mouse fibroblasts to mouse hepatocyte-like cells, the same methodologies may not necessarily be successful when applied to human cells. Scientific literature has indicated that factors and processes necessary and sufficient for altering the differentiation state of mouse cells may be distinct from that necessary and sufficient for altering the differentiation state of human cells. (See, *e.g.*, Yi et al., (2012) Cell Research 22:616-619; Gonzalez *et al.*, (2011) Nature Reviews Genetics 12:231-242; Pang *et al.*, (2011) Nature 476:220-223; Vierbuchen & Wernig (2011) Nature Biotechnology 29:892-907.)

The generation of human induced hepatocytes from non-hepatocyte cells, without any risk of genomic alteration, offers great promise as a means for treating disease through cell transplantation. Human induced hepatocytes generated from human non-hepatocyte cells obtained from individual patients may enable development of patient-specific therapies which do not have a risk of immune rejection, thus eliminating the need for immunosuppressive procedures. Additionally, generation of human induced hepatocytes from disease-specific non-hepatocyte cells could provide a means to model and study specific disease states and develop therapeutics useful for the treatment of these diseases.

Therefore, there exists a need in the art to identify the key transcription factors necessary and sufficient to reprogram human non-hepatocyte cells into human induced hepatocytes. The present invention meets this need by providing methods and compositions useful for generating human induced hepatocytes from human non-hepatocyte cells.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclosure provides, in part, methods and compositions for reprogramming non-hepatocyte cells to induced hepatocytes, populations of induced hepatocytes, compositions comprising induced hepatocytes, and uses thereof. In some embodiments, the non-hepatocyte cells are non-hepatocyte somatic cells. In other embodiments, the compositions and methods provided herein are useful for producing human induced hepatocytes by reprogramming human non-hepatocyte cells.

In one aspect, there is provided a method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, contacting the non-hepatocyte with an agent which increases or induces the expression or activity of one or more

reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

In one aspect, there is provided a method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid or protein preparation which encodes or provides one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

In one aspect, there is provided a cell population comprising induced hepatocytes obtained by the methods as described herein.

In one aspect, there is provided an isolated induced hepatocyte obtained by the methods as described herein.

In one aspect, there is provided a composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a non-hepatocyte cell to an induced hepatocyte when the composition is introduced into a non-hepatocyte cell.

In one aspect, there is provided a method of screening a test compound, the method comprising contacting an induced hepatocyte obtained by the methods as described herein with a test compound, and determining the effect of the test compound on the induced hepatocyte.

The methods and compositions provided herein are useful for reprogramming a starting cell or starting cell population (*e.g.*, a non-hepatocyte cell or non-hepatocyte cell population) to an induced hepatocyte or a population of induced hepatocytes with high efficiency. In some

embodiments, the starting cell or starting cell population is a human non-hepatocyte cell or a human non-hepatocyte cell population (*e.g.* a non-hepatocyte cell of human origin), and the resulting induced hepatocytes are human induced hepatocytes or a population of human induced hepatocytes.

The present inventors have identified FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4 (and various combinations thereof) as key reprogramming factors that can reprogram human non-hepatocyte cells to human induced hepatocytes. The present inventors have also identified CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A as key reprogramming factors that can reprogram human non-hepatocyte cells to human induced hepatocytes.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, contacting the non-hepatocyte with an agent which increases or induces the expression or activity of one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell, thereby producing an induced hepatocyte from the non-hepatocyte cell. In some embodiments, the agent increases or induces the expression or activity of one or more of the following factors: FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In other embodiments, the agent is a nucleic acid encoding one or more of the following factors: FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In other embodiments, the agent is one or more of the following factors: FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In yet other embodiments, the agent increases or induces the expression or activity of one or more of the following factors: CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In yet other embodiments, the agent is a nucleic acid encoding one or more of the following factors: CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In further embodiments, the agent is one or more of the following factors: CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In some embodiments, the nucleic acid is a nucleic acid capable of expressing a reprogramming factor. In some embodiments, the nucleic acid is RNA or mRNA.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid preparation, wherein the nucleic acid preparation comprises nucleic acid molecules encoding one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell. In some embodiments, the nucleic acid preparation is a mixture of nucleic acids (*e.g.*, a nucleic acid preparation comprising a mixture of different nucleic acid molecules) encoding one or more reprograming factors.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid preparation, wherein the nucleic acid preparation comprises nucleic acid molecules capable of expressing one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell. In some embodiments, the nucleic acid preparation is a mixture of nucleic acids (*e.g.*, a nucleic acid preparation comprising a mixture of different nucleic acid molecules) encoding one or more reprograming factors and capable of expressing said one or more reprogramming factors.

In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA1, FOXA3, HNF1A,

and HNF4A, nucleic acid molecules encoding FOXA1, FOXA2, HNF1A, and HNF4A, or nucleic acid molecules encoding FOXA2, FOXA3, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA1, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA3, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA2, HNF1A, and HNF4A. In some embodiments, the nucleic acid preparation useful for reprogramming non-hepatocyte cells to induced hepatocytes comprises nucleic acid molecules encoding FOXA1, FOXA3, and HNF1A, nucleic acid molecules encoding FOXA1, FOXA2, and HNF1A, or nucleic acid molecules encoding FOXA2, FOXA3, and HNF1A. In certain embodiments, the nucleic acid molecules encoding the reprogramming factors are RNA molecules or mRNA molecules.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid preparation, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, a nucleic acid sequence comprising SEQ ID NO:41, and a nucleic acid sequence comprising SEQ ID NO:43, and culturing the non-hepatocyte cell under conditions suitable for reprogramming, thereby producing an induced hepatocyte from the non-hepatocyte cell. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises

nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, and a nucleic acid sequence comprising SEQ ID NO:39. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid preparation, wherein the nucleic acid preparation comprises nucleic acid molecules encoding polypeptides encoding an amino acid sequence comprising SEQ ID NO:34, an amino acid sequence comprising SEQ ID NO:36, an amino acid sequence comprising SEG ID NO:38, an amino acid sequence comprising SEQ ID NO:40, an amino acid sequence comprising SEQ ID NO:42, and an amino acid sequence

nucleic acid preparation comprises nucleic acid molecules encoding polypeptides encoding an amino acid sequence comprising an amino acid sequence comprising SEG ID NO:34, an amino acid sequence comprising SEQ ID NO:36, and an amino acid sequence comprising SEQ ID NO:40. In some embodiments, the nucleic acid preparation comprises nucleic acid molecules encoding polypeptides encoding an amino acid sequence comprising an amino acid sequence comprising SEG ID NO:36, an amino acid sequence comprising SEQ ID NO:38, and an amino acid sequence comprising SEQ ID NO:40.

In some embodiments, the present ~~invention~~ disclosure provides a method for producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, a method for reprogramming a non-hepatocyte cell to an induced hepatocyte), the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a protein preparation comprising one or more reprogramming factors, culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell. In some embodiments, the protein preparation is a mixture of one or more reprograming factor proteins or polypeptides. In some embodiments, the protein preparation for use in producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, for use in reprogramming a non-hepatocyte cell to an induced hepatocyte) comprises FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In some embodiments, the protein preparation for use in producing an induced hepatocyte from a non-hepatocyte cell (*e.g.*, for use in reprogramming a non-hepatocyte cell to an induced hepatocyte) comprises CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA1, FOXA3, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA1, FOXA2, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA2, FOXA3, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA1, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA3, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA2, HNF1A, and HNF4A. In other embodiments, the protein preparation comprises FOXA1, FOXA3, and HNF1A. In other embodiments, the protein preparation comprises FOXA1, FOXA2, and HNF1A. In other embodiments, the protein preparation comprises FOXA2, FOXA3, and HNF1A.

The present ~~invention~~ disclosure also provides a population of induced hepatocytes, wherein the induced hepatocytes are obtained using any of the methods and compositions described herein. In some embodiments, the population of induced hepatocytes provided by the present ~~invention~~ disclosure is a homogeneous population of induced hepatocytes. In some ~~aspects~~ embodiments, a homogeneous population of induced hepatocytes provided by the present ~~invention~~ disclosure is a population of cells wherein a significant portion of the cell population comprises induced hepatocytes, such as, for example, a population of cells in which more than about 50%, more than about 55%, more than about 60%, more than about 65%, more than about 70%, more than about 75%, more than about 80%, more than about 85%, more than about 90%, more than about 91%, more than about 92%, more than about 93%, more than about 94%, more than about 95%, more than about 96%, more than about 97%, more than about 98%, or more than about 99% of the cells in the population are induced hepatocytes produced by the present methods and compositions.

In other ~~aspects~~ embodiments, the present ~~invention~~ disclosure also provides nucleic acid compositions useful for reprogramming a non-hepatocyte cell to an induced hepatocyte. In some embodiments, the present ~~invention~~ disclosure provides nucleic acid compositions useful for reprogramming a human non-hepatocyte cell to a human induced hepatocyte. In some embodiments, the present ~~invention~~ disclosure provides a composition comprising a nucleic acid preparation useful for reprogramming a non-hepatocyte cell to an induced hepatocyte, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4. In some embodiments, the present ~~invention~~ disclosure provides a composition comprising a nucleic acid preparation useful for reprogramming a non-hepatocyte cell to an induced hepatocyte, wherein the nucleic acid preparation comprises nucleic acid molecules encoding CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA3, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules

encoding FOXA2, FOXA3, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA3, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA2, HNF1A, and HNF4A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA3, and HNF1A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, and HNF1A. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules encoding FOXA2, FOXA3, and HNF1A. In certain embodiments, the nucleic acid molecules encoding the reprogramming factors are RNA molecules or mRNA molecules.

In some embodiments, the present ~~invention~~ disclosure provides a composition comprising a nucleic acid preparation useful for reprogramming a non-hepatocyte cell to an induced hepatocyte, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, a nucleic acid sequence of SEQ ID NO:41, and a nucleic acid sequence comprising SEQ ID NO:43. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic

acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence comprising SEQ ID NO:41. In some embodiments, composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, and a nucleic acid sequence comprising SEQ ID NO:39. In some embodiments, the composition comprising a nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

In various ~~aspects~~ embodiments of the methods provided herein, the non-hepatocyte cell is a human non-hepatocyte cell, and the resulting induced hepatocyte is a human induced hepatocyte. In some ~~aspects~~ embodiments of the methods provided herein, the non-hepatocyte cell is not a stem cell. In other ~~aspects~~ embodiments of the methods provided herein, the non-hepatocyte cell is not a pluripotent cell. In still other ~~aspects~~ embodiments of the methods provided herein, the non-hepatocyte cell is a somatic cell, including a human somatic cell.

In various ~~aspects~~embodiments, the induced hepatocytes obtained by the methods of the present ~~invention~~ disclosure express hepatocyte genes, including albumin, α-fetoprotein, α1-anti-trypsin, cytokeratin 18, and delta-like 1.

The present ~~invention~~ disclosure also provides cell compositions comprising induced hepatocytes produced by the methods of the present ~~invention~~disclosure. In some embodiments, various compositions of induced hepatocyte, such as cell cultures or cell populations which are substantially free of cells other than hepatocytes are provided by the present ~~invention~~disclosure. In addition, compositions, such as cell cultures or cell populations that are enriched, isolated, or purified for hepatocytes are provided.

Compositions of induced hepatocytes provided by the present ~~invention~~ disclosure may comprise a homogeneous population of induced hepatocytes. In some ~~aspects~~embodiments, the present ~~invention~~ disclosure provides a composition comprising induced hepatocytes, wherein the composition comprises a homogeneous population of induced hepatocytes. In some embodiments, the present ~~invention~~ disclosure provides a composition of induced hepatocytes, wherein the percentage of cells within the composition comprises about 1%, about 5%, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% induced hepatocytes. In some embodiments, the percentage of cells in a composition comprising induced hepatocytes is 100%.

The present ~~invention~~ disclosure provides induced hepatocytes or populations of induced hepatocytes useful in various research and therapeutic applications. In some embodiments, the induced hepatocytes of the present ~~invention~~ disclosure are used as a model of various human diseases and disorders. In other embodiments, the induced hepatocytes of the present ~~invention~~ disclosure are used as models for hepatitis and other liver disease studies. The present ~~invention~~ disclosure provides induced hepatocytes useful for treating various degenerative liver diseases or inherited or acquired deficiencies of liver function. For example, the present ~~invention~~ disclosure provides methods for providing a cell-based therapy to a patient in need thereof, by administering to the patient a population of induced hepatocytes obtained by the methods disclosed herein. In certain

embodiments, the patient in need has liver disease or a liver disorder such as, for example, liver fibrosis, cirrhosis, liver failure, hepatitis, liver cancer, etc.

The present ~~invention~~ disclosure also provides methods for screening a drug candidate for toxicity (e.g., hepatotoxicity) using the induced hepatocytes obtained by the methods described herein. In some embodiments, the present ~~invention~~ disclosure provides methods of screening a drug candidate for toxicity or hepatotoxicity by contacting a population of induced hepatocytes with the drug candidate and monitoring the induced hepatocytes for toxicity or hepatotoxicity, thereby identifying whether the drug candidate is toxic.

The present ~~invention~~ disclosure further provides a reprogramming cell culture media, wherein the reprogramming cell culture media comprises DMEM/F12+Glutamax media, 10% fetal bovine serum (FBS), 1% Insulin-Transferrin-Selenium, 1% MEM Non-Essential Amino Acids, 5 mM HEPES buffer, 20 ng/ml human hepatocyte growth factor (HGF), 20 ng/ml epidermal growth factor (EGF), 20 ng/ml fibroblast growth factor 2 (FGF2), 200 ng/mL B18R, and 0.1 μM dexamethasone. In various ~~aspects~~ embodiments of the present methods, the non-hepatocyte cells are cultured under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, wherein the cell culture media is the reprogramming media described above.

In other ~~aspects~~embodiments, the present ~~invention~~ disclosure provides methods for identifying a factor that promotes reprogramming of non-hepatocyte cells into induced hepatocytes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 sets forth data showing expression and cellular localization of green fluorescent protein (GFP) and nuclear GFP (NLS GFP) in human neonatal foreskin fibroblasts transfected with various amounts of mRNA encoding GFP or nuclear GFP.

Figure 2 sets forth data showing reprogramming factor expression is maintained in human neonatal foreskin fibroblasts transfected daily for 9 days with a mixture of mRNAs encoding FOXA1, FOXA2, FOXA3, HNF4A, HNF1A, and GATA4.