Finally, cryopreservation of hepatocytes is a key in cell therapy for emergency transplantation in patients with ALF and ACLF. However, the difficulty with cryopreservation is due to cells being subjected to damaging conditions during both freezing and thawing steps leading to decreased cell viability (Terry *et al.*; 2010).

Therefore, there is a need to counteract the shortage of liver donors for liver transplantation and to provide means to easily generate hepatic cells that may be administered in a subject in need of a liver therapy, in particular, within limited amount of time, with high yield and of high quality. There is also a need to provide highly quantitative and qualitative cells that are compatible for regenerating and/or repairing a diseased liver, in particular in a liver undergoing a fulminant liver disorder, more particularly ALF or ACLF. There is further a need for providing a non-limited source of hepatic cells, irrespective of whether there is a shortage of liver donors, allowing both autologous and allogenic (heterologous) transplantation therapies. There is also a need for providing hepatic cells that resist to storage conditions, including cryopreservation. There is further a need to provide hepatic cells that could be administered without co-administration of immunosuppressive agents, as they would be universally transplantable. Finally, there is a need to provide new approaches to generate highly valuable therapeutic hepatic cells, in particular, approaches that are complying with the Good Manufacturing Practice (GMP).

SUMMARY

A first aspect of the ~~invention~~disclosure relates to a population of cells, in particular an isolated population of cells, comprising at least 5% of hepatic stem-like cells expressing the alpha-fœtoprotein marker (AFP+) and not expressing the albumin marker (ALB-), or an extract thereof. In another aspect, the present disclosure refers to a population of cells, in particular an isolated population of cells, comprising at least 5% of hepatic stem-like cells expressing the alpha-fœtoprotein marker (AFP+) and expressing Cytokeratin-19 (CK19+), and not expressing the albumin marker (ALB-), or an extract thereof.

In certain examples~~embodiments~~, the hepatic stem-like cells are further expressing the T-Box Transcription Factor 3 marker (TBX3+) and/or the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+), preferably the T-Box Transcription Factor 3 marker (TBX3+) and the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+).

In some examples~~embodiments~~, the hepatic stem-like cells are cryopreserved.

Another aspect of the ~~invention~~disclosure relates to a particle, in particular a spheroid, comprising a population of cells comprising hepatic stem-like cells, or an extract thereof, according to the instant ~~invention~~disclosure.

In one aspect, the ~~invention~~disclosure relates to a suspension comprising a population of cells comprising hepatic stem-like cells, or an extract thereof, according to the instant ~~invention~~disclosure.

A further aspect of the ~~invention~~disclosure pertains to a pharmaceutical composition comprising (i) a population of cells comprising hepatic stem-like cells, or an extract thereof, and/or at least one particle, and/or a suspension, according to the instant ~~invention~~disclosure, and (ii) a pharmaceutically acceptable vehicle.

A still further aspect of the ~~invention~~disclosure relates to a medical device comprising a population of cells comprising hepatic stem-like cells, or an extract thereof, and/or at least one particle and/or a suspension, and/or a pharmaceutical composition, according to the instant ~~invention~~disclosure.

In some aspect, the ~~invention~~disclosure also relates to a non-human animal model comprising a heterologous population of cells comprising hepatic stem-like cells, or an extract thereof, according to the instant ~~invention~~disclosure.

Another aspect of the ~~invention~~disclosure relates to a hepatic stem-like cell, or an extract thereof, as defined in the instant disclosure, or the population of cells comprising hepatic stem-like cells, or an extract thereof, or the particle, or the suspension, or the pharmaceutical composition according to the instant ~~invention~~disclosure, for use as a medicament.

A further aspect of the ~~invention~~disclosure relates to a use of the hepatic stem-like cell, or an extract thereof, as defined in the instant disclosure, or the population of cells comprising hepatic stem-like cells, or an extract thereof, or the particle, or the suspension, or the pharmaceutical composition, or the medical device according to the instant ~~invention~~disclosure, in the manufacture of a medicament for ~~use in~~ preventing and/or treating a ~~fulminant~~ liver disorder.

In one exampleaspect, the inventiondisclosure relates to the hepatic stem-like cell, or an extract thereof, as defined in the instant disclosure, or the population of cells comprising hepatic stem-like cells, or an extract thereof, or the particle, or the suspension, or the pharmaceutical composition, or the medical device, for use according to the instant inventiondisclosure, wherein the fulminant liver disorder is an acute liver failure (ALF) or an acute chronic liver failure (ACLF).

A still further exampleaspect of the inventiondisclosure relates to the hepatic stem-like cell, or an extract thereof, as defined in the instant inventiondisclosure, or the population of cells comprising hepatic stem-like cells, or an extract thereof, or the particle, or the suspension, or the pharmaceutical composition, or the medical device, for use according to the instant inventiondisclosure, and wherein the ACLF is associated with a liver disease selected in the group consisting of the non-alcoholic steatohepatitis (NASH); alcoholic hepatitis; viral-induced hepatitis; a cryptogenic liver disease; a malignant liver disease, such as hepatocellular carcinoma and cholangiocarcinoma; autoimmune hepatitis, a vascular liver disease, such as Budd-Chiari syndrome; a cholestatic liver disease; and an inherited metabolic liver disease, such as, Wilson's disease and an urea cycle disorder.

In one further aspect, the inventiondisclosure relates to the use of a cryopreserved population of cells comprising hepatic stem-like cells, or an extract thereof, according to the instant inventiondisclosure, for preparing a particle, as defined herein.

Another aspect of the inventiondisclosure relates to an *in vitro* method for screening a drug, said method comprising the steps of:

a) providing at least one hepatic stem-like cell, or an extract thereof, as defined herein, and/or the population of cells comprising hepatic stem-like cells, or an extract thereof, and/or particle, and/or suspension according to the instant inventiondisclosure;

b) contacting said at least one cell or an extract thereof, and/or said population of cells or extract thereof, and/or said particle, and/or said suspension, from step a), with a drug candidate;

c) measuring one or more biological parameter(s) and optionally comparing said one or more biological parameter(s) with one or more reference parameter(s);

d) determining whether the drug candidate is of therapeutic and/or diagnostic interest.

In one aspect, the ~~invention~~disclosure relates to a kit for treating and/or preventing a fulminant liver disorder, said kit comprising:

a) hepatic stem-like cells, or an extract thereof, as defined herein, or a population of cells comprising hepatic stem-like cells, or an extract thereof, or particle, or suspension, or pharmaceutical composition according to the instant ~~invention~~disclosure; and

b) a mean to administer said cells or extract thereof, population or extract thereof, or particle, or suspension or pharmaceutical composition.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- "**About**" preceding a figure encompasses plus or minus 10%, or less, of the value of said figure. It is to be understood that the value to which the term "about" refers is itself also specifically, and preferably, disclosed.
- "**Comprise**" is intended to mean "**contain**", "**encompass**" and "**include**". In some embodiments, the term "**comprise**" also encompasses the term "**consist of**".
- "**Fulminant liver disorder**" refers to a rapid and severe liver deterioration condition in an individual, with or without known pre-existing or diagnosed liver disease. It is to be understood that a fulminant liver disorder is itself a non-chronic disease, but may arise from a chronic liver condition. As used herein, a fulminant liver disorder refers to any liver disease prioritized for liver transplantation when using a scoring system for organ allocation such as the Model for End- stage Liver Disease (MELD)

CLAIMS

1. A population of cells, in particular an isolated population of cells, comprising at least 5% of hepatic stem-like cells expressing the alpha-fœtoprotein marker (AFP+) and expressing Cytokeratin-19 (CK19+), and not expressing the albumin marker (ALB-), or an extract thereof.

2. The population of cells according to claim **1**, wherein the hepatic stem-like cells are further expressing at least one other marker, in particular at least two markers, selected from the Epithelial Cell Adhesion Molecule (EPCAM+) and the Transthyretin (TTR+).

~~**2.**~~**3.** The population of cells according to claim **1** or **2**, wherein the hepatic stem-like cells are further expressing the T-Box Transcription Factor 3 marker (TBX3+) and/or the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+), preferably the T-Box Transcription Factor 3 marker (TBX3+) and the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+).

4. The population of cells according to claim **1** or **2**, wherein the hepatic stem-like cells are further expressing EPCAM (EPCAM+), Forkhead Box A2 (FOXA2+), the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+), antigen KI76 (KI67+), and the SRY-Box Transcription Factor 17 (SOX17+).

5. The population of cells according to claim **1** or **2**, wherein the hepatic stem-like cells are further expressing the T-Box Transcription Factor 3 marker (TBX3+), the Hepatocyte Nuclear Factor 4 Alpha marker (HNF4A+), Epithelial Cell Adhesion Molecule (EPCAM+), and Transthyretin (TTR+).

6. The population of cells according to any one of claims **1** to **5**, wherein the hepatic stem-like cells are further expressing AFP, APOA1, APOA2, APOB, APOE, CD 164, CD24, DPP4, EPCAM, G1A1, GSTA2, KRT18, KRT19, SEPP1, SOD1, SPARC, TTR, VIM and VTN, and not expressing ALB.

7. The population of cells according to claim **6**, wherein the hepatic stem-like cells are further expressing APOA4, BMP2, BMP4, DLK1, GATA4, GATA6, GSTA1, HNF1B, HNF4A, SMAD7 and TBX3.

8. The population of cells according to claim **6** or **7**, wherein the hepatic stem-like cells does not express ABCBll, ASGR1, CYP1A2, CYP2A6, CYP2B6, CYP2B7P, CYP2C9, CYP2E1, CYP3A4, CYP3A7, F9, NAGS, UGT1A1 and PDX1.

~~3.~~**9.** The population of cells according to any one of claims **1** to **8**~~or 2~~, wherein the hepatic stem-like cells are cryopreserved.

10. The population of cells according to any one of claims **1** to **9**, wherein the extract comprises extracellular vesicles, in particular exosomes, or exosome-like particles, or a mix thereof.

~~4.~~**11.** A particle, in particular a spheroid, comprising a population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **10**~~3~~.

~~5.~~**12.** A suspension comprising a population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **10**~~3~~.

~~6.~~**13.** A pharmaceutical composition comprising **(i)** a population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **10**~~3~~, and/or at least one particle according to claim **11**~~4~~, and/or a suspension according to claim **12**~~5~~, and **(ii)** a pharmaceutically acceptable vehicle.

~~7.~~**14.** A medical device comprising a population of cells comprising hepatic stem-like cells, or an extract thereof according to any one of claims **1** to **10**~~3~~, and/or at least one particle according to claim **11**~~4~~ and/or a suspension according to claim **12**~~5~~, and/or a pharmaceutical composition according to claim **13**~~6~~.

~~8.~~**15.** A non-human animal model comprising a heterologous population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **10**~~3~~.

~~9.~~16. The hepatic stem-like cells, or an extract thereof, as defined in any one of claims **1** to ~~10~~3, or the population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to ~~10~~3, or the particle according to claim ~~11~~4, or the suspension according to claim ~~12~~5, or the pharmaceutical composition according to claim ~~13~~6, for use as a medicament.

~~10.~~17. Use of ~~T~~the hepatic stem-like cells, or an extract thereof, as defined in any one of claims **1** to ~~10~~3, or the population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to ~~10~~3, or the particle according to claim ~~11~~4, or the suspension according to claim ~~12~~5, or the pharmaceutical composition according to claim ~~13~~6, or the medical device according to claim ~~14~~7, in the manufacture of a medicament for ~~use in~~ preventing and/or treating a ~~fulminant~~ liver disorder.

18. The use according to claim **17**, wherein the liver disorder is a fulminant liver disorder.

~~11.~~19. ~~The hepatic stem-like cells, or an extract thereof, as defined in any one of claims **1** to **3**, or the population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **3**, or the particle according to claim **4**, or the suspension according to claim **5**, or the pharmaceutical composition according to claim **6**, or the medical device according to claim **7**, for~~ The use according to claim ~~18~~10, wherein the fulminant liver disorder is an acute liver failure (ALF) or an acute chronic liver failure (ACLF).

~~12.~~20. The ~~hepatic stem-like cells, or an extract thereof, as defined in any one of claims **1** to **3**, or the population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to **3**, or the particle according to claim **4**, or the suspension according to claim **5**, or the pharmaceutical composition according to claim **6**, or the medical device according to claim **7**, for~~ use according to claim ~~19~~11, ~~and~~ wherein the ACLF is associated with a liver disease selected in the group consisting of the non-alcoholic steatohepatitis (NASH); alcoholic hepatitis; viral-induced hepatitis; a cryptogenic liver disease; a malignant liver

disease, such as hepatocellular carcinoma and cholangiocarcinoma; autoimmune hepatitis, a vascular liver disease, such as Budd-Chiari syndrome; a cholestatic liver disease; and an inherited metabolic liver disease, such as, Wilson's disease and an urea cycle disorder.

~~13.~~21. Use of a cryopreserved population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to ~~10~~3, for preparing a particle, as defined in claim ~~11~~4.

~~14.~~22. An *in vitro* method for screening a drug, said method comprising the steps of:

a) providing at least one hepatic stem-like cell, or an extract thereof, as defined in any one of claims **1** to ~~10~~3, and/or the population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to ~~10~~3, and/or particle according to claim ~~11~~4, and/or suspension according to claim ~~12~~5;

b) contacting said at least one cell or an extract thereof, and/or said population of cells or extract thereof, and/or said particle, and/or said suspension, from step a), with a drug candidate;

c) measuring one or more biological parameter(s) and optionally comparing said one or more biological parameter(s) with one or more reference parameter(s);

d) determining whether the drug candidate is of therapeutic and/or diagnostic interest.

~~15.~~23. A kit for treating and/or preventing a fulminant liver disorder, said kit comprising:

a) hepatic stem-like cells, or an extract thereof, as defined in any one of claims **1** to ~~10~~3, or a population of cells comprising hepatic stem-like cells, or an extract thereof, according to any one of claims **1** to ~~10~~3, or particle according to claim ~~11~~4, or suspension according to claim ~~12~~5, or pharmaceutical composition according to claim ~~13~~7; and

b) a mean to administer said cells or extract thereof, population or extract thereof, or particle, or suspension or pharmaceutical composition.