# INDUCED HEPATOCYTES AND USES THEREOF

## CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 62/085,185, filed on November 26, 2014, which is incorporated herein by reference in its entirety.

## INCORPORATION BY REFERENCE

**[0002]** All publications, patents, and patent applications disclosed herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term disclosed herein and a term in an incorporated reference, the term herein controls.

## SUMMARY

**[0003]** In one of many examples, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte *in vitro*, comprising: contacting the trophoblast stem cell with a conditioned medium (e.g., for sufficient time) to induce differentiation of the trophoblast stem cell into an induced hepatocyte, wherein the condition medium comprises a fibroblast growth factor (FGF), a steroid, and a cytokine. In some examples, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte *in vitro*, which comprises (a) contacting the trophoblast stem cell in a conditioned medium comprising a fibroblast growth factor (FGF), a steroid, and a cytokine; and (b) incubating the cell for sufficient time to induce differentiation of the trophoblast stem cell into an induced hepatocyte. In some examples, the method further comprises contacting the trophoblast stem cell with the FGF prior to addition of the steroid and the cytokine to the conditioned medium. In some examples, the trophoblast stem cell is contacted with FGF for at least 2 hours, at least 4 hours, at least 6 hours, 8 hours, at least 12 hours, at least 16 hours, at least 20 hours, or at least 24 hours prior to addition of the steroid and the cytokine to the conditioned medium. In some examples, the method further comprises incubating the trophoblast stem cell for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days. In some examples, the steroid and the cytokine are added simultaneously or sequentially into the conditioned medium.

**[0003a]** In another example, disclosed herein is an isolated induced hepatocyte derived from a trophoblast stem cell, wherein the hepatocyte expresses human leukocyte antigen G (HLA-G) and one or more biomarkers selected from

the group consisting of C-X-C chemokine receptor type 4 (CXCR4), bile salt export pump (BSEP), cytochrome P450 7A1 (CYP7A1), serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 1 (SERPINA1), ATP-binding cassette sub-family C (ABCC2), CCAAT-enhancer-binding protein beta (C/EBPβ), and hepatocyte nuclear factor 1-alpha (HNF1α).

**[0003b]** In yet another example, disclosed herein is a pharmaceutical composition comprising the isolated hepatocyte disclosed herein and a pharmaceutically acceptable carrier.

**[0003c]** In yet another example, disclosed herein is use of the isolated hepatocyte disclosed herein in the manufacture of a medicament for the treatment of a liver-associated disease or disorder in a subject, wherein the liver-associated disease or disorder is characterized by acute hepatic failure; or deficient or impaired LDL uptake, glycogen storage, or cytochrome P450 (CYP) enzymatic activity.

**[0003d]** In yet another example, disclosed herein is the isolated hepatocyte as disclosed herein for use in therapy.

**[0004]** In some examples, an induced hepatocyte herein is a hepatic progenitor cell. In some examples, FGF upregulates miRNA-124a in the TS cell. In some examples, elevated level of miRNA-124a initiates definitive endoderm (DE) specification in the TS cell. In some

## CLAIMS

**WHAT IS CLAIMED IS:**

1. An isolated induced hepatocyte derived from a trophoblast stem cell, wherein the hepatocyte expresses human leukocyte antigen G (HLA-G) and one or more biomarkers selected from the group consisting of C-X-C chemokine receptor type 4 (CXCR4), bile salt export pump (BSEP), cytochrome P450 7A1 (CYP7A1), serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin) member 1 (SERPINA1), ATP-binding cassette sub-family C (ABCC2), CCAAT-enhancer-binding protein beta (C/EBPβ), and hepatocyte nuclear factor 1-alpha (HNF1α).
2. The hepatocyte of claim 1, wherein the hepatocyte recruits $CD4^{+}Foxp3^{+}$ Treg cells.
3. The hepatocyte of claim 1, wherein the hepatocytes form tissue of a 3-dimensional structure.
4. The hepatocyte of claim 1, wherein the hepatocytes cluster or aggregate.
5. The hepatocyte of claim 1, wherein the hepatocytes form a crescent cell mass.
6. The hepatocyte of claim 1 or 2, wherein the hepatocyte further expresses one or more markers selected from the group consisting of C-kit, CK19, CK18, ALB, α-AFP, betatrophin, ADH1, APOF, CPS1, GATA4, CYP1A1, CYP2B6, ASGR1, MRP2, Cx32, G6PC, HNF4α, SOX17, and TTR.
7. A pharmaceutical composition comprising the isolated hepatocyte of claim 1 and a pharmaceutically acceptable carrier.
8. Use of the isolated hepatocyte of claim 1 in the manufacture of a medicament for the treatment of a liver-associated disease or disorder in a subject, wherein the liver-associated disease or disorder is characterized by acute hepatic failure; or deficient or impaired LDL uptake, glycogen storage, or cytochrome P450 (CYP) enzymatic activity.
9. The use of claim 8, wherein the medicament comprises the isolated hepatocyte of claim 1 in a pharmaceutically acceptable carrier.
10. The use of claim 9, wherein the pharmaceutically acceptable carrier comprises a phosphate buffer saline.
11. The use of claim 10, wherein the medicament is formulated for administration as an intravenous injection.
12. The use of claim 8, wherein the disease or disorder is liver failure.
13. The isolated hepatocyte of claim 1 for use in therapy.