Adult stem cell media (ASCM) composition

Basal media
- Advance DMEM
- HEPEs
- L-Glutamine
- N-2 supplement
- B-27 supplement

Key components added
- EGF
- R-spondin1
- Nicotinamide
- Cholera endotoxin
- Dasatinib

Component	Regulated pathways
EGF	Activated EGFR and downstream signaling pathway
R-Spondin 1	Amplifies Wnt signaling pathway activation
Nicotinamide	Generates NAD+ and NADP+ which are coenzymes in enzymatic oxidation-reduction reactions in cellular processes such as glycolysis, citric acid cycle and electron transport chain
Cholera endotoxin	Activates adenylate cyclase and induces cAMP/PKA pathway
Dasatinib	Dual inhibitor of ABL and SRC family kinases

Figure 1

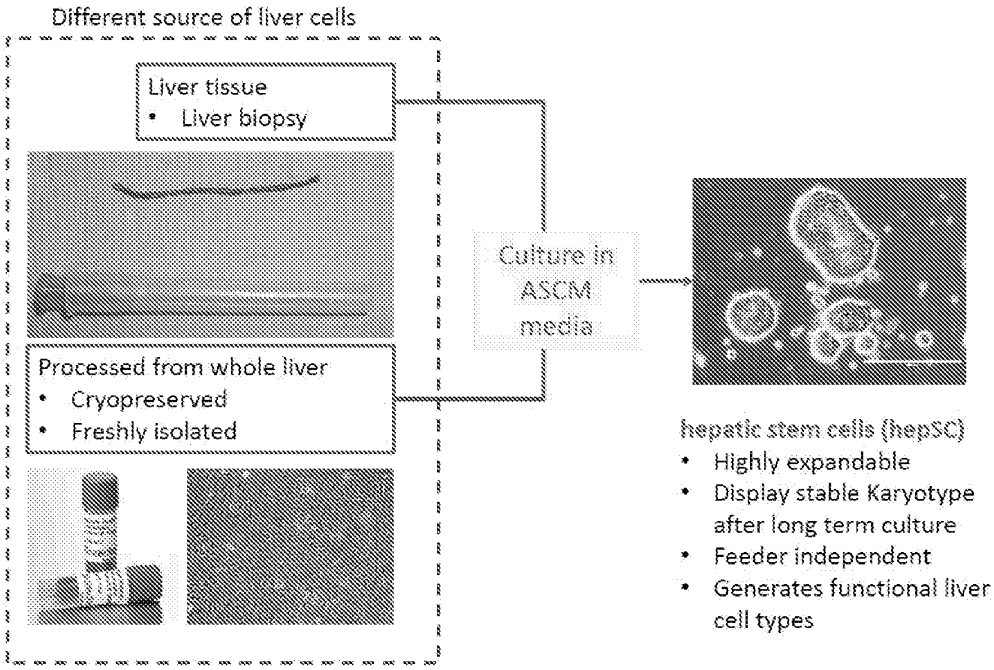

Different source of liver cells
Liver tissue
• Liver biopsy
Processed from whole liver
• Cryopreserved
• Freshly isolated
Culture in ASCM media
hepatic stem cells (hepSC)
• Highly expandable
• Display stable Karyotype after long term culture
• Feeder independent
• Generates functional liver cell types

Figure 2

Protocol overview: Derivation of stem cells from adult liver tissue biopsy (human and mouse)

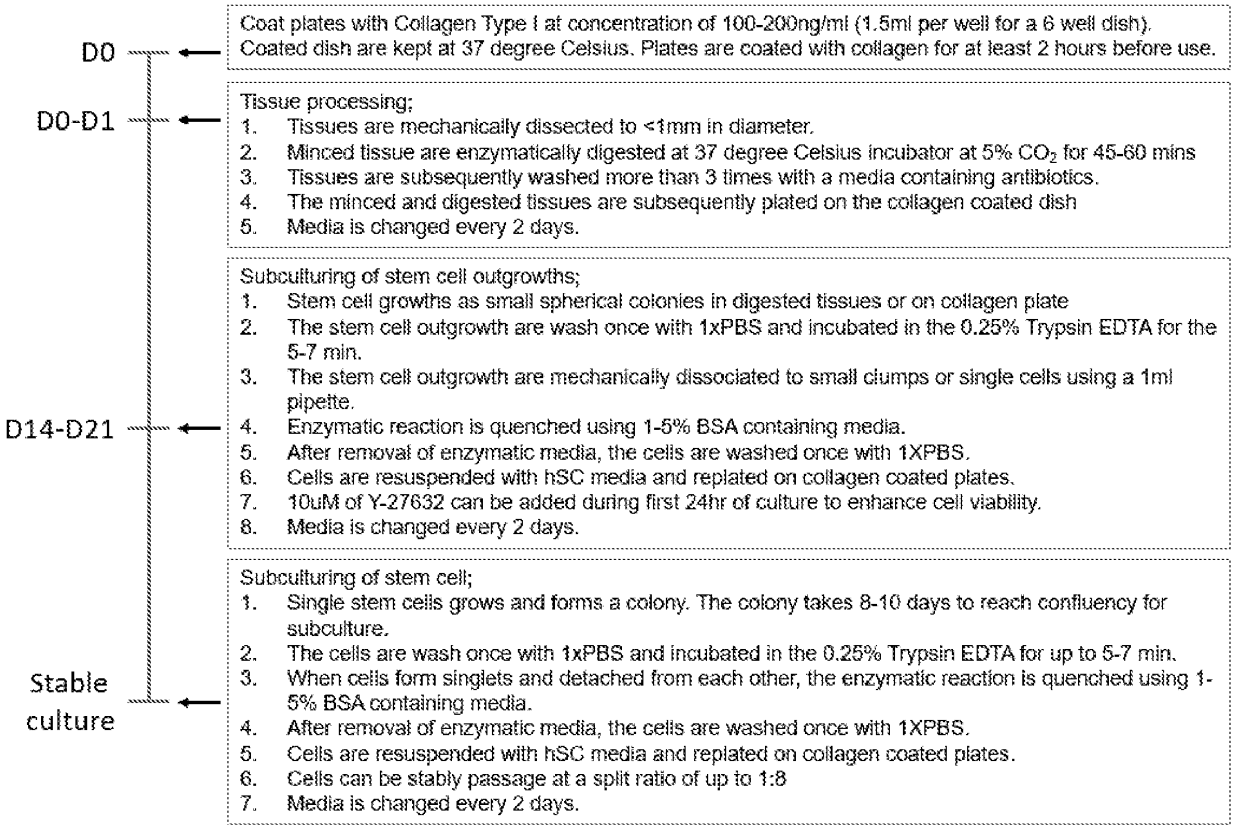


Figure 3

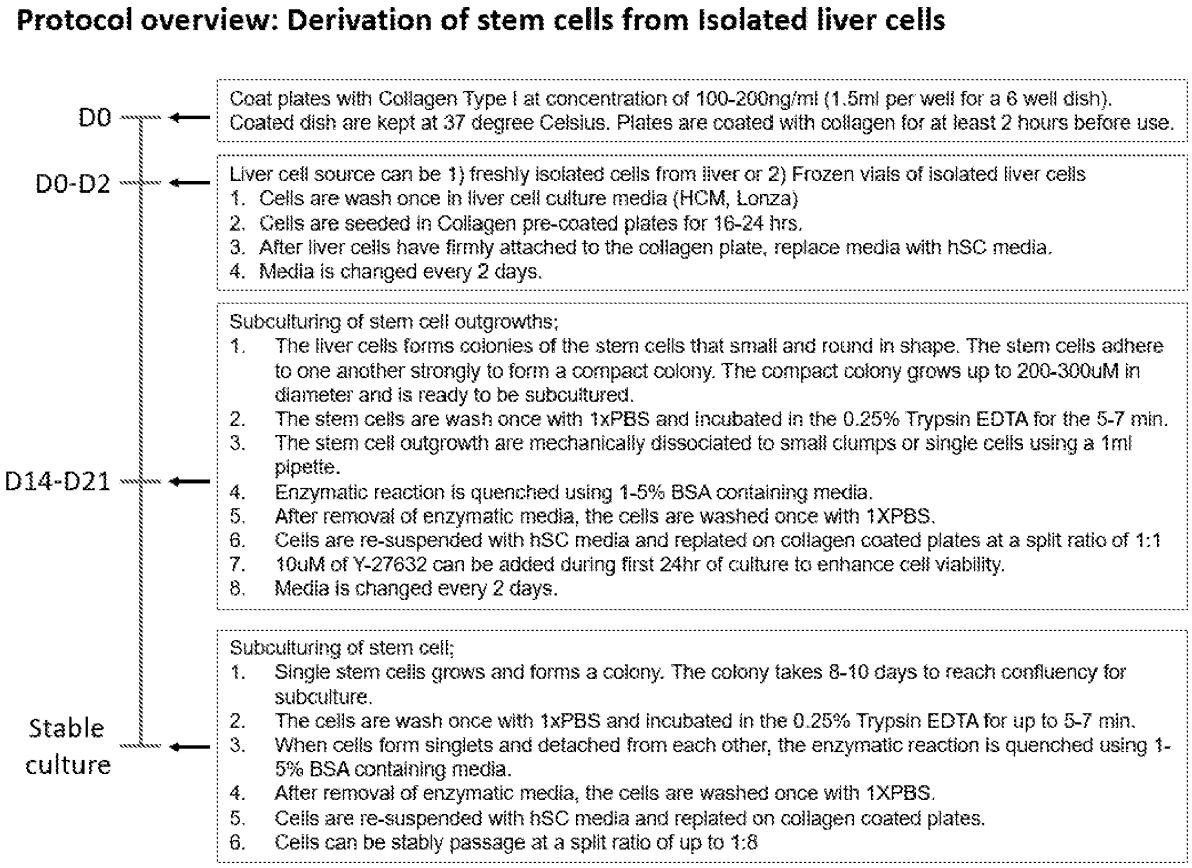

Protocol overview: Derivation of stem cells from Isolated liver cells
D0
Coat plates with Collagen Type I at concentration of 100-200ng/ml (1.5ml per well for a 6 well dish). Coated dish are kept at 37 degree Celsius. Plates are coated with collagen for at least 2 hours before use.
D0-D2
Liver cell source can be 1) freshly isolated cells from liver or 2) Frozen vials of isolated liver cells
1. Cells are wash once in liver cell culture media (HCM, Lonza)
2. Cells are seeded in Collagen pre-coated plates for 16-24 hrs.
3. After liver cells have firmly attached to the collagen plate, replace media with hSC media.
4. Media is changed every 2 days.
D14-D21
Subculturing of stem cell outgrowths;
1. The liver cells forms colonies of the stem cells that small and round in shape. The stem cells adhere to one another strongly to form a compact colony. The compact colony grows up to 200-300uM in diameter and is ready to be subcultured.
2. The stem cells are wash once with 1xPBS and incubated in the 0.25% Trypsin EDTA for the 5-7 min.
3. The stem cell outgrowth are mechanically dissociated to small clumps or single cells using a 1ml pipette.
4. Enzymatic reaction is quenched using 1-5% BSA containing media.
5. After removal of enzymatic media, the cells are washed once with 1XPBS.
6. Cells are re-suspended with hSC media and replated on collagen coated plates at a split ratio of 1:1
7. 10uM of Y-27632 can be added during first 24hr of culture to enhance cell viability.
8. Media is changed every 2 days.
Stable culture
Subculturing of stem cell;
1. Single stem cells grows and forms a colony. The colony takes 8-10 days to reach confluency for subculture.
2. The cells are wash once with 1xPBS and incubated in the 0.25% Trypsin EDTA for up to 5-7 min.
3. When cells form singlets and detached from each other, the enzymatic reaction is quenched using 1-5% BSA containing media.
4. After removal of enzymatic media, the cells are washed once with 1XPBS.
5. Cells are re-suspended with hSC media and replated on collagen coated plates.
6. Cells can be stably passage at a split ratio of up to 1:8

Figure 4

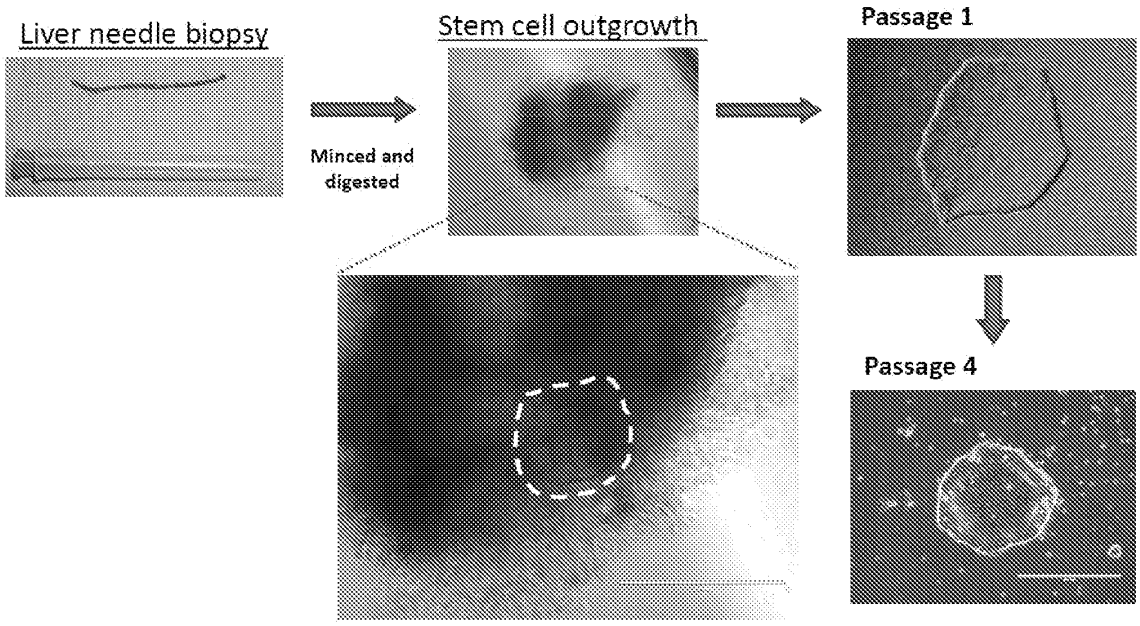

Liver needle biopsy
Minced and digested
Stem cell outgrowth
Passage 1
Passage 4

Figure 5

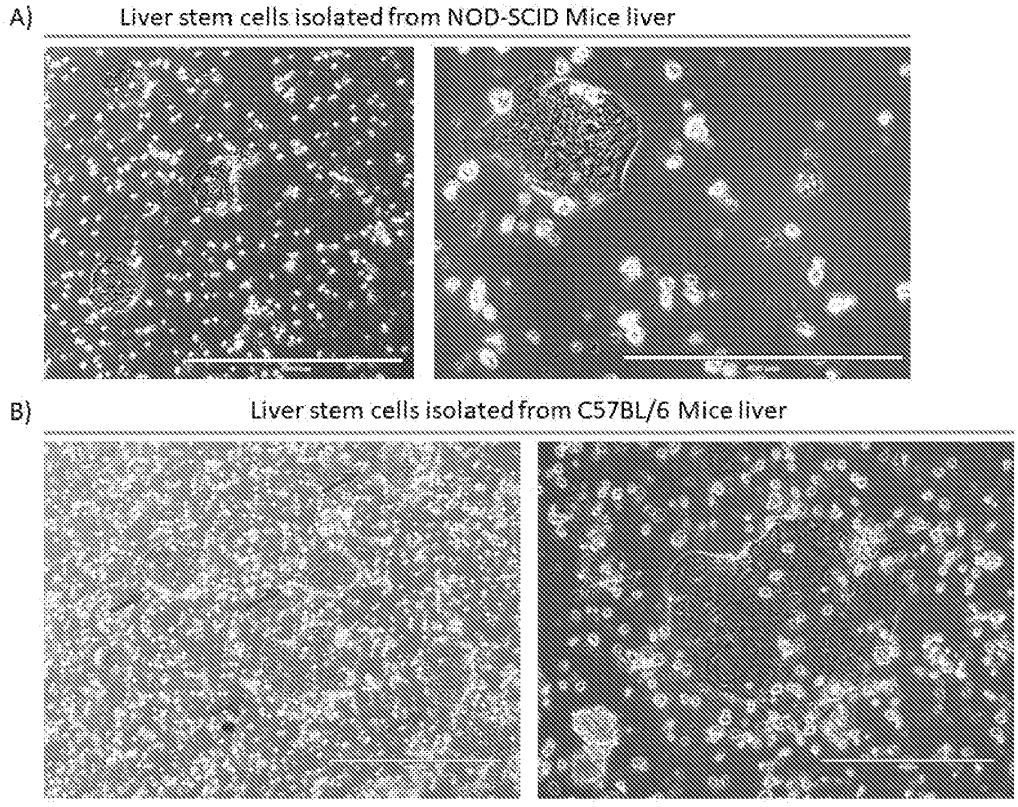

A)
Liver stem cells isolated from NOD-SCID Mice liver
B)
Liver stem cells isolated from C57BL/6 Mice liver

Figure 6

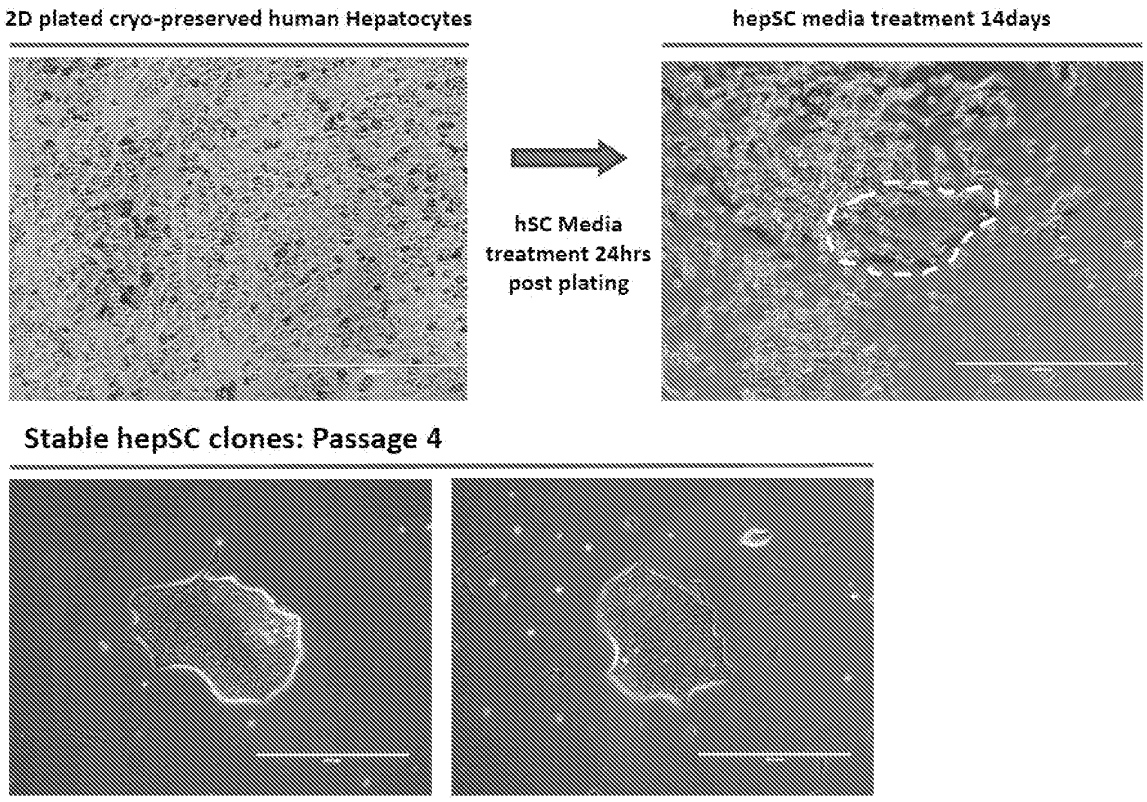

2D plated cryo-preserved human Hepatocytes
hepSC media treatment 14days
hSC Media
treatment 24hrs
post plating
Stable hepSC clones: Passage 4

Figure 7

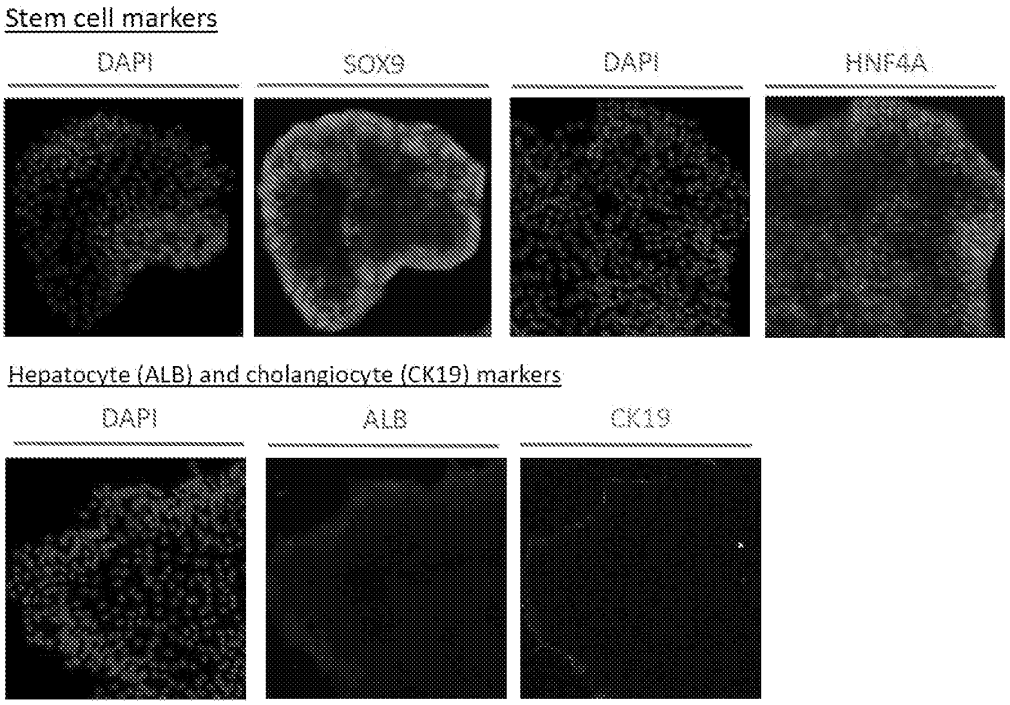

Stem cell markers
DAPI
SOX9
DAPI
HNF4A
Hepatocyte (ALB) and cholangiocyte (CK19) markers
DAPI
ALB
CK19

Figure 8

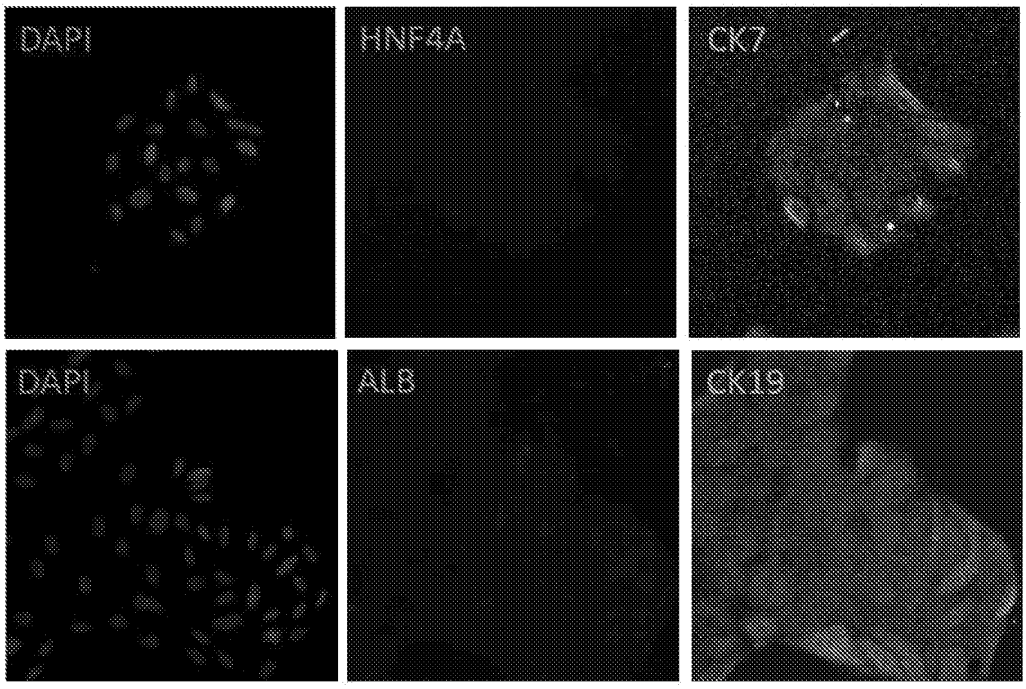


Figure 9

Chemicals	Pathway targeted
Dasatinib	BCR-ABL tyrosine kinase inhibitor, also reported to inhibit SRC, c-KIT, PDGF and EPHA
Imatinib	BCR-ABL tyrosine kinase inhibitor, also reported to inhibit c-KIT and PDGF
Nilotinib	BCR-ABL tyrosine kinase inhibitor, also reported to inhibit c-KIT and PDGF

Figure 10

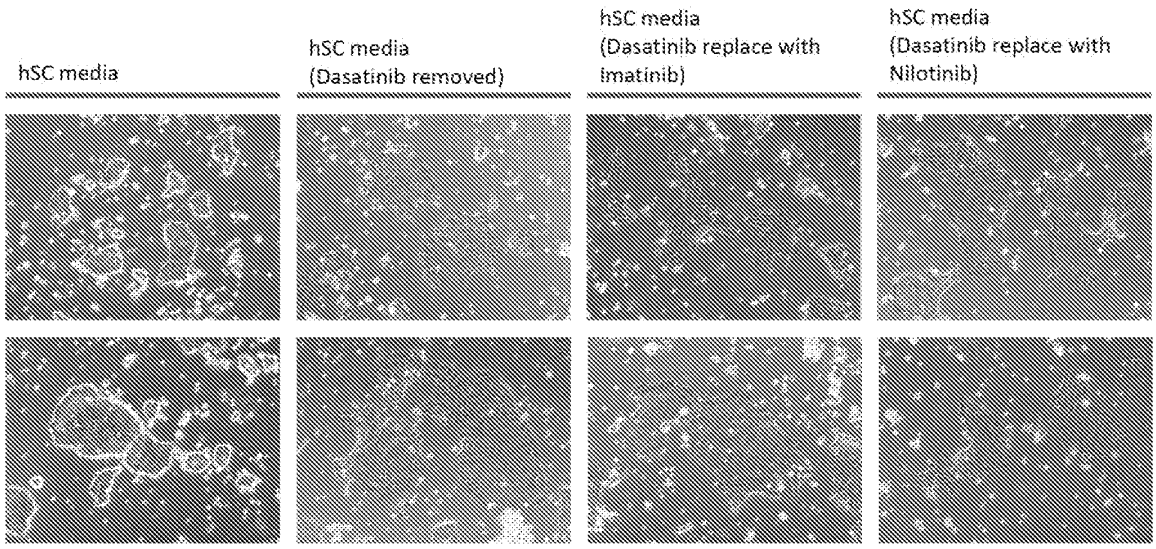

hSC media
hSC media
(Dasatinib removed)
hSC media
(Dasatinib replace with
Imatinib)
hSC media
(Dasatinib replace with
Nilotinib)

Figure 11

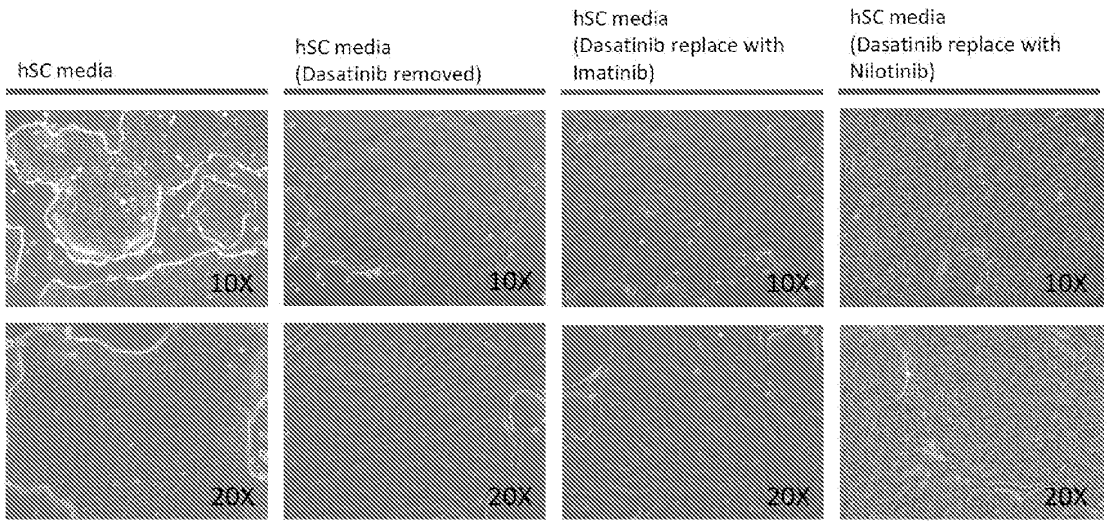

hSC media
hSC media
(Dasatinib removed)
hSC media
(Dasatinib replace with
Imatinib)
hSC media
(Dasatinib replace with
Nilotinib)
10X
10X
10X
10X
20X
20X
20X
20X

Figure 12

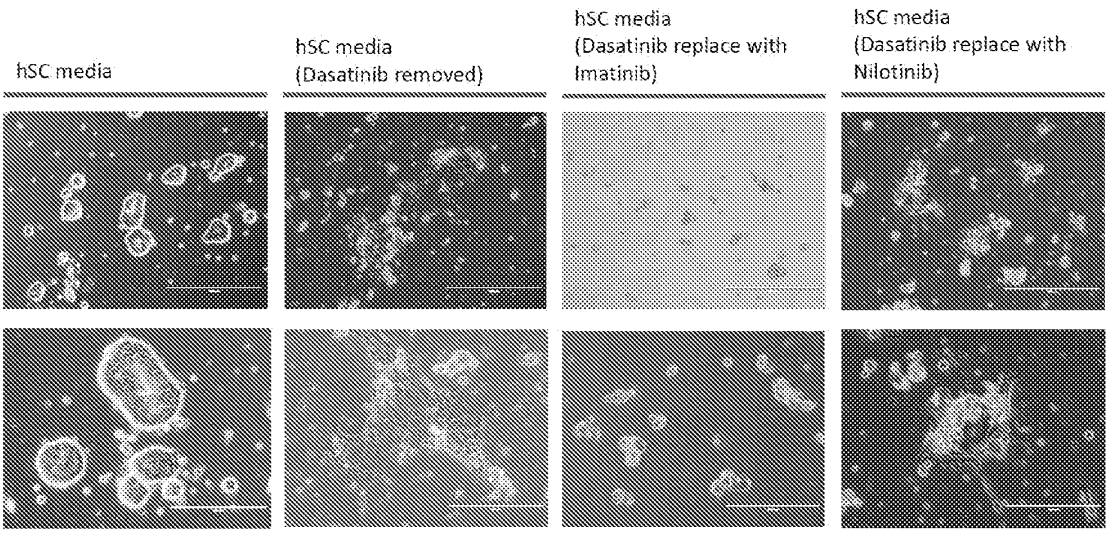

hSC media
hSC media
(Dasatinib removed)
hSC media
(Dasatinib replace with
Imatinib)
hSC media
(Dasatinib replace with
Nilotinib)

Figure 13

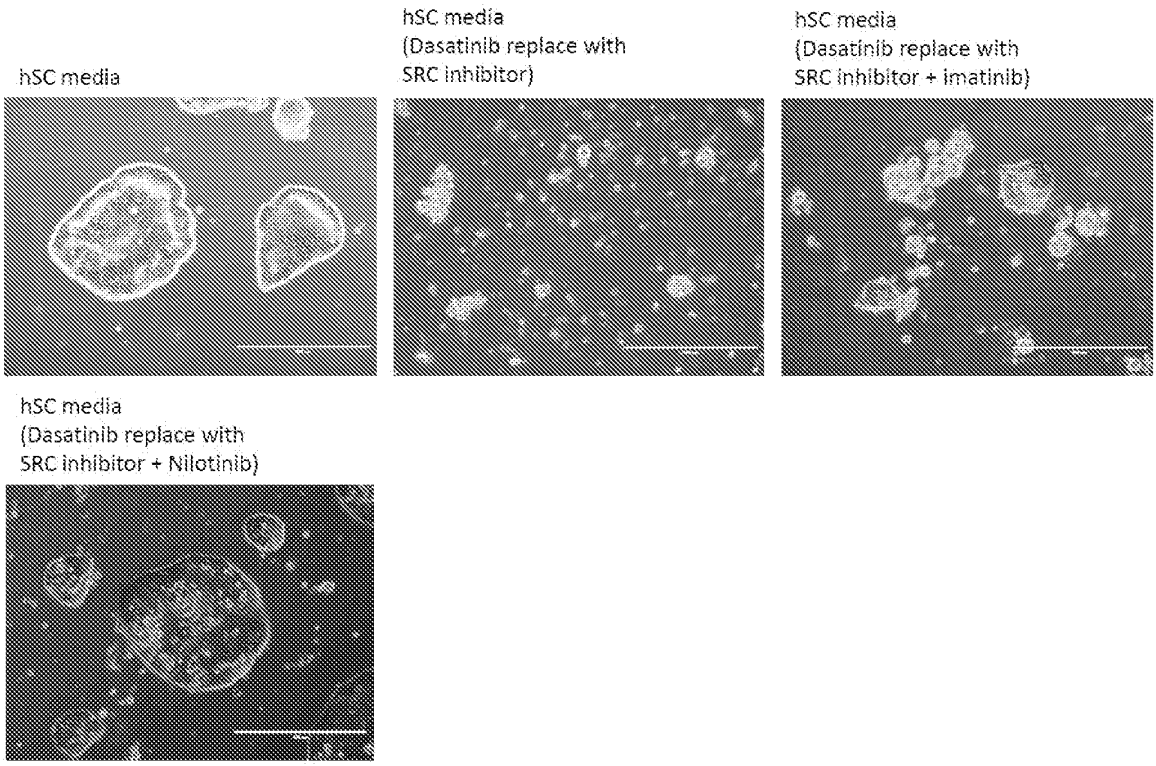

hSC media
hSC media
(Dasatinib replace with
SRC inhibitor)
hSC media
(Dasatinib replace with
SRC inhibitor + imatinib)
hSC media
(Dasatinib replace with
SRC inhibitor + Nilotinib)

Figure 14

Basal media
- Advance DMEM
- HEPEs
- L-Glutamine
- N-2 supplement
- B-27 supplement

Key components added
- EGF
- R-spondin1
- Nicotinamide
- Cholera endotoxin
- Dasatinib

Component	Regulated pathways
EGF	Activated EGFR and downstream signaling pathway
R-Spondin 1	Amplifies Wnt signaling pathway activation
Nicotinamide	Generates NAD+ and NADP+ which are coenzymes in enzymatic oxidation-reduction reactions in cellular processes such as glycolysis, citric acid cycle and electron transport chain
Cholera endotoxin	Activates adenylate cyclase and induces cAMP/PKA pathway
Dasatinib	Dual inhibitor of ABL and SRC family kinases

Figure 15

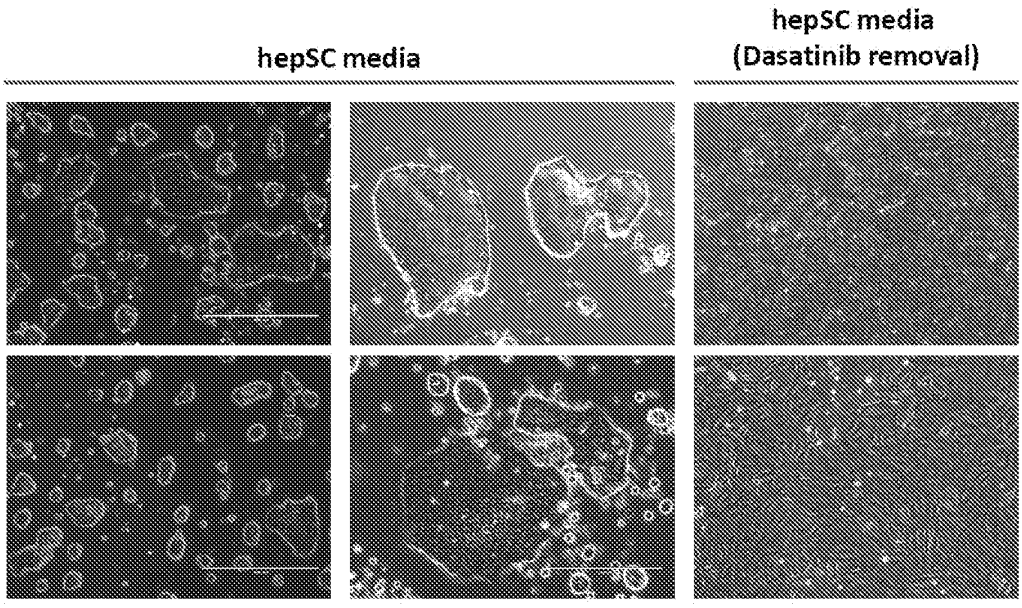


Figure 16

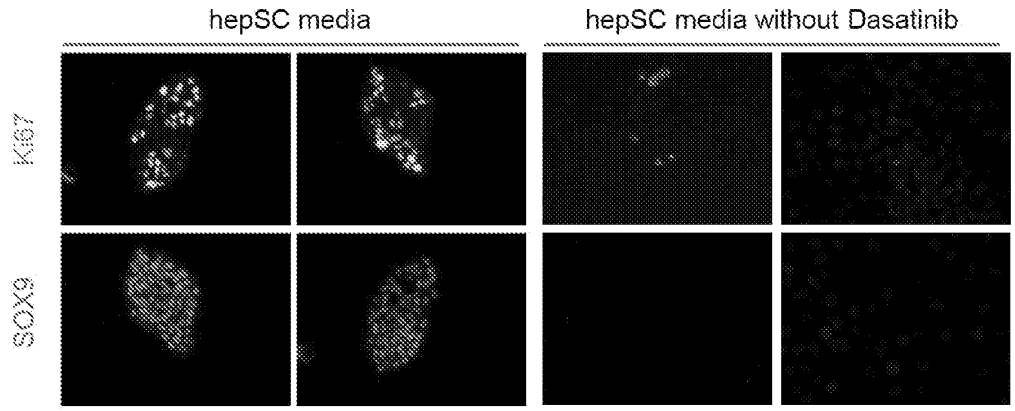


Figure 17

Basal media
- Advance DMEM
- HEPEs
- L-Glutamine
- N-2 supplement
- B-27 supplement

Key components added
- EGF
- R-spondin1
- Nicotinamide
- Cholera endotoxin
- Dasatinib

Optional molecules
- CHIR-99014

Component	Regulated pathways
EGF	Activated EGFR and downstream signaling pathway
R-Spondin 1	Amplifies Wnt signaling pathway activation
Nicotinamide	Generates NAD+ and NADP+ which are coenzymes in enzymatic oxidation-reduction reactions in cellular processes such as glycolysis, citric acid cycle and electron transport chain
Cholera endotoxin	Activates adenylate cyclase and induces cAMP/PKA pathway
Dasatinib	Dual inhibitor of ABL and SRC family kinases

Figure 18

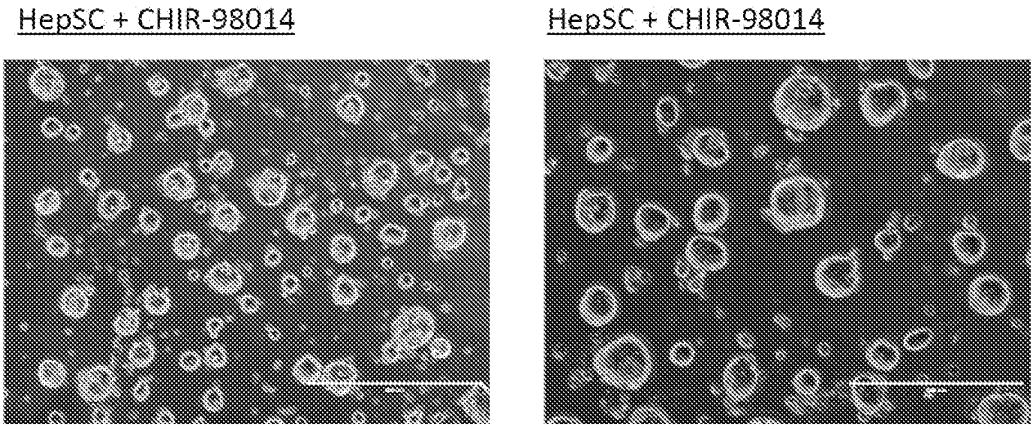


Figure 19