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Death from liver disease continues to increase in contrast to other chronic conditions.\(^1,2\) Cirrhosis of the liver is the result of prolonged injury to the liver arising from multiple etiologies. Cirrhosis-related deaths accounted for over a million deaths globally in 2010 with mortality rates increasing substantially in the United Kingdom.\(^3,4\) In the United Kingdom, chronic liver disease accounts for the majority of liver transplantations, with a relatively lower incidence of transplantations to treat acute liver failure (ALF) (NHS Interim Report on Liver Transplantation, 2018). ALF is a relatively rare but life-threatening critical illness with acetaminophen (APAP) poisoning alone accounting for half of ALF cases in the United States equating to nearly 500 deaths annually.\(^5,6\) Liver failure arising from either acute or chronic injury is limited to orthotopic liver transplantation (OLT) as the only curative option. Liver transplantation alone is inadequate with demand for grafts outweighing supply of suitable organs. Furthermore, the surgical procedure carries significant morbidity and mortality, and patients are committed to life-long immunosuppression.\(^7\) Therefore, there is an urgent requirement for the development of alternative therapies for acute and chronic liver diseases. Despite the relative success of therapeutic interventions for specific etiologies (e.g., novel antiviral therapy for hepatitis C virus infection, alcohol abstinence for alcoholic liver disease), patients often present to medical attention late when cirrhosis and related complications have already occurred.\(^8\) Therefore, there is a need to explore novel therapies for both acute failure and chronic liver disease to provide additional therapeutic options beside organ transplantation.

**Cell Therapies for Liver Disease**

Cell therapies herald a new era in medicine, offering alternative strategies to promote the functional recovery of diseased or injured tissues. Cell therapies are an attractive therapeutic approach because they promote the repair of a patient’s own tissue using a fully defined (i.e., Good Manufacturing Practices [GMP]-compliant) cellular product that can be produced at scale, and delivered to patients. In the context of liver disease, several cell types have been tested in both preclinical animal models and human trials with varying degrees of success in terms of safety and efficacy (see ►Table 1). The first evidence of the feasibility of cellular therapy for liver disease was gained from hepatocyte transplantation for metabolic liver disease.\(^9-11\) Two studies performing intrasplenic transplantation of allogenic hepatocytes in ALF patients with hepatic encephalopathy grade > 3 showed minimal improvement in survival.\(^12\) In the group listed for OLT, hepatocyte transplantation improved cardiovascular stability but did not significantly
Table 1 Cell therapy strategies that have been trialed for liver disease

| Cell type     | Use                  | Safety                  | Efficacy                                                                 | Strengths and limitations                        |
|---------------|----------------------|-------------------------|--------------------------------------------------------------------------|---------------------------------------------------|
| Hepatocytes   | Humans (small RCTs)  | High risk of thrombosis | Reduction of LDL (familial hypercholesterolemia)                          | Poor engraftment                                  |
|               |                      |                         | Reduction of need of factor VII replacement (congenital deficiency)       |                                                   |
|               |                      |                         | Reduction of ALT and BIL (biliary atresia)                                |                                                   |
|               |                      |                         | No efficacy proven in ALF and other congenital and metabolic diseases     |                                                   |
|               |                      |                         | (PFIC, OTC, and ASL deficiency)                                            |                                                   |
| iPSCs         | Animals              | High teratogenic risk   | Improvement in survival (50 vs. 0% survival at day 3 in treatment vs. control) | Teratogenic                                      |
|               |                      |                         | Reduction of fibrosis (50% reduction at Sirius red staining)               | Generate organ buds                               |
| Fetal hepatocytes | Animals              | Safe in rats with no evidence of oncogenesis at 3 mo | Improvement in bilirubin of around 50% in Gunn's rats | Isolation from aborted fetus                      |
|               |                      |                         | Improvement in survival                                                   | Poor expansion                                    |
| HPCs          | Animals              | Little risk of thrombosis | Not proven                                                                | Poor engraftment                                  |
| hBTSC         | Humans (2 case reports) | Safe                  | Improvement of MELD (from 24 to 20) Improvement of CP score (from 12 to 10) but not sustained at 1 y | Multipotent                                       |
| HSCs          | Humans (RCT and pilot study) | Safe (no SAR or SUSAR in RCT) | Transient nonstatistically significant improvement in bilirubin in pilot study. No evidence of improvement in MELD in large RCT | Poor engraftment                                  |
| FLSPCs        | Animals              | Safe in rats (small size, can be used in low numbers) | Improvement in albumin                                                   | Require regenerative stimuli to proliferate       |
|               |                      |                         |                                                                          | Pluripotential                                    |
|               |                      |                         |                                                                          | Good cryopreservation                             |
| MSCs          | Humans (RCT)         | Safe (no SAR or SUSAR in RCT) | Among all markers only Alb improved with statistically significance compared with controls (from 30 to 35) | Easy to expand in vitro                           |
|               |                      |                         |                                                                          | Immunomodulatory                                  |
| Macrophages   | Animals On-going phase 1/2 clinical trial | Safe (no evidence of cytokine storm in mice models) | Improvement in albumin (~5–10% in treatment group) Reduction of fibrosis (reduction of 25% of collagen I and hydroxyproline staining in treatment vs. control) | Immunomodulatory Antifibrotic                      |

Abbreviations: ALF, acute liver failure; ALT, alanine aminotransferase; ASL, argininosuccinate lyase; CP score, Child–Pugh score; FLSPC, fetal stem/progenitor cell; hBTSC, human biliary tree stem cell; HPC, hepatocyte progenitor cell; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell; LDL, low-density lipoprotein; MELD, Model for End-Stage Liver Disease; MSC, mesenchymal stem cell; OTC, ornithine transcarbamylase; PFIC, progressive familial intrahepatic cholestasis; RCT, randomized control trial; SAR, serious adverse reaction; SUSAR, suspected unexpected serious adverse reaction.

ameliore liver function. Therefore, hepatocyte transplantation has only shown utility to bridge to OLT. Experiments in rodents have shown hepatocytes transferred via the hepatic portal vein cause significant thrombosis and ischemia-reperfusion injury. Even when intrasplenic approach is adopted, engraftment of hepatocytes is extremely limited as over 90% of transplanted cells are phagocytosed by Kupffer cells (KCs).

Since adult hepatocytes have limited availability, there is a worldwide effort to produce functional hepatocytes from pluripotent cells to generate a potentially limitless source of hepatocyte-like cells (HLCs) for drug screening and medical use. Recently, there have been great advances producing HLCs that recapitulate the biology of bona fide adult hepatocytes. For instance, HLCs can be derived from induced pluripotent stem
cells (iPSCs) or embryonic stem cells (ESCs). In vitro studies demonstrate that HLCs can be generated in a stepwise protocol from iPSCs.\textsuperscript{18,19} iPSC-derived cells could be sourced from individual patients to allow production of autologous cells for transfer back into the same patient.\textsuperscript{20,21} However, these have not been tested yet in clinical trials for acute or chronic liver disease. In parallel, fetal hepatocytes have been proposed as an alternative strategy to improve engraftment and function. Preclinical studies provided evidence of fetal hepatocyte metabolic function in rats.\textsuperscript{22,23} In patients, allogenic fetal hepatocytes were transplanted intraperitoneally to seven patients with ALF but led to recovery in only three subjects with advanced encephalopathy.\textsuperscript{24} Several limiting factors preclude hepatocyte therapy as an effective therapeutic strategy for liver disease including risk of thrombosis, the unstable ex vivo phenotype, and poor hepatocyte engraftment.\textsuperscript{14,25,26} To mitigate these limitations, alternative cell types have been considered, for example, hepatocyte progenitor cells (HPCs). HPCs are found in adult livers in the canal of Hering and can become activated after liver injury to repopulate the organ with functional hepatocytes or biliary cells.\textsuperscript{27} HPCs are an attractive option for cell transplantation due to their small size (5–15 \( \mu \)m), reduced risk of embolism, ease of cryopreservation, tolerability toward ischemia, and minimal immunogenicity.\textsuperscript{28} However, HPCs are a relatively scarce cell type in adult liver, and engraftment represents a major obstacle for clinical use, although recent work showed coating the cells with hyaluronic acid provided a modest improvement in engraftment in mice.\textsuperscript{28} Furthermore, the differentiation of human HPCs to mature hepatocytes has not yet been convincingly demonstrated. HPCs are present in fetal liver in high numbers and can be easily isolated and cultured. Therefore, fetal human biliary tree stem cells (hBTSCs) have been considered for HPC therapy.\textsuperscript{29} hBTSCs have been tested in two case reports in cirrhotic patients after transplantation of 4 to 6 \( \times 10^7 \) cells via the hepatic artery. Although this technique appeared safe, biochemical improvements were only transitory suggesting limited efficacy.\textsuperscript{30} Another approach involves use of fetal stem/progenitor cells (FLSPCs), highly proliferative precursor cells of endodermal origin with the capacity to form hepatocytes and bile duct epithelial cells (sourced from rats at ED14). Although FLSPCs have not yet been tested in humans, they have been shown to repopulate large areas of rat parenchyma, maintain high proliferation rates, and retain differentiation potential posttransplantation even after cryopreservation.\textsuperscript{31,32} In contrast to the poor replicative capacity of ex vivo hepatocytes, hematopoietic stem cells (HSCs) are highly proliferative with the ability to transdifferentiate into mature hepatocytes thereby representing an appealing cell therapy for liver disease.\textsuperscript{33–35} A randomized-controlled trial of HSC-like bone marrow-derived mononuclear cell transfer to 30 cirrhotic patients on the OLT waiting list suggested transient improvement of albumin in treated subjects. However, this was not statistically significant and the overall liver function did not improve as per the Child–Pugh score.\textsuperscript{36} Moreover, a U.K.-based clinical trial recently reported that CD-133+ HSC therapy in conjunction with granulocyte-colony stimulating factor (G-CSF) did not improve liver function in cirrhotic patients.\textsuperscript{37} Mesenchymal stem cells (MSCs) are multipotent cells that can be readily isolated from adult bone marrow or umbilical cord tissue and can expand in vitro with capacity to differentiate into several lineages including hepatocytes.\textsuperscript{38–40} MSCs are known to exhibit immunomodulatory functions and have been shown to reduce inflammation, reduce injury, and protect against hepatocyte apoptosis in several liver injury (acute liver injury [ALI]) models.\textsuperscript{41–45} MSCs have also been tested in cirrhotic patients demonstrating minor improvements in liver synthetic function and Model for End-Stage Liver Disease (MELD) score, although MSCs require further evaluation in larger clinical trials with predefined primary endpoints.\textsuperscript{46–49} Studies to date have involved small numbers of patients with short-term endpoints, thus evidence of long-term benefit and a clear understanding of the mechanism of action of MSCs is required.\textsuperscript{50} In summary, various cell therapies hold potential to provide new strategies to treat liver disease. However, many cell therapy candidates are not yet ready to be tested in clinical trials. Engraftment represents a major obstacle in diseased liver tissue for various cell therapies designed to improve hepatic function. Further work to elucidate the mechanisms of action of each cell therapy candidate will help design early clinical trials to test safety and efficacy. Data from clinical trials are currently at an early stage and have only showed limited success at best so far.\textsuperscript{49,51–53}

**Role of Macrophages in Liver Disease**

The liver contains the largest population of tissue resident macrophages in the body in the form of KCs located within the hepatic sinusoids.\textsuperscript{54,55} In the steady state, KCs provide important hepatic innate immunity by efficiently phagocytosing gut-derived pathogens (e.g., *Escherichia coli* and bacterial products) from portal blood, thereby providing an immunological barrier between the gut and the systemic circulation.\textsuperscript{56} KCs are also highly adapted to remove apoptotic debris (principally dead erythrocytes), particulate matter, and are involved in the clearance of several serum proteins (\textsuperscript{57,58} Fig. 1A and B). KCs have been shown to be implicated in the early activation of the innate immune system after a hepatotoxic event, for example, during APAP overdose. Hepatocyte necrosis releases a plethora of proinflammatory signals, including several danger-associated molecular patterns (DAMPs), chemokines, and cytokines that can activate resident macrophages via Toll-like receptor signaling resulting in the recruitment of circulating monocytes and other inflammatory cells to the liver.\textsuperscript{59,60} However, recent studies have shown in ALI, there is a substantial loss of KCs at peak injury leading to a deficit in hepatic innate immunity.\textsuperscript{61,62} During APAP-induced liver injury, uncontrolled inflammation resulting from massive hepatocyte necrosis coupled with KC loss results in a sepsis-like condition termed systemic inflammatory response syndrome (SIRS)—a major determinant of clinical outcome in patients with APAP-induced ALI.\textsuperscript{63} Likewise, in chronic end-stage liver disease, when KC-mediated barrier function is diminished, patients are prone to developing bacterial and fungal infections representing a major trigger of acute-on-chronic liver failure.\textsuperscript{64,65} Clearly, liver resident KCs are critical in maintaining important
Kupffer cells (KCs) are liver resident macrophages located within the hepatic sinusoids that comprise part of the mononuclear phagocyte system. (B) KCs possess several important functions in the steady state including providing barrier function against gut-derived bacteria, scavenging of damaged/aged erythrocytes, and clearance of several serum proteins. (C) During chronic liver disease, hepatic myofibroblasts (activated hepatic stellate cells) deposit excessive amounts of collagen replacing hepatocytes impacting liver function. (D) Transfer of bone marrow-derived macrophages (BMDMs)/monocyte-derived macrophages (MDMs) have shown efficacy in liver fibrosis models with evidence of collagen regression, myofibroblast apoptosis, and enhanced recruitment of innate immune cells. (E) During acute liver injury, a transient loss of KCs occurs alongside massive hepatocyte necrosis causing a deficit in barrier function. (F) Supplementing the macrophage pool during acute liver injury is a potential strategy to restore hepatic barrier function. Alternatively activated macrophages (AAMs) in particular possess a high capacity for efferocytosis to resolve necrosis and in turn reduce inflammation and promote liver repair.
innate immunity and are fundamentally implicated in the pathology of liver disease.

**Macrophage Therapy for Liver Fibrosis**

Chronic liver disease is fundamentally distinct pathology from ALI resulting from long-term iterative injury with an inflammatory basis arising from various etiologies. Ultimately, chronic liver disease results in the deposition of large quantities of extracellular matrix (ECM). The substitution of healthy parenchyma with scar tissue (ECM proteins comprising mainly collagens) can develop into cirrhosis characterized by reduced liver function, portal hypertension, and related complications. Human and mouse macrophages express several members of the matrix metalloproteinase (MMP) family of endopeptidases, including MMP8, MMP9, and MMP12. A subset of MMPs have properties allowing them to unwind and cleave collagen helices affording them collagenolytic activity, a natural process that occurs in development and wound healing. Therefore, macrophages represent a key cell type implicated in the metabolism of ECM and tissue remodeling. The deranged architecture, inflammatory niche, and excessive ECM in fibrotic liver provide significant barriers for the engraftment and long-term functionality of transplanted cells. Novel strategies that target the existing hepatic scar tissue directly, using either cells or biologics, are gaining attention as an alternative to hepatocyte transplantation. Novel carrier systems (e.g., liposomes or mannosylated conjugated proteins) have been developed to target endogenous KCs to modulate macrophage function in situ (for expert review on targeting endogenous macrophages for liver disease, refer to Tacke). In parallel, transfer of exogenous macrophages (e.g., syngeneic, autologous, or allogeneic cells) represents an alternative technique to modulate the hepatic microenvironment. Intravenous injection of bone marrow-derived macrophages (BMDMs) to mice with established carbon tetrachloride (CCL4)-induced liver fibrosis resulted in less collagen deposition, fewer hepatic myofibroblasts (activated hepatic stellate cells), and enhanced recruitment of host monocytes and neutrophils—a further source of MMP9. Importantly, liver synthetic function was improved in fibrotic mice after BMDM delivery evidenced by increased serum albumin levels. In this study, macrophages were injected via the hepatic portal vein, a dosing route that may be unsuitable in cirrhotic patients due to associated coagulopathy and portal hypertension. However, a more recent study showed that transfer of classically activated macrophages (CAMs) administered to fibrotic mice via tail vein also resolved collagen efficiently. Further mechanistic insight from this study revealed that recruited natural killer cells were a major source of tumor necrosis factor-related apoptosis-inducing ligand that promoted myofibroblast apoptosis. In addition, intravenous delivery of murine ESC-derived macrophages recapitulated fibrosis resolution observed with primary macrophages, although a greater number of cells were required to achieve efficacy. These studies provide evidence that disease-modifying macrophages can be administered peripherally. Translational studies have since demonstrated that primary human monocyte-derived macrophages (MDMs) sourced from healthy donors have antifibrotic activity after intrasplenic cell transfer to fibrotic immunocompromised mice. However, safety concerns exist using myeloid cell transfer approaches given that several groups have reported injurious responses after transplanting immature cell types in disease models, for example, bone marrow precursor cells and monocytes. These findings underscore the importance of using defined protocols that yield highly enriched populations of fully mature cells qualified by a robust set of maturity markers.

In contrast to the emergency setting of ALF, the relatively slow progression of compensated liver cirrhosis (median survival > 12 years) provides a therapeutic window available over a longer timeframe assuming complications can be managed and disease-inducing factors controlled (e.g., antiviral medication, cessation of alcohol consumption). Therefore, this timeframe allows the collection of a patient’s own monocytes for macrophage differentiation (typically 7 days) before infusion back into the patient, that is, autologous cell therapy. Moore et al demonstrated that MDMs sourced from cirrhotic patients are phenotypically similar to healthy donor-derived macrophages in terms of MMP expression and surface marker composition. Furthermore, intrasplenic transplantation of healthy human MDMs to an immunocompromised mouse model of liver fibrosis elicited regression of collagen, and reduction of liver injury markers. This work provided the platform to build a GMP-compatible pipeline to generate clinical-grade human MDMs for potential therapeutic applications. A differentiation protocol now exists with clear release criteria for functionally mature human macrophages (25F9hi, CD206hi, CCR2hi) using a defined serum-free, antibiotic-free method. Safety and efficacy studies of autologous macrophage therapy are now underway in a Phase I/II first-in-human clinical trials for the treatment of liver cirrhosis. Exogenous macrophage delivery has shown promise in preclinical models to elicit collagen regression and stimulate hepatic function.

**Macrophage Efferocytosis and Acute Liver Injury**

Macrophages are exquisitely adapted to recognize and remove dead or dying cells from the system. Macrophages, including KCs, express a repertoire of cell surface receptors including Mer, phosphatidylserine receptors, lectins, and scavenger receptors that recognize motifs on dying cells to initiate and facilitate their internalization and degradation. Macrophage-mediated removal of dead cells, known as efferocytosis coined from the Latin term “efferre”: “to bury,” is thought to be a prerequisite for the resolution of inflammation by clearing the inflammatory source to allow the restitution of injured tissue. During liver injury (e.g., during APAP overdose), there is a sudden and massive chemical insult to the liver, causing widespread hepatocyte necrosis that occurs rapidly after drug ingestion. In the clinic, severe toxicity can be mitigated via the timely infusion of N-acetylcysteine (NAC; a sulfhydryl donor that boosts hepatocyte antioxidant capacity to prevent hepatocyte

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*Macrophages as a Cell-Based Therapy for Liver Disease* Starkey Lewis et al.
Macrophages as a Cell-Based Therapy for Liver Disease

Macrophages are an inherently plastic cell type capable of acquiring a spectrum of phenotypes in response to stimuli from the microenvironment. Traditionally, this phenotypic axis was defined simplistically as “M1” macrophages (classical-activation with enhanced bactericidal properties), versus “M2” macrophages (alternative-activation with enhanced tissue remodeling properties), which has provided a useful framework despite calls for a more nuanced nomenclature. Functional analysis of different macrophage phenotypes can be achieved by polarizing cells in vitro using defined factors (e.g., lipopolysaccharide and interferon γ to produce CAMs, or IL-4/-13 to produce AAMs). As discussed earlier, CAMs out-performed standard BMDMs in terms of their role in collagen regression. Polarized macrophages may offer greater efficacy or improved safety profiles since a polarized cell population is phenotypically more uniform. One safety concern that exists with macrophage therapy is the risk of transplanted macrophages acquiring a potentially deleterious phenotype in response to microenvironmental cues in a diseased organ. Polarizing macrophages ex vivo using high concentrations of recombinant cytokines prior to transplant may reduce this risk since there is some evidence that polarized macrophages can retain their phenotype epigenetically.

Safety studies that test macrophage phenotype and persistence in relevant disease models are warranted. In the setting of ALI, efficient efferocytosis of necrotic material is required to suppress...
inflammation. Numerous groups have reported that AAMs have enhanced phagocytic function versus standard BMDMs or CAMs.\textsuperscript{60,117,118} Therefore, transferring AAMs or by promoting hepatic macrophage phagocytosis may represent a therapeutic strategy in the setting of ALI.

Highly defined macrophages with desired characteristics may indeed provide more precise therapy. Tissue-resident macrophages are known to display unique gene expression profiles with considerable diversity among macrophage populations.\textsuperscript{119} While macrophages share a common set of functions, tissue-specific functions do exist, for example, osteoclasts perform efficient bone resorption in contrast to microglia, which support neuronal circuit development.\textsuperscript{120,121} In the liver, KCs are highly specialized at removing damaged erythrocytes from the circulation.\textsuperscript{122} KCs express several genes involved with lipid and iron metabolism including several scavenger receptors, which are enriched in KCs versus other macrophage populations.\textsuperscript{123} Tissue-resident macrophages, including KCs, develop from embryonic precursors with the capacity to proliferate and self-renew.\textsuperscript{124,125} During APAP-ALL, approximately half of KCs are lost at peak injury but recover through proliferation over several days.\textsuperscript{62} During this time, circulating inflammatory monocytes infiltrate the liver and differentiate into short-lived MDMs. Blood monocytopenia resulting from massive influx of circulating monocytes into the liver has been associated with poor prognosis in patients.\textsuperscript{126} It has been shown experimentally that MDMs can repopulate the liver and acquire self-renewal properties, but only under specific conditions.\textsuperscript{123} ESC-derived macrophages may resemble tissue-resident macrophages more closely with lower expression levels of Myb (a HSC transcription factor) compared with BMDMs.\textsuperscript{80} The source of exogenous macrophages may have implications on the phenotype, function, and persistence of these cells in tissues after administration.

In summary, evidence suggests that macrophages play a key role in the initiation and resolution phases of both acute and chronic liver disease. The barrier function provided by KCs is essential to prevent bacteria and systemic inflammation. Supplementing hepatic macrophage populations using exogenous cell transfer or by cytokine-induced endogenous macrophage expansion are clinically relevant strategies that have the potential to augment hepatic innate immunity during liver disease. Methods to generate clinical-grade primary human macrophages have recently been described allowing these cells to be evaluated in prospective clinical trials.

**Main Concepts and Learning Points**

- **Kupffer cells** are liver resident macrophages that possess several important functions in liver tissue, including providing barrier function against gut-derived pathogens.
- **Macrophages** play distinct roles in the initiation and resolution phases of liver injury, therefore are intrinsically implicated in liver disease pathophysiology.
- **Patients** with both acute and chronic liver disease have a perturbed phagocytic system thereby being at risk of developing serious bacterial/fungal infections.
- **Strategies** that restore hepatic innate immunity during liver disease through direct cell transfer or cytokine-induced macrophage replacement are gaining attention.
- **Primary human macrophages** can now be manufactured to meet GMP standards and clinical trials to test safety and efficacy in liver disease are underway.

**Conflict of Interest**\textsuperscript{93}

Dr. Starkey Lewis has a patent PCT/GB2017/052769 pending. Dr. Forbes has a patent PCT/GB2017/052769 pending, and a patent UK application ref 1804255.6 pending.

**References**

1. Murray CJ, Richards MA, Newton JN, et al. UK health performance: findings of the Global Burden of Disease Study 2010. Lancet 2013;381(9871):997–1020
2. Shielis MS, Chernyavskiy P, Anderson WF, et al. Trends in premature mortality in the USA by sex, race, and ethnicity from 1999 to 2014: an analysis of death certificate data. Lancet 2017;389(10073):1043–1054
3. Leon DA, McCambridge J. Liver cirrhosis mortality rates in Britain from 1950 to 2002: an analysis of routine data. Lancet 2006;367(9504):52–56
4. Mokdad AA, Lopez AD, Shapira S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med 2014;12:145
5. Bernal W, Wendon J. Acute liver failure. N Engl J Med 2013;369(26):2525–2534
6. Lee WM. Acetaminophen (APAP) hepatotoxicity–Isn’t it time for APAP to go away? J Hepatol 2017;67(06):1324–1331
7. Goldberg D, French B, Trotter J, et al. Underreporting of liver transplant waitlist removals due to death or clinical deterioration: results at four major centers. Transplantation 2013;96(02):211–216
8. D’Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. J Hepatol 2006;44(01):217–231
9. Matas AJ, Sutherland DE, Stefles MW, et al. Hepatocellular transplantation for metabolic deficiencies: decrease of plasmas bilirubin in Gunn rats. Science 1976;192(4242):892–894
10. Fox JJ, Chowdbyrne JR, Kaufman SS, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med 1998;338(20):1422–1426
11. Sokal EM, Smets F, Bourgois A, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation 2003;76(04):735–738
12. Strom SC, Fisher RA, Thompson MT, et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 1997;63(04):559–569
13. Bilir BM, Guinet D, Kar rer F, et al. Hepatocyte transplantation in acute liver failure. Liver Transpl 2000;6(01):32–40
14. Weber A, Groyer-Picard MT, Franco D, Dagher I. Hepatocyte transplantation in animal models. Liver Transpl 2009;15(01):7–14
15. Joseph B, Malhi H, Bhargava KK, Palestro CJ, McCuskey RS, Gupta S. Kupffer cells participate in early clearance of syngeneic hepatocytes transplanted in the rat liver. Gastroenterology 2002;123(05):1677–1685
16. Zakikhani K, Pournasr B, Vosough M, Nassiri-Asl M. In vitro generated hepatocyte-like cells: a novel tool in regenerative medicine and drug discovery. Cell J 2017;19(02):204–217
17. Zhang K, Zhang L, Liu W, et al. In vitro expansion of primary human hepatocytes with efficient liver repopulation capacity. Cell Stem Cell 2018;23(06):806–819.e4
Macrophages as a Cell-Based Therapy for Liver Disease

Starkey Lewis et al.

18 Sullivan GJ, Hay DC, Park IH, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 2010;51(01):329–335
19 Si-Tayeb K, Noto FK, Nagaoka M, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51(01):297–305
20 Takayama K, Akita N, Mimura N, et al. Generation of safe and therapeutically effective human induced pluripotent stem cell-derived hepatocyte-like cells for regenerative medicine. Hepatol Commun 2017;1(10):1058–1069
21 Liu H, Kim Y, Sharkis S, Marchionni L, Jiang Y. In vivo liver regeneration potential of human induced pluripotent stem cells from diverse origins. Sci Transl Med 2011;3(82):82ra39
22 Kokudo N, Okui H, Azuma T, et al. Long-term effects of intrapleurally transplanted adult hepatocytes and fetal liver in hyperbilirubinemic Gunn rats. Transpl Int 1995;8(04):262–267
23 Lilja H, Arkadopoulos N, Blanc P, et al. Fetal rat hepatocytes: isolation, characterization, and transplantation in the Nagase analbuminemic rats. Transplantation 1997;64(09):1248–1254
24 Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation 1994;58(08):951–952
25 Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc 1992;24(06):3052–3053
26 Hansel MC, Gramolini R, Skvorak K, et al. The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. Curr Protoc Toxicol 2014;62:14
27 Susich R, Moss N, Kubota H, et al. Hepatic progenitors and strategies for liver cell therapies. Ann N Y Acad Sci 2001;944:398–419
28 Nevi L, Carpino G, Costantini D, et al. Hyaluronic acid improves liver engraftment of transplanted human biliary tree stem/progenitor cells. Stem Cell Res Ther 2017;8(01):68
29 Semeraro R, Carpino G, Cardinale V, et al. Multipotent stem/progenitor cells in the human foetal biliary tree. J Hepatol 2012;57(05):987–994
30 Cardinale V, Carpino G, Gentile R, et al. Transplantation of human fetal biliary tree stem/progenitor cells into two patients with advanced liver cirrhosis. BMC Gastroenterol 2014;14:204
31 Yovchev MI, Xue Y, Shafritz DA, Locker J, Oertel M. Repopulation of the fibrotic/cirrhotic rat liver by transplanted hepatic stem/progenitor cells and mature hepatocytes. Hepatology 2014;59(01):284–295
32 Oertel M, Menthen A, Chen YQ, Shafritz DA. Properties of cryopreserved fetal liver stem/progenitor cells that exhibit long-term repopulation of the normal rat liver. Stem Cells 2006;24(10):2244–2251
33 Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. Hepatology 2000;32(01):11–16
34 Moore JK, Stuchfield BM, Forbes SJ. Systematic review: the effects of autologous stem cell therapy for patients with liver disease. Aliment Pharmacol Ther 2014;39(07):673–685
35 Czyz J, Wiese C, Rollettschek A, Blyszczuk P, Cross M, Wobus AM. Potential of embryonic and adult stem cells in vitro. Biol Chem 2003;384(10–11):1391–1409
36 Lyra AC, Soares MB, da Silva LF, et al. Infusion of autologous bone marrow mononuclear cells through hepatic artery results in a short-term improvement of liver function in patients with chronic liver disease: a pilot randomized controlled study. Eur J Gastroenterol Hepatol 2010;22(01):33–42
37 Newsome PN, Fox R, King AL, et al. Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. Lancet Gastroenterol Hepatol 2018;3(01):25–36
38 Chen AK, Revenyu S, Oh SK. Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: achievements and future direction. Biotechnol Adv 2013;31(07):1032–1046
39 Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284(5411):143–147
40 Ji R, Zhang N, You N, et al. The differentiation of MSCs into functional hepatocyte-like cells in a liver biomatrix scaffold and their transplantation into liver-fibrotic mice. Biomaterials 2012;33(35):8995–9008
41 Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006;107(04):1484–1490
42 Jung J, Choi JH, Lee Y, et al. Human placenta-derived mesenchymal stem cells promote hepatic regeneration in CCl4- and/or LPS-injured rat liver model via increased autophagic mechanism. Stem Cells 2013;31(08):1584–1596
43 Zhu X, He B, Zhou X, Ren J. Effects of transplanted bone-marrow-derived mesenchymal stem cells in animal models of acute hepatitis. Cell Tissue Res 2013;351(03):477–486
44 Kanazawa H, Fujimoto Y, Teratani T, et al. Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. PLoS One 2011;6(04):e19195
45 Salomone F, Barbagallo I, Puzzo L, Piazza C, Li Volti G. Efficacy of adipose tissue-mesenchymal stem cell transplantation in rats with acetaminophen liver injury. Stem Cell Res (Amst) 2013;11(03):1037–1044
46 Zhang Z, Lin H, Shi M, et al. Human umbilical cord mesenchymal stem cells improve liver function and ascsites in decompensated liver cirrhosis patients. J Gastroenterol Hepatol 2012;27(Suppl 2):112–120
47 Owen A, Newsome PN. Mesenchymal stromal cell therapy in liver disease: opportunities and lessons to be learnt? Am J Physiol Gastrointest Liver Physiol 2015;309(10):G791–G800
48 Shi M, Zhang Z, Xu R, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl Med 2012;1(10):725–731
49 Mohammadnejad M, Alimoghaddam K, Bagheri M, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. Liver Int 2013;33(10):1490–1496
50 Zhao L, Chen S, Shi X, Cao H, Li L. A pooled analysis of mesenchymal stem cell-based therapy for liver disease. Stem Cell Res Ther 2018;9(01):72
51 Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. J Hepatol 2015;62(1 Suppl):S157–S169
52 Huebert RC, Rakela J. Cellular therapy for liver disease. Mayo Clin Proc 2014;89(03):414–424
53 El-Ansary M, Abdel-Aziz I, Mogawer S, et al. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in Egyptian patients with HCV induced liver cirrhosis. Stem Cell Rev 2012;8(03):972–981
54 Bilzer M, Rogel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver Int 2006;26(10):1175–1186
55 Ju C, Tacke F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. Cell Mol Immunol 2016;13(03):316–327
56 Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. Hepatology 2008;47(02):729–736
57 Dixon LJ, Barnes M, Tang H, Pritchard MT, Nagy LE. Kupffer cells in the liver. Compr Physiol 2013;3(02):785–797
58 Rady ZA, Koza-Taylor PH, Bell RR, et al. Increased serum enzyme levels associated with Kupffer cell reduction with no signs of hepatic or skeletal muscle injury. Am J Pathol 2011;179(01):240–247
59 Martin-Murphy BV, Holt MP, Ju C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. Toxicol Lett 2010;192(03):387–394
Haideri SS, McKinnon AC, Taylor AH, et al. Injection of embryonic stem cell derived macrophages ameliorates fibrosis in a murine model of liver injury. Nat Regen Med 2017;2(01):14

Moore JK, Mackinnon AC, Wojtacha D, et al. Phenotypic and functional characterization of macrophages with therapeutic potential generated from human cirrhotic monocytes in a cohort study. Cytotherapy 2015;17(11):1604–1616

Heymann F, Hammerich L, Storch D, et al. Hepatic macrophage migration and differentiation critical for liver fibrosis is mediated by the chemokine receptor C-C motif chemokine receptor 8 in mice. Hepatology 2012;55(03):898–909

Mossanen JC, Krenkel O, Ergen C, et al. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatology 2016;64(05):1667–1682

Fraser AR, Pass C, Burgoyne P, et al. Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: a novel cellular therapeutic for liver cirrhosis. Cytotherapy 2017;19(09):1113–1124

Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 2001;411(6834):207–211

Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 2000;405(6872):85–90

Carlsson A, Christenson K, Matlak M, et al. Galectin-3 functions as an opsonin and enhances the macrophage clearance of apoptotic neutrophils. Glycobiology 2009;19(01):16–20

Platt N, Suzuki H, Kurihara Y, Kodama T, Gordon S. Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro. Proc Natl Acad Sci U S A 1996;93(22):12456–12460

deCathelineau AM, Henson PM. The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. Essays Biochem 2003;39:105–117

Greenlee-Wacker MC. Clearance of apoptotic neutrophils and resolution of inflammation. Immunol Rev 2016;273(01):357–370

James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatoxicity. Drug Metab Dispos 2003;31(12):1499–1506

Prescott LF, Park J, Ballantyne A, Adriaenssens P, Proudfoot AT. The role of monocyte-derived macrophages restricts hepatic dissemination of intraperitoneal bacteria by neutrophil recruitment. Immunity 2017;47(02):374–388.e6

Sierro F, Evrard M, Rizzetto S, et al. A liver capsular network of inflammation and neutrophil influx in arthritis. Cell Reports 2014;9(02):618–632

Fallowfield JA, Mizuno M, Kendall TJ, et al. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 2007;178(08):5288–5295

Newby AC. Metalloproteinase production from macrophages - a perfect storm leading to atherosclerotic plaque rupture and myocardial infarction. Exp Physiol 2016;101(11):1327–1337

Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biol 2015;44-46:224–231

Madsen DH, Leonard D, Masdeuksas A, et al. MZ-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway. J Cell Biol 2013;202(06):951–966

Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nat Rev Immunol 2014;14(03):181–194

Surewaard BG, Deniset JF, Zemp FJ, et al. Identification and treatment of the Staphylococcus aureus reservoir in vivo. J Exp Med 2016;213(07):1141–1151

Melgert BN, Olinga P, Van Der Laan JM, et al. Targeting deoxymethasone to Kupffer cells: effects on liver inflammation and fibrosis in rats. Hepatology 2001;34(4 Pt 1):719–728

Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol 2017;66(06):1300–1312

Thomas JA, Pope C, Wojtacha D, et al. Macrophage therapy for murine liver fibrosis recruits host effector cells improving fibrosis, regeneration, and function. Hepatology 2011;53(06):2003–2015

Ma PF, Gao CC, Yi J, et al. Cytotherapy with M1-polarized macrophages ameliorates liver fibrosis by modulating immune microenvironment in mice. J Hepatol 2017;67(04):770–779

Haideri SS, McKinnon AC, Taylor AH, et al. Injection of embryonic stem cell derived macrophages ameliorates fibrosis in a murine model of liver injury. NJR Regen Med 2017;2(01):14

Moore JK, Mackinnon AC, Wojtacha D, et al. Phenotypic and functional characterization of macrophages with therapeutic potential generated from human cirrhotic monocytes in a cohort study. Cytotherapy 2015;17(11):1604–1616

Heymann F, Hammerich L, Storch D, et al. Hepatic macrophage migration and differentiation critical for liver fibrosis is mediated by the chemokine receptor C-C motif chemokine receptor 8 in mice. Hepatology 2012;55(03):898–909

Mossanen JC, Krenkel O, Ergen C, et al. Chemokine (C-C motif) receptor 2-positive monocytes aggravate the early phase of acetaminophen-induced acute liver injury. Hepatology 2016;64(05):1667–1682

Fraser AR, Pass C, Burgoyne P, et al. Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: a novel cellular therapeutic for liver cirrhosis. Cytotherapy 2017;19(09):1113–1124

Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 2001;411(6834):207–211

Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 2000;405(6872):85–90

Carlsson A, Christenson K, Matlak M, et al. Galectin-3 functions as an opsonin and enhances the macrophage clearance of apoptotic neutrophils. Glycobiology 2009;19(01):16–20

Platt N, Suzuki H, Kurihara Y, Kodama T, Gordon S. Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes in vitro. Proc Natl Acad Sci U S A 1996;93(22):12456–12460

deCathelineau AM, Henson PM. The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. Essays Biochem 2003;39:105–117

Greenlee-Wacker MC. Clearance of apoptotic neutrophils and resolution of inflammation. Immunol Rev 2016;273(01):357–370

James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatoxicity. Drug Metab Dispos 2003;31(12):1499–1506

Prescott LF, Park J, Ballantyne A, Adriaenssens P, Proudfoot AT. The role of monocyte-derived macrophages restricts hepatic dissemination of intraperitoneal bacteria by neutrophil recruitment. Immunity 2017;47(02):374–388.e6

Rolando N, Philpott-Howard J, Williams R. Bacterial and fungal infection in acute liver failure. Semin Liver Dis 1996;16(04):389–403

Rolando N, Harvey F, Brahm J, et al. Prospective study of bacterial infection in acute liver failure: an analysis of fifty patients. Hepatology 1990;11(01):49–53

Rolando N, Harvey F, Brahm J, et al. Fungal infection: a common, unrecognised complication of acute liver failure. J Hepatol 1991;12(01):1–9

Karvellas CJ, Cavazos J, Battenhouse H, et al; US Acute Liver Failure Study Group. Effects of antimicrobial prophylaxis and blood stream infections in patients with acute liver failure: a retrospective cohort study. Clin Gastroenterol Hepatol 2014;12(11):1942–9.e1

Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. Hepatology 2000;32(4 Pt 1):734–739
Macrophages as a Cell-Based Therapy for Liver Disease

Starkey Lewis et al

101 Possamai LA, Thurs MR, Wendon JA, Antoniades CG. Modulation of monocyte/macrophage function: a therapeutic strategy in the treatment of acute liver failure. J Hepatol 2014;61(02):439–445

102 Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. Cold Spring Harb Perspect Biol 2014;6(06):a021857

103 Stuchfield BM, Antoine DJ, Mackinnon AC, et al. CSF1 restores innate immunity after liver injury in mice and serum levels indicate outcomes of patients with acute liver failure. Gastroenterology 2015;149(07):1896–1909.e14

104 Holt MP, Yin H, Ju C. Exacerbation of acetaminophen-induced disturbances of liver sinusoidal endothelial cells in the absence of Kupffer cells in mice. Toxicol Lett 2010;194(1-2):34–41

105 You Q, Holt M, Yin H, Li G, Hu CJ, Ju C. Role of hepatic resident and infiltrating macrophages in liver repair after acute injury. Biochem Pharmacol 2013;86(06):836–843

106 Holt MP, Cheng L, Ju C. Identification and characterization of infiltrating macrophages in acetaminophen-induced liver injury. J Leukoc Biol 2008;84(06):1410–1421

107 Ju C, Reilly TP, Bourdi M, et al. Protective role of Kupffer cells in acetaminophen-induced hepatic injury in mice. Chem Res Toxicol 2002;15(12):1504–1513

108 Bourdi M, Masubuchi Y, Reilly TP, et al. Protection against acetaminophen-induced liver injury and lethality by interleukin 10; role of inducible nitric oxide synthase. Hepatology 2002;35(02):289–298

109 Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Büschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 1995;22(02):226–229

110 Ehling J, Bartneck M, Wei X, et al. CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. Gut 2014;63(12):1960–1971

111 Boulter L, Guest RV, Kendall TJ, et al. WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. J Clin Invest 2015;125(03):1269–1285

112 Preziosi M, Okabe H, Poddar M, Singh S, Monga SP. Endothelial Wnts regulate β-catenin signaling in murine liver zonation and regeneration: a sequel to the Wnt-Wnt situation. Hepatol Commun 2018;2(07):845–860

113 Yang J, Mowry LE, Nejak- Bowen KN, et al. β-catenin signaling in murine liver zonation and regeneration: a Wnt-Wnt situation I. Hepatology 2014;60(03):964–976

114 Mukaro VR, Bylund J, Hodge G, et al. Lectins offer new perspectives in the development of macrophage-targeted therapies for COPD/emphysema. PLoS One 2013;8(02):e56147

115 Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014;6:13

116 Liu HC, Zheng MH, Du YL, et al. N9 microglial cells polarized by LPS and IL4 show differential responses to secondary environmental stimuli. Cell Immunol 2012;278(1-2):84–90

117 Chinetti-Gbaguidi G, Baron M, Bouchel MA, et al. Human atherosclerotic plaque alternative macrophages display low cholesterol handling but high phagocytosis because of distinct activities of the PPARγ and LXRα pathways. Circ Res 2011;108(08):985–995

118 Mendoza-Coronel E, Ortega E. Macrophage polarization modulates FcγR- and CD13-mediated phagocytosis and reactive oxygen species production, independently of receptor membrane expression. Front Immunol 2017;8:303

119 Gautier EL, Shay T, Miller J, et al; Immunological Genome Consortium. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 2012;13(11):1118–1128

120 Blair HC, Teitelbaum SL, Ghiselli R, Gluck S. Osteoclast bone resorption by a polarized vascular proton pump. Science 1989;245(4920):855–857

121 Zhan Y, Paolicelli RC, Sforazzini F, et al. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci 2014;17(03):400–406

122 Terpstra V, van Berkel TJ. Scavenger receptors on liver Kupffer cells mediate the in vivo uptake of oxidatively damaged red blood cells in mice. Blood 2000;95(06):2157–2163

123 Scott CL, Zheng F, De Baetselier P, et al. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. Nat Commun 2016;7:10321

124 Sheng J, Ruedi C, Karjalainen K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. Immunity 2015;43(02):382–393

125 Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 2015;518(7540):547–551

126 Moore JK, MacKinnon AC, Man TY, Manning JR, Forbes SJ, Simpson KJ. Patients with the worst outcomes after paracetamol (acetaminophen)-induced liver failure have an early monocytopenia. Aliment Pharmacol Ther 2017;45(03):443–454
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