell as defined in any one of claims 23 to 25, wherein the three or more transcription factors are integrated into the genome of the cell.

27. The cell as defined in claim 26, wherein the three or more transcription factors are integrated into a target site of the cell.

28. A cell as defined in any one of claims 23 to 27, for use in therapy.

29. A kit for differentiating a cell into a hepatic cell comprising:

(i) a source cell and an agent that activates or increases the expression or amount of at least three or more transcription factors; and/or

(ii) one or more expression cassette(s) encoding at least three or more transcription factors,

wherein the three or more transcription factors is selected from the group consisting of: FOXA1 or FOXA3; NR1I2 or NR1I3; HHEX; CREB3L3; GATA4; KLF9; ATF5; MLXIPL; FOXA2; AR; ARID3C; CEBPA; CUX2; EPAS1; HLF; HNF1A; HNF4A; HNF4G; KLF15;

NCOA2; NR0B2; NR1H4; NR5A2; ONECUT1; ONECUT2; PPARA; PROX1; RORA; RORC; RXRA; SALL1; SMAD1; SREBF1; STAT3; TSHZ2; XBP1; ZBTB16; and variants thereof.

30. Use of a kit as defined in claim 29, for differentiating a cell into a hepatic cell.

31. A method of drug screening comprising contacting a hepatic cell generated using the method as defined in any one of claims 1 to 21, or a hepatic cell as defined in any one of claims 23 to 28, with the drug and observing a change in the hepatic cells induced by the drug, or a change in the metabolism of the drug.

32. Use of hepatic cells generated using the method as defined in any one of claims 1 to 21 or hepatic cells as defined in any one of claims 23 to 28 in the manufacture of a medicament for ~~A method for~~ treating a subject having or at risk of a liver disease or dysfunction~~ comprising administering to the subject a therapeutically effective amount of hepatic cells generated using the method as defined in any one of claims 1 to 21, or hepatic cells as defined in any one of claims 23 to 28~~.