

Claims

1. A method for expanding cholangiocytes in vitro comprising:
 - (i) providing a population of cholangiocytes and;
 - (ii) culturing the population in the presence of a farnesoid X receptor (FXR) agonist to produce an expanded population of FXR-treated cholangiocytes.
2. A method according to claim 1 wherein the farnesoid X receptor (FXR) agonist is chenodeoxylic acid (CDA) or obeticholic acid (OCA)
- ~~3. A method according to claim 1 or claim 2 wherein the cholangiocytes are primary cholangiocytes or cholangiocytes differentiated from iPSCs.~~
- ~~43.~~ A method according to ~~any one of the preceding claims~~claim 1 or claim 2 wherein the population is cultured in an expansion medium comprising the farnesoid X receptor (FXR) agonist, an epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt signalling potentiator, to produce the expanded population of FXR-treated cholangiocytes.
- ~~5. A method according to any one of the preceding claims wherein the FXR-treated cholangiocytes form organoids in the expansion medium.~~
- ~~64.~~ A method according to any one ~~of the preceding claims~~of claims 1 to 3 wherein the cholangiocytes are extrahepatic cholangiocytes.
- ~~7. A method according to any one of claims 1 to 5 wherein the cholangiocytes are, intrahepatic cholangiocytes.~~
- ~~8. A method according to any one of claims 1 to 5 wherein the cholangiocytes are or gallbladder cholangiocytes.~~
- ~~9. A method according to any one of the preceding claims wherein the expansion medium is a nutrient medium which comprises an FXR agonist, epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt signalling potentiator.~~
- ~~105.~~ A method according to any one ~~of claims 1 to 4 of the preceding claims~~ wherein the non- canonical Wnt signalling potentiator is a Wnt agonist, optionally
- ~~11. A method according to claim 10 wherein the Wnt agonist is R-spondin.~~

~~126.~~ A method according to any one of claims 1 to 5 ~~the preceding claims~~ wherein the canonical Wnt signalling inhibitor is Dickkopf-related protein 1 (DKK-1).

~~13.~~ ~~A method according to any one of the preceding claims wherein the primary cholangiocytes may be obtained or isolated from primary bile tissue from a donor individual.~~

~~7.~~ ~~14.~~ A method according to any one of claims 1 to 7 ~~the preceding claims~~ wherein population is cultured in 3D culture in the expansion medium, optionally

~~15.~~ ~~A method according to claim 14 wherein the expansion medium further comprises a scaffold matrix, optionally~~ ~~tionally within the scaffold matrix is~~ a decellularised human or non-human tissue extracellular matrix.

~~16.~~ ~~A method according to any one of the preceding claims wherein expansion medium comprises a scaffold matrix and a nutrient medium supplemented with (i) EGF, (ii) the canonical Wnt inhibitor (iii) the non-canonical Wnt potentiator and (iv) the FXR agonist.~~

~~17.~~ ~~A method according to claim 15 wherein expansion medium consists of a scaffold matrix and a nutrient medium supplemented with (i) EGF, (ii) the canonical Wnt inhibitor (iii) the non-canonical Wnt potentiator and (iv) the FXR agonist..~~

~~18.~~ ~~A method according to any one of the preceding claims wherein the nutrient medium is chemically defined.~~

~~19.~~ ~~A method according to claim 18 wherein the chemically defined nutrient medium is William's E medium, optionally supplemented with one or more of nicotinamide, sodium bicarbonate, 2-phospho-L-ascorbic acid trisodium salt, sodium pyruvate, glucose, HEPES, ITS+ premix, dexamethasone, glutamax, penicillin and streptomycin.~~

~~20.~~ ~~A method according to any one of the preceding claims wherein cholangiocytes are human~~

~~218.~~ A method according to any one of claims 1 to 7 ~~the preceding claims~~ wherein the population of cholangiocytes is devoid of stem cells

~~922.~~ A method according to any one of claims 1 to 8 ~~the preceding claims~~ wherein the cholangiocytes in

the expanded population

(i) express CK7, CK18, CK19, HNF1B, Gamma Glutamyl-Transferase (GGT), CFTR, SCR, SSTR2, Apical Salt and Bile Transporter (ASBT), Aquaporin 1 and Anion Exchanger 2, FGF19, SOX17;

(ii)

~~23. A method according to any one of the preceding claims wherein the cholangiocytes in the expanded population~~ display ALP activity, GGT activity, MDR1 mediated secretion, physiological responses to secretin and somatostatin, export of bile acids, CFTR mediated chloride transfer, physiological responses to ATP and acetylcholine and increased proliferation in response to VEGF; and/or.

(iii)

~~24. A method according to any one of the preceding claims wherein the cholangiocytes in the expanded population~~ do not express MHC antigens or express MHC antigens at levels lower than primary cholangiocytes.

~~2510.~~ A method according to any one of claims 1 to 9 ~~the preceding claims~~ wherein the cholangiocytes are cultured in the expansion medium for 20 or more passages.

~~26. A method according to any one of the preceding claims wherein the method comprises disrupting cholangiocyte organoids such that the expanded population comprises individual cells.~~

~~2711.~~ A method according to any one of claims 1 to 10 ~~the preceding claims~~ comprising seeding the expanded population of FXR-treated cholangiocytes into a biocompatible scaffold, optionally within the scaffold matrix is a decellularised human or non-human tissue extracellular matrix.

~~28. A method according to claim 26 comprising culturing the biocompatible scaffold in an expansion medium comprising an RXR agonist, epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt/ signalling potentiator, such that the cholangiocytes populate the scaffold.~~

~~2912.~~ A method according to claim 11-27 ~~or 28~~ wherein the cholangiocytes form a biliary epithelium in the scaffold.

~~3013.~~ A method according to any one of claims 1 to 12 ~~the preceding claims~~ comprising storing the expanded population of FXR-treated cholangiocytes or scaffold and/or.

~~31. A method according to any one of the preceding claims comprising admixing the~~

expanded population or scaffold with a therapeutically acceptable excipient.

~~32~~14. An isolated population of FXR-treated cholangiocytes produced by a method of any one of claims 1 to ~~34~~13.

~~33. — A population according to claim 32 wherein the FXR treated cholangiocytes are in the form of organoids, sub-organoid clusters or individual cells.~~

~~34~~15. A population according to claim ~~14~~32 or 33 wherein the FXR treated cholangiocytes are within a biocompatible scaffold, optionally wherein the decellularised scaffold matrix is a human or non-human tissue extracellular matrix.

~~35~~16. A population according to ~~any one of claims 32 to 34~~claim 14 or claim 15 wherein the FXR treated cholangiocytes

(i) express CK7, CK18, CK19, HNF1B, Gamma Glutamyl-Transferase (GGT), CFTR, SCR, SSTR2, Apical Salt and Bile Transporter (ASBT), Aquaporin 1 and Anion Exchanger 2, FGF19, SOX17;

(ii)

~~36. — A population according to any one of claims 32 to 35 wherein the FXR treated cholangiocytes in the expanded population~~ do not express MHC antigens or express low levels of MHC antigens, and/or :

(iii)

~~37. — A population according to any one claims 32 to 36 wherein the FXR treated cholangiocytes~~ display ALP activity, GGT activity, MDR1 mediated secretion, physiological responses to secretin and somatostatin, export of bile acids, CFTR mediated chloride transfer, physiological responses to ATP and acetylcholine and increased proliferation in response to VEGF

~~17~~38. A biocompatible scaffold comprising an isolated population according to any one claims ~~14~~32 to ~~37~~16.

~~39~~18. Use of A~~n~~ isolated population of FXR-~~treated~~ cholangiocytes according to any one of claims ~~14~~32 to ~~37~~16 or a scaffold according to claim ~~38~~17 in the manufacture of a medicament for ~~use in~~ the treatment of a biliary disorder.

~~40. — A method of treating a patient with a biliary disorder comprising;~~

~~administering a population of isolated FXR treated cholangiocytes according to any one of claims 32 to 37 or a scaffold according to claim 38 to an individual in need thereof.~~

~~41. — A method according to claim 40 further comprising administering an FXR agonist to the individual.~~

~~42. — A method of treating a patient with a biliary disorder comprising; administering a population of isolated cholangiocytes to an individual in need thereof and; administering an FXR agonist to the individual.~~

~~43. — A method according to claim 42 wherein the cholangiocytes are isolated FXR treated cholangiocytes according to any one of claims 32 to 37.~~

~~44. — A method of preparing liver tissue for transplantation comprising exposing the liver tissue to an FXR agonist ex vivo.~~

~~45. — A method of treating a patient with a biliary disorder comprising; exposing liver tissue obtained from the patient to an FXR agonist ex vivo; and administering the liver tissue to the patient.~~

~~46. — A method according to claim 45 further comprising administering an FXR agonist to the individual.~~

1947. A method of screening a compound comprising;
contacting a population of isolated FXR treated cholangiocytes according to any one of claims 14 to 16 ~~32 to 37~~ or a scaffold according to claim ~~38~~ 17 with a test compound, and;
determining the effect of the test compound on said the FXR treated cholangiocytes or scaffold and/or the effect of said FXR treated cholangiocytes or scaffold on the test compound.

~~48. — A method according to claim 47,~~
optionally wherein the effect of the test compound on one or more of proliferation, ALP activity, GGT activity, MDR1 mediated secretion, secretin and/or somatostatin responsiveness, bile acid export, CFTR mediated chloride transfer, and ATP, acetylcholine and VEGF responsiveness is determined.

4920. A method of determining the susceptibility of a patient to a therapeutic compound comprising;
(i) providing a population of isolated cholangiocytes having a disease phenotype from an individual with a disease condition;
(ii) culturing the population in an expansion medium comprising an FXR receptor,

epidermal growth factor (EGF), a canonical Wnt signalling inhibitor and a non-canonical Wnt signalling potentiator, to produce an expanded population of cholangiocytes displaying a disease phenotype,

(iii) contacting the expanded population of FXR treated cholangiocytes produced by a method described herein with a therapeutic compound, and;

(iv) determining the effect of the therapeutic compound on said FXR treated cholangiocytes,

wherein an amelioration of the disease phenotype of the FXR treated cholangiocytes is indicative that the individual is susceptible to treatment with the therapeutic compound.

5021. A kit for production of FXR treated cholangiocyte organoids comprising an expansion medium comprising an FXR receptor, epidermal growth factor (EGF), a non- canonical Wnt/PCP signalling potentiator and a canonical Wnt signalling inhibitor.

5122. Use of a culture medium for the *in vitro* production of FXR treated cholangiocyte organoids, wherein the culture medium comprises an FXR receptor, epidermal growth factor (EGF), a non- canonical Wnt/PCP signalling potentiator and a canonical Wnt signalling inhibitor.