

such as liver disease, by utilizing the MSC to produce factors beneficial in the treatment of liver disease. Such factors can include cytokines, for example IL-6. IL-6 is a pleiotropic cytokine, exerting a variety of effects on inflammation, liver regeneration, and defense against infections by regulating adaptive immunity. Due to its high abundance in inflammatory settings, IL-6 is often viewed as a detrimental cytokine. However, accumulating evidence supports the view that IL-6 has a beneficial impact in numerous liver pathologies, due to its roles in liver regeneration and in promoting an anti-inflammatory response in certain conditions. IL-6 promotes proliferation, angiogenesis and metabolism, and downregulates apoptosis and oxidative stress; together these functions are critical for mediating hepatoprotection. IL-6 is also an important regulator of adaptive immunity where it induces T cell differentiation and regulates autoimmunity. It can augment antiviral adaptive immune responses and mitigate exhaustion of T cells during chronic infection.

[0006] Disclosed examples comprise compositions for treating a patient, for example a human or non-human mammal, suffering from liver disease or symptoms thereof, said compositions comprising MSC derived from progenitor cells isolated from, for example, adipose tissue, umbilical cord, placental tissue, bone marrow, dental tissue, testicle tissue, uterine tissue, umbilical cord tissue, or skin tissue, that are allogeneic or autologous to a target patient; and a saline solution, wherein the composition can prevent, reduce, or eliminate the symptoms of liver disease in a target patient.

[0007] Disclosed examples comprise therapeutic use of “activated” MSC. For example, examples comprise purifying MSC with different abilities to maximize their therapeutic benefit for specific use, for example using cell-sorting procedures such as Magnetic-Activated Cell Sorting (MACS) or Fluorescence-Activated Cell Sorting (FACS). Further examples comprise activating MSC with specific stimulatory agents including, for example, cytokines, reactive proteins, chemicals, small molecules, and combinations thereof. These stimulatory agents can enhance or suppress MSC function; for example, immunosuppression by MSC can be induced by proinflammatory cytokines.

[0008] Disclosed examples comprise MSC that have been frozen and thawed. The MSC can comprise unactivated or activated MSC. In examples comprising the

use of activated then frozen MSC, the MSC can be activated again. In examples comprising the use of activated then frozen MSC, the activated MSC do not require additional activation.

[0009] Further examples comprise the use of MSC in combination treatments, for example the use of MSC in combination with a drug or pharmaceutical active agent or pharmaceutical composition. For example, disclosed examples comprise administration of MSC in combination with, for example, exosomes, such as purified exosomes.

[0009a] In one aspect, the present disclosure provides use of mesenchymal stem cells (MSC) in the manufacture of a medicament for treating alcoholic hepatitis wherein activated MSC is to be administered to a patient in need thereof, wherein administration of said activated MSC to the patient increases B cell lymphoma 2 (BCL2) expression of hepatocytes.

[0009b] In another aspect, the present disclosure provides an *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with at least one of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.

[0009c] In yet another aspect, the present disclosure provides an *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with at least two of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.

[0009d] In yet another aspect, the present disclosure provides an *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell

lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 shows MSC treatment rescued mortality of humanized FRG mice with alcoholic hepatitis (Overall survival). Alcohol binging Cohort shows a higher survival rate in MSC treated mice compared to PBS control group. Log-rank (Mantel-Cox) test $P < 0.0001$. Gehan-Breslow-Wilcoxon test $P < 0.0001$.

[0011] FIG. 2 depicts ALT and AST from Cohort 1; ALT and AST were measured by biochemical assays. Both AST and ALT levels were reduced after non-activated MSC repetitive injection into mice with alcoholic hepatitis. Student's T-test analyses demonstrated that post-treatment ALT and AST showed significantly lower values in MSC repetitive injection groups while PBS injection did not significantly alter the ALT and AST values.

[0012] FIG. 3 depicts activated MSC treatment rescued mortality of humanized FRG mice with alcoholic hepatitis (Overall Survival); FRG mouse survival data in presence / absence of MSC injection are shown. Log-rank (Mantel-Cox) test P value was 0.0130, log-rank test for trend P value was 0.0032. Gehan-Breslow-Wilcoxon test P value was 0.0270.

[0013] FIG. 4 depicts histological findings at death or sacrifice of humanized FRG mice with alcoholic hepatitis;

[0014] FIG. 4A. PBS control mice showing mild (1+) steatosis, HE x 100x.

[0015] FIG. 4B. PBS control mice showing mild (1+ steatosis), HE x 100 x.

What is claimed is:

- 1) Use of mesenchymal stem cells (MSC) in the manufacture of a medicament for treating alcoholic hepatitis wherein activated MSC is to be administered to a patient in need thereof, wherein administration of said activated MSC to the patient increases B cell lymphoma 2 (BCL2) expression of hepatocytes.
- 2) The use of claim 1 wherein said activated MSC comprise MSC activated with at least one of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17).
- 3) The use of claim 1 wherein said activated MSC comprise MSC activated with at least two of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17).
- 4) The use of claim 1 wherein said activated MSC comprise MSC activated with interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17).
- 5) The use of claim 1 wherein said patient is a mammal.
- 6) The use of claim 5 wherein said mammal is a human.
- 7) The use of claim 1, wherein said administration comprises at least one of subcutaneous, intra-lesional, intravenous, intra-peritoneal or intramuscular administration
- 8) The use of claim 7, wherein said MSC are autologous.
- 9) The use of claim 7, wherein said MSC are allogenic.
- 10) The use of claim 8 or 9 wherein said MSC are to be administered in a dose between 1×10^3 cells and 1×10^{12} cells.
- 11) The use of claim 10, wherein said dose comprises at least two doses.
- 12) The use of claim 11, wherein said dose comprises at least three doses.
- 13) The use of claim 12, wherein said dose comprises at least four doses.

- 14) The use of claim 13, wherein said dose comprises at least five doses.
- 15) An *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with at least one of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.
- 16) An *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with at least two of interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.
- 17) An *in-vitro* method of activating a cytokine-producing MSC, comprising stimulating the MSC with interferon gamma (IFN γ), Tumor Necrosis Factor alpha (TNF α), and interleukin-17 (IL-17), wherein administration of said activated MSC to a patient with alcohol hepatitis increases B cell lymphoma 2 (BCL2) expression of hepatocytes, thereby enhancing cell viability and promoting liver regeneration in the patient.
- 18) The method of any of claims 15-17, wherein said cytokine comprises at least one of IL-6, IL-17A, IFN gamma, TNF α , TGF β , MCP1, HGF, IL-8, TIMP-1, TIMP-2, VEGF, IDO, MIP-1b, and IL-10.