# Hepatic Stem Cell

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**Abstract**: The liver in an animal adult healthy body maintains a balance between cell renew and cell loss. The cell damage can stimulate the new cell growth and in liver it is hepatocyte self-replication. The transplanted hepatocyte can undergo clonal expansion which shows that the hepatocytes themselves can play the function as stem cells in the liver. Severe liver injury can activate a potential stem cell compartment located within the intrahepatic biliary tree, giving rise to cords of bipotential within the lobules that can differentiate into hepatocytes and biliary epithelial cells. A third population of stem cells with hepatic potential resides in the bone marrow; these haematopoietic stem cells can contribute to regeneration and restore normal liver function.
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The liver in an animal adult healthy body maintains a balance between cell renew and cell loss. The cell damage can stimulate the new cell growth and in liver it is hepatocyte self-replication. The transplanted hepatocyte can undergo clonal expansion which shows that the hepatocytes themselves can play the function as stem cells in the liver. Severe liver injury can activate a potential stem cell compartment located within the intrahepatic biliary tree, giving rise to cords of bipotential (oval cells) within the lobules that can differentiate into hepatocytes and biliary epithelial cells. A third population of stem cells with hepatic potential resides in the bone marrow; these haematopoietic stem cells can contribute to regeneration and restore normal liver function (Forbes, et al, 2012). The hepatic stem cell is also called liver stem cell.

Bone marrow is the site of hematopoiesis and bone marrow transplant has been successfully used for decades as a means of treating various hematological malignancies in which the recipient hematopoietic compartment is replaced by donor-derived stem cells. Progenitor cells in bone marrow are capable to differentiate into other tissues, such as cardiac tissue. Clinical trials have been conducted demonstrating beneficial effects of bone marrow infusion in cardiac patients. It is believed that injured tissue, whether neural tissue after a stroke, or injured cardiac tissue, has the ability to selectively attract bone marrow stem cells, perhaps to induce regeneration. Bone marrow has therapeutic effect in conditions ranging from liver failure, to peripheral artery disease, and the possibility of using bone marrow stem cells in kidney failure has been relatively understudied (Ma et al. 2009). Liver stem cell, or oval cells, differentiate into both hepatocytes and cholangiocytes during chronic liver injury.

Firstly, under certain circumstances, bone marrow engraftment in tubules can be dramatically increased. Held et al. made use of a transgenic fumarylacetoacetate (FAH)-/- mouse, in which discontinuation of the rescue drug NTBC leads to acute tubular necrosis (Held et al. 2006). After transplanting bone marrow from wild-type mice into FAH-/- mice, a few bone marrow-derived tubular cells are noted. In a subset of the FAH-/- mice, there is, in addition, loss of heterozygosity (LOH) in the liver for homogentistic acid hydrogenase, which induces a more severe, ongoing form of acute tubular necrosis. In FAH-/- animals with additional hepatic LOH, up to 50% of tubular cells are bone marrow-derived cells. Engraftment of these wild-type bone marrow-derived cells leads to morphological resolution of ATN and to disappearance of the aminoaciduria present in control mice. In this model, the bone marrow cells have a strong survival advantage over native tubular cells, due to their ability to metabolise toxic products. It is possible that this strong positive selective pressure is necessary for regeneration to occur through wild-type bone marrow cells. Interestingly, most of the bone marrow-derived tubular cells are derived from cell fusion between bone marrow cells and tubular cells. This is supported by a study by Li et al. in which fusion of bone marrow cells to tubular cells account for part of bone marrow-derived tubular cells after ischaemia/reperfusion (I/R) injury, but not all. In this model without selective pressure, the percentage of bone marrow-derived tubular cells is low (1.8%) (Li et al. 2007b).

In addition, injection of stem cells into the kidney or the bloodstream can lead to an improvement of renal function, although this does not always seem to be mediated by transdifferentiation into renal cells. Current views favour a predominant role for the

delivery of a cocktail of angiogenic and immunomodulatory mediators as the main means by which bone marrow cells enhance epithelial and endothelial cell survival. As far as engraftment of bone marrow cells as renal parenchymal cells is concerned, proving functionality of the engrafted bone marrow-derived cells is crucial in order to assign to them a role in improved renal function, rather than relying on morphological observations alone.

Primary liver cells (hepatocytes) are the liver's chief functional cells that perform most of the liver's complex metabolic tasks. It is possible to grow hepatocytes that could provide significant biomedical advantages, including cell therapy, and the liver stem cells are good candidate. For the patients suffering from liver cancer, the liver stem cells could be safe and credible source of liver cells as an alternative to liver transplant. Growing liver cells form liver stem cells could also be useful in detecting safe drug screening methods.

Stem cell therapy is already a popular intervention for treating blood and immune system conditions, as well as numerous skin diseases. It involves a careful medical procedure of introducing adult cells that are grown from early cells or stem cells, into damaged tissues. Liver stem cells are the precursors for a subset of liver cancer, hepatocellular carcinoma.

The possibility of liver stem cell therapy, especially for those with chronic liver diseases, could definitely ease the worry of hundreds of people anxiously waiting for liver transplants. Selecting CD45 negative cells is used in the isolation of liver stem cells. Magnetic antibody metod is applied in the isolation liver stem cells.

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocellular carcinoma (ICC) are common liver tumors. Originating from one pluripotent liver stem cell both tumor entities can occur in a cirrhotic liver. According to Wengert et al report, several risk factors have been identified as causative for both carcinomas. Surgical resection, interventional procedures and transplantation are available as curative treatment options when diagnosed in time. The common characteristic features and morphology in cross-sectional imaging by ultrasound (US), multidetector computed tomography (CT) and magnetic resonance imaging (MRI) were used. It showed a better understanding of the carcinogenesis model of both liver tumors originating from one pluripotent liver stem cell (Wengert, et al, 2014).

The precursor to oval cells is considered to be a facultative liver stem cell (LSC). Recent lineage tracing experiments indicated that the LSC is SRY-related HMG box transcription factor 9 positive (Sox9(+) ) and can replace the bulk of hepatocyte mass in several settings. Quantitative analysis showed that Sox9(+) cells contributed only minimally (<1%) to the hepatocyte pool, even in classic oval cell injury models (Tarlow, et al, 2014).

Glycogen storage disease type I (GSDI), an inborn error of carbohydrate metabolism, is caused by defects in the glucose-6-transporter/glucose-6-phosphatase complex, which is essential in glucose homeostasis. With the feasibility of novel cell-based therapies, including hepatocyte transplantations and liver stem cell transplantations, it is essential to consider long term outcomes of liver replacement therapy (Boers, et al, 2014).

A method for the feeder-independent culture of PICM-19 pig liver stem cell line was recently devised, but the cell line's growth was finite and the cells essentially ceased dividing after approximately 20 passages over a 1 year culture period. Talbot and Caperna reported the isolation, continuous culture, and initial characterization of a spontaneously arising feeder-independent PICM-19 subpopulation, PICM-19FF, that maintained replication rate and hepatocyte functions over an extended culture period. Accordign to Talbot and Caperna's report, PICM-19FF cells grew to 90-98 % confluency after each passage at 2 week intervals, and the cells maintained a high cell density after 2 years and 48 passages in culture (average of 2.6 × 10(6) cells/T25 flask or 1 × 10(5) cells/cm(2)). Morphologically, the PICM-FF cells closely resembled the finite feeder-independent PICM-19 cultures previously reported, and, as before, no spontaneous formation of 3D multicellular ductules occurred in the cells' monolayer. Their bipotent stem cell nature was therefore not evident (Talbot and Caperna, 2014).

Saito, et al studied a 43-year-old man patient with chronic hepatitis B without history of hepatocellular carcinoma (HCC) and first diagnosed with thrombosis in right portal vein trunk and portal vein branches and ruptured esophageal varices in October 2011, and underwent endoscopic variceal ligation, but ruptured repeatedly. Computed tomography (CT) scan showed that portal vein thrombosis had low density from early to late phase. This is the first case of HCC only in portal vein without liver parenchyma tumor nodules, with difficult differential diagnosis from a non-malignant portal vein thrombosis (Saito, et al, 2013).

Various stem cell populations have been described in distinct models of liver regeneration (Dahlke, et al, 2004).

One example of the protocols for the liver stem cells analysis (Rountree, et al, 2015):

**1. Parenchymal and non-parenchymal separation from whole liver**

1) Prepare the enzyme solution for digestion using a 15 cc tube with screw top in 10 ml sterile PBS containing 5 mg collagenase, 5 mg pronase, and 1 mg DNAse.
2) Euthanize mouse using institution approved (Institutional Animal Care and Use Committee) method such as $CO_2$ asphyxiation. Wipe abdominal area of euthanized animals with 70% ethanol solution. Using sterile instruments, open abdominal cavity and explant liver en-block. Remove gall-bladder from explanted liver. Transfer whole liver to laminar flow hood in closed sterile dish.
3) This procedure is completed in a laminar flow hood. Using a sterile razor blade, mince liver with combination of multiple horizontal and vertical cuts for 1 minute in sterile dish. Place ¼ of minced liver pulp in 15 cc tube with 10 cc PBS with collagenase, pronase, and DNAse from step 1.1 above. Repeat with each ¼ minced liver.
4) Place tubes with ¼ minced liver pulp and enzymes into water bath at 37°C for 45 minutes, with shaker at 1-2 cycles/second. Wipe tubes with 70% ethanol after removal from water bath prior to transfer to laminar flow hood.
5) This procedure is completed in a laminar flow hood. Strain digested liver pulp through 70 micron mesh filter to collect into a sterile dish. Using 2 ml aliquots of sterile DMEM:F12 media with 10% heat inactivated fetal bovine serum, rinse the filter and use the rubber end of a syringe plunger to mash the digested pulp through the filter. Repeat 5 times to make total volume of filtrate approximately 20 mL. Divide the filtrate into 2 equal 15 mL tubes.
6) All transfers should be completed in laminar flow hood, and use refrigerated centrifuge at 4°C if available. Centrifuge at 50 x g for 1 minute. Save supernatant #1 and discard parenchymal pellet Centrifuge supernatant #1 at 50 x g for 1 minute. Save supernatant #2 and discard pellet. Centrifuge supernatant #2 at 50 x g for 1 minute. Save supernatant #3 and discard pellet. Centrifuge final supernatant #3 for 180 x g for 8 minute to obtain non-parenchymal fraction.

**2. Red cell lysis**

Work in laminar flow hood, keep cells cold, and use solutions cooled to 4°C.

1) The night before the procedure, prepare red cell lysis buffer by diluting 10X concentration stock BD Pharm Lyse buffer with a 1:10 dilution with sterile distilled water. 1X solutionshould be stored for 30 days at 4°C.
2) The night before the procedure, prepare Miltenyi buffer using sterile PBS, 0.5% bovine serum *Albumin*, and 2mM EDTA. Filter solution using vacuum filter unit with 0.45 micron filter. Cover top of tilter unit with original plastic lid and secure with plastic wrap. Storeentire filter unit at 4°C for 12 hours to degas EDTA. Filter unit can be replaced with standard sterile cap after 12 hours.
3) This procedure is completed in a laminar flow hood. Using a 5 ml sterile tube, re-suspend non-parenchymal pellet from step 1.6 above into 1 mL of 1X diluted red blood cell lysis buffer from 2.1 above. Cap the tube for transfer out of the laminar flow hood.
4) Gently vortex for 5seconds and incubate for 15 minutes at 4°C protected from light.
5) Centrifuge at 200 x g for 5 minutes.
6) This procedure is completed in a laminar flow hood. Discard lysed RBCs in supernatant, and re-suspend pellet in 1 ml ice-cold and sterile Miltenyi buffer.
7) Centrifuge at 200 x g for 5 minutes.
8) This procedure is completed in a laminar flow hood. Discard supernatant and re-suspend pellet in 1 ml ice-cold and sterile Miltenyi buffer.
9) Remove 10 µl of PBS cell suspension and add 10 µl tryan blue. Count remainingnon-parenchymal cells using hemocytometer.

**3. CD45 hematopoietic cell depletion from non-parenchymal fraction**

Work in laminar flow hood, keep cells cold, and use solutions cooled to 4°C.

1) Suspend cells in 100 µL of Miltenyi buffer per 107 cells up to 108 total cells.
2) Apply 20 µL Miltenyi CD45 microbead antibody for each 107 cells and incubate at 4°C for 15 minutes.
3) Add additional 2 ml Miltenyi buffer and centrifuge at 200 x g for 5 minutes. Remove supernatant. Re-suspend cell pellet (up to 108 total cells) in 1 ml Miltenyi buffer.
4) In laminar flow hood, filter cells using Miltenyi LD magnetic column. Start by placing column in magnetic holder (Miltenyi MidiMACS or QuadroMACS). Place sterile 5 ml tube below filter to catch filtrate. Prepare column by loading 2 ml Miltenyi buffer.
5) Once pre-filter wash is complete, load cells onto LD column. Once the cell suspension is within the column, add 1 ml Miltenyi buffer and repeat 1 ml Miltenyi buffer wash 2 additional times. Do NOT use plunger provided with column to increase speed of filtration. ONLY collect filtrate when the filter in placed in the magnetic holder.

6) Centrifuge the collected filtrate of approximately 5 ml (the column holds the remaining 1 ml) of CD45-depleted non-parenchymal cells at 200 x g for 5 minutes. Discard the column with the retained CD45 positive cells.

**4. Flow cytometry isolation of CD133 positive cells**

1) Prepare oval cell media. Use 1:1 DMEM:F12 medium with 10 % heat inactivated fetal calf serum as base, and add insulin (1 µg/ml), HEPES (5 mol/L), and Penicillin/Streptomycin (1% volume/volume). Filter solution using vacuum filter unit with 0.45 micron filter.
2) Re-suspend cells in Miltenyi buffer at 100 µL per $10^7$ cells. Add 2 µL of CD133-PE conjugated antibody. Using a second group of cells, incubate with IgG-PE conjugated antibody as a control. Retain a third group of cells without staining as an unstained control for FACS.
3) Incubate at 4°C for 15 minutes in the dark. Re-suspend in 2 ml staining buffer. Centrifuge at 200 x g for 5 minutes. Discard supernatant and re-suspend pellet in 1 ml Miltenyi buffer.
4) This step is conducted using standard flow cytometry cell sorting procedures, which may be institution specific. Using unstained cells and IgG PE stained cells, adjust sorting parameters for optimized gating of CD133+ cell population. PE (R-Phytoerythrin) can generally be used with any flow cytometer that has a laser that emits at 488 nm. The peak emission for PE is 575 nm and is detected in the FL-2 channel. Note: Using a BD FACS Calibur or BD FACS Vantage machines, with the Cell Quest program for data collection, we use Forward Scatter and Side Scatter view in the log scale to identify cell populations, with side scatter set to 250. FL1 and FL2 are both in log scale and set to 550. These parameters provide an initial starting place to view liver non-parenchymal cells, and are adjusted as needed based on staining intensity of positive and negative populations.
5) Isolate the CD133+ cell population using CD133+ gate and collect the cells in sterile filtered cell media.

**5. Cell culture methods**

1) Centrifuge FACS collected cells at 200 x g for 5 minutes. Re-suspend cell pellet in oval cell media with approximately 5000 cells per ml. May start with higher concentrations, up to 50,000 cells/ml for initial experiments, and reduce as technique improves and overall cell viability and yield improves.
2) Plate cells onto BD Biocoat Laminin coated 96 well plates using 1000 cells/$cm^2$. Place in humidified cell culture incubator at 37°C, with 5% $CO_2$. After 24 hours, add Hepatocyte Growth Factor (50 ng/nl) and Epidermal Growth Factor (20 ng/ml).
3) For single cells, isolate cells directly into 50 µl of oval cell media in each well of a 96 well Laminin coated plate. Use single cell FACS settings for strict selection of one positive cell only. After 24 hours, add 50 µl of oval cell media with HGF and EGF as above in step 5.2. Change media fully after 5-7 days.
4) Once the expanding colonies are greater than 50% confluent, which typically occurs after 2 weeks, depending on total number of cells plated and cell viability, the cells may be split 1:3 as below.
5) Split cells using Trypsin 0.05%-EDTA. Apply just enough to cover well bottom, 50-100 µL/well on 96 well plate Place in incubator at 37°C for 3-5 minutes.
6) Add 100 µL of media to each well and transfer all liquid to 5 ml tube. Add 1 mL media to each tube and centrifuge at 200 x g for 5 minutes.
7) Re-suspend cells in media plate in laminin coated dish, using cells from 1 well to plate into 3 new wells (1:3 ratio).

**6. Confirmation of bi-potential status using RT-PCR**

This procedure is detailed in the RNeasy protocol handbook, which is supplied with the RNeasy Kit.

1) Use RNeasy micro columns for 96 well plate colonies. Aspirate culture media from each well. Add 75 µl Buffer RLT (from RNeasy Kit) directly to each well. Scrape plate bottom with sterile rubber policeman. Pipettelysate into micro-centrifuge tube and vortex mixture for 1 minute. Add 70% ethanol to lysates and mix by pipetting.
2) Transfer the solution to RNeasy column placed in a 2 ml collection tube (as supplied in RNeasy Kit) and centrifuge in micro-centrifuge for 15 seconds at 10,000 rpm. Discard eluted filtrate.
3) Re-use the collection tube. Add 350 µL Buffer RW1 (RNeasy Kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the column membrane. Discard eluted wash.
4) Add 10 µL DNase I stock solution to 70 µL Buffer RDD (both supplied in RNeasy Kit).Add the 80 µL DNase I Buffer RDD incubation mix to the RNeasy column membrane and incubate for 15 minutes at room temperature.
5) Add 350 µL Buffer RW1 (RNeasy kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the membrane. Discard eluted wash and collection tube.

6) Place the RNeasy column in a new 2 ml collection tube (supplied in RNeasy kit). Add 500 µL Buffer RPE(RNeasy Kit) to the RNeasy column and centrifuge for 15 seconds at 10,000 rpm to wash the membrane. Discard eluted wash.
7) Prepare 80% ethanol using RNase-free water (RNeasy Kit). Add 500 µL of 80% ethanol to the RNeasy columnand centrifuge for 2 minutes at 10,000 rpm. Discard eluted wash.
8) Place the RNeasy column in a new 2 ml collection tube (RNeasy Kit) and centrifuge at full speed for 5 minutes. Discard any eluted wash and collection tube.
9) Place the RNeasy column in a new 1.5 ml collection tube (RNeasy kit). Add 14 µL RNase-free water (RNeasy Kit) to the center of the column membrane and centrifuge for 1 minute at full speed. Collect filtrate with purified RNA and transfer to ice if creating cDNA immediately or store at minus 80°C for future use.
10) Reverse transcription using Omniscript.Dilute RNase inhibitor to a final concentration of 10 units/ µL in ice-cold 1x Buffer RT Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Prepare a fresh master mix on ice according to page 13 of Omniscript protocol. Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Recommend to prepare a volume of mastermix 10% greater than that required for the total required for all reactions.
11) Add template RNA to the individual tubes containing master mix. Vortex for 5 seconds, and pulse centrifuge for 5 seconds. Incubate for 60 min at 37°C.
12) PCR amplification of hepatocyte (*Albumin*) and cholangiocyte specific genes using HotStarTaq DNA polymerase Prepare reaction mix per page 15 of HotStarTaq protocol book. Recommend diluting stock primers to concentration of 20 pm/µl, and with 1 µL/reaction tube used for forward and reverse primers. Depending on number of cells initially used for RNA extraction, we recommend dividing final cDNA among 3 reaction tubes (β-actin for loading control, *Albumin*, and KRT19) to start.
13) PCR primer design is listed in Table 1.
14) Place reaction tubes into thermocycler. Recommend following program for initial experiments: 95°Cfor 15 minutes x 1 cycle followed by 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds x 35 cycles, followed by 72°C for 10 minutes.
15) PCR products are analyzed using ethidium bromide impregnated agarose gel.

**7. Confirmation of tumor potential of CD133+ stem cells**

1) This procedure can be done with freshly isolated cells from step 4 or using cells that have been cultured from step 5. We recommend performing initial experiments with cultured cells, as freshly isolated cells will have reduced viability and reduced yield.
2) Trypsinize cells using Trypsin 0.05%-EDTA from step 5.5 above. After 3-5 minute incubation at 37°C, add 100 µL of media to each well and transfer all liquid from each well to individual 5 ml tubes (1 well = 1 tube). Add 1 ml media to each tube and centrifuge at 200 x g for 5 minutes.
3) Re-suspend cells in 1 ml ice cold PBS. Remove 10 µl of PBS cell suspension and add 10 µl trypan blue Using trypan blue exclusion, determine numbers of live cells. If using FACS isolated cells, cell number will be determined by FACS isolation count.
4) Centrifuge cells at 200 x g for 5 minutes and re-suspended in PBS at a concentration of 1 x $10^6$ live cells/100 µl. Add 100 µl Matrigel. Six-week-old immune-deficient Nude mice were injected subcutaneously using 28 gague needle. Inject 1 x $10^6$ cells in 200 µl per site.
5) Mice are monitored for tumor growth daily. Once tumors form, typically after 3-4 weeks incubation, tumor volume is measured using calipers (height x length x width).

**8. Representative Results:**

From normal, healthy murine liver, the expected cellular yield of CD133+liver stem cells is 1,000 to 5,000 per liver. These cells are relatively rare in quiescent liver and will not expand well in culture. We do not recommend using single cell analysis on normal liver and the yield of viable cells that will expand is extremely low.

Isolation of single cells from chronic injury models will yield several (3-9 colonies/96 well plate) colonies that expand from single cells once the procedure is mastered, and cell viability is ensured. Confirmation of bi-potential status is conducted using *Albumin* and Krt19 RT-PCR. Colonies from expanded single cells will demonstrate both expression for markers of hepatocytes (*Albumin*) and cholangiocytes (*Krt19*).

CD133+ stem cells from normal liver and chemically induced liver injury (e.g. DDC 0.1% diet for 6 weeks) will not form tumors in nude mice. CD133+ stem cells from specific genetic models (MAT1a$^{-/-}$ or liver specific Pten$^{-/-}$ mice) will form tumors in nude mice if isolated late in late pre-tumor chronic injury phase. This tumor forming phenotype is currently identified as a cancer stem cell.[2-4]For

example, the MAT1a$^{-/-}$ mice form spontaneous liver tumors at 18 weeks of age. CD133+ liver stem cells isolated at 15-16 weeks, during a late chronic injury phase of liver disease, will form tumors in nude mice.

Major markers present on the surface of hepatic stem cell include EpCAM, E-cadherin, CD29 and CD133. Epithelial cell adhesion molecule (EpCAM) is a transmembrane glycoprotein mediating $Ca^{2+}$-independent homotypic cell-cell adhesion in epithelia. EpCAM is also involved in cell signaling, migration, proliferation and differentiation. Additionally, EpCAM has oncogenic potential via its capacity to upregulate c-myc, e-fabp, and cyclins A & E. Since EpCAM is expressed exclusively in epithelia and epithelial-derived neoplasms, EpCAM can be used as diagnostic marker for various cancers. It appears to play a role in tumorigenesis and metastasis of carcinomas, so it can also act as a potential prognostic marker and as a potential target for immunotherapeutic strategies. E-cadherin, or epithelial cadherin, is an cell-cell adhesion glycoprotein important to cellular processes such as morphology, polarity, development, tissue integrity and migration. E cadherin's extracellular domain interacts with other E-cadherin protein on adjacent epithelial cells to establish adhesion between them. E cadherin has also been identified as a tumor and metastasis suppressor, and its mutations have been linked to various forms of cancer. Integrin beta-1 (ITGB1, CD29, VLA-beta) is the beta subunit found in the integrin families, forming a heterodimer integrin receptor through non-covalent bonding with various integrin alpha subunits. Integrin heterodimer containing Integrin beta-1 binds to various cell surface and extracellular proteins (CD49a-f, CD51) to mediate cell to cell and cell to matrix adhesion (1). Integrin beta-1 plays a critical role in the cell adhesion and recognition in embryogenesis, hemostasis, immune response, tissue repair, metastatic diffusion of tumor cells and development. Prominin 1 (CD133) is expressed on primitive hematopoietic stem and progenitor cells, retinoblastoma, hemangioblasts, and neural stem cells as well as on developing epithelium. The CD133 positive fraction of human bone marrow, cord blood and peripheral blood have been shown to efficiently engraft in xenotransplantation models, and have been shown to contain the majority of the granulocyte/macrophage precursors, NOD/SCID repopulating cells and CD34 + dendritic cell precursors (Novus, 2014).

**References**


1. Barreda DR, Hanington PC, Belosevic M. Regulation of myeloid development and function by colony stimulating factors. Dev Comp Immunol 2004, 28:509-554.
2. Boers SJ, Visser G, Smit PG, Fuchs SA(1). Liver transplantation in glycogen storage disease type I. Orphanet J Rare Dis. 2014 Apr 9;9:47. doi: 10.1186/1750-1172-9-47.
3. C. Bart Rountree, Wei Ding, Hein Dang, Colleen VanKirk, Gay M. Crooks. Isolation of CD133+ Liver Stem Cells for Clonal Expansion. http://www.jove.com/video/3183/isolation-of-cd133-liver-stem-cells-for-clonal-expansion. 2015.
4. Dahlke MH(1), Popp FC, Larsen S, Schlitt HJ, Rasko JE. Stem cell therapy of the liver--fusion or fiction? Liver Transpl. 2004 Apr;10(4):471-9.
5. Gupta S, Verfaillie C, Chmielewski D, et al. A role for extrarenal cells in the regeneration following acute renal failure. *Kidney Int* 2002*;*62*:*1285-1290.
6. Herrera MB, Bussolati B, Bruno S, et al. Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. *Kidney Int* 2007*;*72*:*430-441.
7. Imasawa T, Nagasawa R, Utsunomiya Y, et al. Bone marrow transplantation attenuates murine IgA nephropathy: role of a stem cell disorder. *Kidney Int* 1999*;*56*:*1809-1817.
8. Imasawa T, Utsunomiya Y, Kawamura T, et al. The potential of bone marrow-derived cells to differentiate to glomerular mesangial cells. *J Am Soc Nephrol* 2001*;*12*:*1401-1409.
9. Ma H, Stephen Lee S, Nair S, Chou SY. Reduction of renal interstitial fibrosis and protection of renal function by pentoxifylline in chronic partial ureteral obstruction. *FASEB Journal.* 2008;22:917.4.
10. Nair S, Maini A, Ma H, Chou SY. Pentoxifylline Ameliorates Tubulointerstitial Fibrosis and Protects Renal Function in Chronic Partial Ureteral Obstruction. J Am Soc Nephrol 2007;18:400A-401A.
11. Poulsom R, Forbes SJ, Hodivala-Dilke K, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 2001*;*195*:*229-235.
12. Raval M, Ma H, Chung E, Suwangomolkul A, Chou SY. Hemodialysis Decreases Expression of the Platelet P2Y12 Adenosine Diphosphate Receptor. 28th Annual Dialysis Conference. Hemodialysis International. 2008;12(1):139.
13. Saito M(1), Seo Y, Yano Y, Uehara K, Hara S, Momose K, Hirano H, Yokozaki H, Yoshida M, Azuma T. Portal venous tumor growth-type of hepatocellular carcinoma without liver parenchyma tumor nodules: a case report. Ann Hepatol. 2013 Nov-Dec;12(6):969-73.
14. Talbot NC(1), Caperna TJ. A feeder-cell independent subpopulation of the PICM-19 pig

liver stem cell line capable of long-term growth and extensive expansion. Cytotechnology. 2014 Jan;66(1):1-7. doi: 10.1007/s10616-013-9541-y. Epub 2013 Feb 9.
15. Tarlow BD(1), Finegold MJ, Grompe M. Clonal tracing of Sox9+ liver progenitors in mouse oval cell injury. Hepatology. 2014 Jul;60(1):278-89. doi: 10.1002/hep.27084. Epub 2014 May 28.
16. Terrier B, Joly D, Ghez D, et al. Reversible paraparesis in multiple myeloma with renal failure. *Nephrol Dial Transplant* 2006;21:1439-1440.
17. Wengert GJ(1), Bickel H, Breitenseher J, Ba-Ssalamah A. Primary liver tumors: Hepatocellular versus intrahepatic cholangiocellular carcinoma. Radiologe. 2015 Jan 11. [Epub ahead of print].
18. Ying QL, Nichols J, Evans EP, et al. Changing potency by spontaneous fusion. *Nature* 2002;416:545-548.
19. Zhang S, Wang D, Estrov Z, et al. Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes *in vivo*. *Circulation* 2004;110:3803-3807.
20. Zhang W, Bevins MA, Plantz BA, Smith LA, Meagher MM. Modeling Pichia pastoris growth on methanol and optimizing the production of a recombinant protein, the heavy-chain fragment C of botulinum neurotoxin, serotype A. Biotechnol Bioeng 2000, 70:1-8.
21. Forbes S, Vig P, Poulsom R, Thomas H, Alison M. J Pathol. Hepatic stem cells. 2002 Jul;197(4):510-8. http://www.ncbi.nlm.nih.gov/pubmed/12115866.
22. Novus. http://www.novusbio.com/research-areas/stem-cell-markers/hepatic-stem-cell-markers.html. 2014
23. Alison MR. Stem cells in pathobiology and regenerative medicine. J Pathol 2009;217(2):141-143.
24. Alison MR, Choong C, Lim S. Application of liver stem cells for cell therapy. Semin Cell Dev Biol 2007;18(6):819-826.
25. Broekema M, Harmsen MC, van Luyn MJ, Koerts JA, Petersen AH, van Kooten TG, van Goor H, Navis G, Popa ER. Bone marrow-derived myofibroblasts contribute to the renal interstitial myofibroblast population and produce procollagen I after ischemia/reperfusion in rats. J Am Soc Nephrol 2007;18(1):165-175.
26. Bruce SJ, Rea RW, Steptoe AL, Busslinger M, Bertram JF, Perkins AC. In vitro differentiation of murine embryonic stem cells toward a renal lineage. Differentiation 2007;75(5):337-349.
27. Bussolati B, Bruno S, Grange C, Buttiglieri S, Deregibus MC, Cantino D, Camussi G. Isolation of renal progenitor cells from adult human kidney. Am J Pathol 2005;166(2):545-555.
28. Bussolati B, Hauser PV, Carvalhosa R, Camussi G. Contribution of stem cells to kidney repair. Curr Stem Cell Res Ther 2009;4(1):2-8.
29. Dekel B, Burakova T, Arditti FD, Reich-Zeliger S, Milstein O, Aviel-Ronen S, Rechavi G, Friedman N, Kaminski N, Passwell JH, Reisner Y. Human and porcine early kidney precursors as a new source for transplantation. Nat Med 2003;9(1):53-60.
30. Dekel B, Zangi L, Shezen E, Reich-Zeliger S, Eventov-Friedman S, Katchman H, Jacob-Hirsch J, Amariglio N, Rechavi G, Margalit R, Reisner Y. Isolation and characterization of nontubular sca-1+lin- multipotent stem/progenitor cells from adult mouse kidney. J Am Soc Nephrol 2006;17(12):3300-3314.
31. Gupta S, Verfaillie C, Chmielewski D, Kren S, Eidman K, Connaire J, Heremans Y, Lund T, Blackstad M, Jiang Y, Luttun A, Rosenberg ME. Isolation and characterization of kidney-derived stem cells. J Am Soc Nephrol 2006;17(11):3028-3040.
32. Held PK, Al-Dhalimy M, Willenbring H, Akkari Y, Jiang S, Torimaru Y, Olson S, Fleming WH, Finegold M, Grompe M. In vivo genetic selection of renal proximal tubules. Mol Ther 2006;13(1):49-58.
33. Humphreys BD, Bonventre JV. Mesenchymal stem cells in acute kidney injury. Annu Rev Med 2008;59:311-325.
34. Imai E, Iwatani H. The continuing story of renal repair with stem cells. J Am Soc Nephrol 2007;18(9):2423-2424.
35. Imberti B, Morigi M, Tomasoni S, Rota C, Corna D, Longaretti L, Rottoli D, Valsecchi F, Benigni A, Wang J, Abbate M, Zoja C, Remuzzi G. Insulin-like growth factor-1 sustains stem cell mediated renal repair. J Am Soc Nephrol 2007;18(11):2921-2928.
36. Kim D, Dressler GR. Nephrogenic factors promote differentiation of mouse embryonic stem cells into renal epithelia. J Am Soc Nephrol 2005;16(12):3527-3534.
37. Kobayashi T, Tanaka H, Kuwana H, Inoshita S, Teraoka H, Sasaki S, Terada Y. Wnt4-transformed mouse embryonic stem cells differentiate into renal tubular cells. Biochem Biophys Res Commun 2005;336(2):585-595.
38. Kunter U, Rong S, Djuric Z, Boor P, Muller-Newen G, Yu D, Floege J. Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. J Am Soc Nephrol 2006;17(8):2202-2212.

39. Kurdi M, Booz GW. G-CSF-based stem cell therapy for the heart--unresolved issues part B: Stem cells, engraftment, transdifferentiation, and bioengineering. Congest Heart Fail 2007;13(6):347-351.
40. Lazzeri E, Crescioli C, Ronconi E, Mazzinghi B, Sagrinati C, Netti GS, Angelotti ML, Parente E, Ballerini L, Cosmi L, Maggi L, Gesualdo L, Rotondi M, Annunziato F, Maggi E, Lasagni L, Serio M, Romagnani S, Vannelli GB, Romagnani P. Regenerative potential of embryonic renal multipotent progenitors in acute renal failure. J Am Soc Nephrol 2007;18(12):3128-3138.
41. Li J, Deane JA, Campanale NV, Bertram JF, Ricardo SD. The contribution of bone marrow-derived cells to the development of renal interstitial fibrosis. Stem Cells 2007a;25(3):697-706.
42. Li L, Truong P, Igarashi P, Lin F. Renal and bone marrow cells fuse after renal ischemic injury. J Am Soc Nephrol 2007b;18(12):3067-3077.
43. Lin F, Moran A, Igarashi P. Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. J Clin Invest 2005;115(7):1756-1764.
44. Little MH. Regrow or repair: potential regenerative therapies for the kidney. J Am Soc Nephrol 2006;17(9):2390-2401.
45. Ma AC, Chung MI, Liang R, Leung AY. The role of survivin2 in primitive hematopoiesis during zebrafish development. Leukemia 2009.
46. Rad FH, Ulusakarya A, Gad S, Sibony M, Juin F, Richard S, Machover D, Uzan G. Novel somatic mutations of the VHL gene in an erythropoietin-producing renal carcinoma associated with secondary polycythemia and elevated circulating endothelial progenitor cells. Am J Hematol 2008;83(2):155-158.
47. Roufosse C, Cook HT. Stem cells and renal regeneration. Nephron Exp Nephrol 2008;109(2):e39-45.
48. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, Ronconi E, Meini C, Gacci M, Squecco R, Carini M, Gesualdo L, Francini F, Maggi E, Annunziato F, Lasagni L, Serio M, Romagnani S, Romagnani P. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol 2006;17(9):2443-2456.
49. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 2000;97(21):11307-11312.
50. Steenhard BM, Isom KS, Cazcarro P, Dunmore JH, Godwin AR, St John PL, Abrahamson DR. Integration of embryonic stem cells in metanephric kidney organ culture. J Am Soc Nephrol 2005;16(6):1623-1631.
51. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A 2006;103(19):7321-7326.
52. Suzuki A, Iwatani H, Ito T, Imai E, Okabe M, Nakamura H, Isaka Y, Yamato M, Hori M. Platelet-derived growth factor plays a critical role to convert bone marrow cells into glomerular mesangial-like cells. Kidney Int 2004;65(1):15-24.
53. Vigneau C, Polgar K, Striker G, Elliott J, Hyink D, Weber O, Fehling HJ, Keller G, Burrow C, Wilson P. Mouse embryonic stem cell-derived embryoid bodies generate progenitors that integrate long term into renal proximal tubules in vivo. J Am Soc Nephrol 2007;18(6):1709-1720.
54. Vogetseder A, Picard N, Gaspert A, Walch M, Kaissling B, Le Hir M. Proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells. Am J Physiol Cell Physiol 2008;294(1):C22-28.
55. Witzgall R. Are renal proximal tubular epithelial cells constantly prepared for an emergency? Focus on "the proliferation capacity of the renal proximal tubule involves the bulk of differentiated epithelial cells". Am J Physiol Cell Physiol 2008;294(1):C1-3.
56. Xian CJ, Foster BK. Repair of injured articular and growth plate cartilage using mesenchymal stem cells and chondrogenic gene therapy. Curr Stem Cell Res Ther 2006;1(2):213-229.
57. Yamamoto M, Cui L, Johkura K, Asanuma K, Okouchi Y, Ogiwara N, Sasaki K. Branching ducts similar to mesonephric ducts or ureteric buds in teratomas originating from mouse embryonic stem cells. Am J Physiol Renal Physiol 2006;290(1): F52-60.