

Liver regeneration - mechanisms and models to clinical application

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1 **Liver regeneration: mechanisms and models to clinical application**

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3
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16
17
18 **Keywords:** liver regeneration, liver failure, hepatic progenitor cells, cell therapy, cirrhosis, hepatocellular
19 cancer.

20
21 **Abbreviations:** APOLT, auxiliary partial orthotopic liver transplantation; HPCs, Hepatic progenitor cells;
22 LSEC, liver sinusoidal endothelial cells; MSCs, mesenchymal stem cells; OLT, Orthotopic liver
23 transplantation; VEGF, vascular endothelial growth factor;

24
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1
2 **Key points**

- 3 • **Liver regeneration is well described and occurs efficiently in the normal liver to restore normal**
4 **architecture, size and function**
- 5 • **Chronic injury severely impairs liver regeneration through excess inflammation, scarring and**
6 **epithelial abnormalities, this is less well studied but clinically important.**
- 7 • **Zebrafish are an excellent new tool to study liver regeneration and enable large scale chemical**
8 **screening assays.**
- 9 • **There is a gap between the generally utilised animal models of liver regeneration and clinically**
10 **important scenarios of severe liver injury and impaired liver regeneration**
- 11 • **Understanding and promoting regeneration and repair of the failing liver is a key challenge of**
12 **major clinical significance**
- 13 • **Modern imaging techniques will allow non-invasive real-time assessment of liver structure and**
14 **function.**
- 15 • **Cell therapies that have been successful in animal models are now being trialled in the more**
16 **challenging clinical arena.**
- 17
18

19 **Introduction**

20 **Why the clinical need to understand measure and promote liver regeneration?**

21 Although the normal liver has a fantastic regenerative capacity following acute injury or resection this
22 regenerative ability becomes overwhelmed in two important scenarios: (1) in the setting of severe acute liver
23 injury or (2) when there is severe chronic liver injury with aberrant liver architecture and marked liver
24 fibrosis. These are clinically relevant scenarios that often result in serious morbidity and mortality. Whilst
25 there have been decades of excellent and clinically informative research into understanding the signals that
26 control regeneration of the normal liver¹ the mechanisms at play when the abnormal liver attempts
27 regeneration are less well described². Understanding how regeneration fails or is impaired in the severely
28 damaged liver is an important goal. Lessons learned from relevant animal models may have a have
29 importance in the clinical setting and aid the development of new therapies to either promote regeneration or
30 prevent complications that arise during the period of liver regeneration. A clinical scenario where an
31 improved understanding of regeneration of the compromised liver includes liver transplantation, where the
32 increasingly common use of partial livers such as split livers and living donor transplants relies upon
33 regeneration of the donor graft to reach the correct liver mass. Failure of regeneration in these settings
34 results in poor or delayed graft function, prolonged intensive care stays, occasionally a requirement for re-
35 transplantation or ultimately even death of the recipient. By understanding the pathological mechanisms

1 driving these adverse conditions it is hoped that the period of regeneration can be more predictable and the
2 associated clinical complications ultimately preventable. The ability to predict or improve liver regeneration
3 when the liver is compromised, for example in the setting of cirrhosis when surgical resection of
4 hepatocellular cancer (HCC) is commonly performed, or following the resection of colorectal hepatic
5 metastasis, when the liver has received prior chemotherapy, would allow clinicians to optimise cancer
6 resection approaches. Furthermore by understanding the mechanisms underlying normal liver regeneration
7 and aberrant liver regeneration in chronic liver injury it is hoped that we will be able to promote “healthy
8 regeneration” or remodelling in chronic liver disease. Such a scenario would be liver cirrhosis where the
9 initial insult may now be directly treated such as viral hepatitis or autoimmune hepatitis, but the liver tissue
10 is left severely damaged and still susceptible to the clinical consequences of liver failure, portal hypertension
11 and an increased risk of HCC. This review cannot be comprehensive but seeks to describe briefly the
12 mechanisms underpinning liver regeneration, the models used to study this and then discuss areas where
13 failed or compromised liver regeneration is clinically relevant – with a view to highlighting areas for future
14 research.

15 **1. Mechanisms and models of Liver regeneration**

17 In the following section we will review some of the animal models that have been used to understand liver
18 regeneration. These have traditionally been in rodents but new models are emerging such as the zebrafish. A
19 general theme is that there has been much work important work understanding how the normal liver
20 regenerates in these models, but there is less information about how the damaged or compromised liver
21 regenerates.

22 **The Rat model of liver regeneration (see **figure 1**)**

24 The normal liver will attempt to retain an appropriate size relative to the rest of the body. Following injury
25 or resection the remaining liver undergoes a rapid series of co-ordinated changes to regain its original
26 volume and structure¹. Interestingly, this need to retain the previous size to body weight ratio appears after
27 liver hypertrophy has been induced by growth factors such as Tri-iodothyronine, when the liver shrinks back
28 to its original size³.

29 The rat partial hepatectomy model is the classic model of liver regeneration and has been studied for
30 decades. In a landmark paper from 1931, Higgins and Anderson reported that removal of the two anterior
31 lobes of the rat liver (the median and left lateral lobes) equated to a 70% reduction in liver size.⁴ This
32 standardised procedure is well tolerated and produces a reliable result. Whilst the normal adult liver is
33 mitotically quiescent with only minor hepatocyte proliferation detectable, following 70% or “two thirds
34 partial hepatectomy” the remaining liver remnant undergoes a series of rapid vascular endothelial,
35 inflammatory and epithelial changes (**See Figure 2A**). The peak of liver regeneration, as measured by the
36 number of hepatocytes in DNA synthetic phase, termed “S phase”, occurs about 24 hours following

1 resection. By 7 to 10 days following hepatectomy the rat has largely regrown a normal sized liver (93%) by
2 hyperplasia of the remnant lobes, and by 20 days following hepatectomy the liver has fully regained its
3 starting volume. This simple and repeatable experimental procedure has enabled many important new
4 insights into regeneration of the normal liver¹. Following such “normal regeneration” the non-parenchymal
5 cells in the liver, namely the stellate cells, liver sinusoidal endothelial cells (LSECS) and macrophages act in
6 a coordinated fashion and help to control the epithelial regenerative response⁵. In a classic parabiosis
7 experiment by Moolten and Bucher, carotid-to-jugular cross circulation was established between a rat that
8 has been subjected to partial hepatectomy and a normal rats. This induced “liver regeneration” in the non-
9 hepatectomized rats with normal livers. This suggested factors were circulating from the hepatectomized rat
10 to the normal rat to induce the regenerative response and thereby pointed to there being circulating blood
11 derived factors that help to stimulate and co-ordinate liver regeneration following partial hepatectomy⁶.
12 Interleukin-6, tumor necrosis factor- α (TNF- α), hepatocyte growth factor (HGF), epidermal growth factor
13 (EGF) and thyroid hormone have been discovered as humoral factors that control liver regeneration^{7, 8}.
14 Whilst the multiple important mechanisms controlling normal liver regeneration that have been identified in
15 the rat, these have been well reviewed elsewhere¹ and we will therefore not write further on this.

16
17 The volume of liver resected in the rat can be increased to 90%, effectively modelling the clinical syndrome
18 termed “small for size”. In both the 90% hepatectomy model and the clinical situation survival is
19 compromised with death from liver failure a significant risk⁹. In the 90% hepatectomy model and the
20 clinical situation, if the volume of resection increases beyond a threshold then the regenerative capacity of
21 the remaining hepatocytes actually begin to fall, thereby contributing to a rapidly developing scenario of
22 liver failure. Understanding why there is a failure of appropriate regeneration by the remaining liver is a
23 clinically important goal. The contributory mechanisms of this failure of regeneration are likely to be
24 multiple but involve vascular shear stress in the livers sinusoids caused by the portal blood passing through a
25 small parenchymal volume which can cause periportal sinusoidal endothelial damage and parenchymal
26 inflammation¹⁰.

27
28 Various strategies have been deployed in this model of 90% hepatectomy to increase liver regeneration and
29 /or improve the survival following this operation. Ren et al. showed that 90% but not 70% hepatectomy
30 increased portal and systemic endotoxin levels. Following this observations they used selective bowel
31 decontamination with Gentamycin and showed that this reduced lipopolysaccharide levels, enhance liver
32 regeneration and increased the survival following 90% hepatectomy from 24% to 56%¹¹. Given that sepsis
33 due to gut related organisms is a major cause of death following major hepatic resection this is a potentially
34 important finding¹². Another potentially clinically relevant treatments for this syndrome is octreotide which
35 has been shown in the rat 90% hepatectomy model to reduce mortality from 63% to 33%¹³. Interestingly,
36 although octreotide had this beneficial effect on mortality it actually reduced early hepatocyte proliferation,

1 however it did reduce liver injury and necrosis and modified the hepatic methionine cycle reaction, causing
2 an increase in methionine and 5'-methylthioadenosine, which was thought to be important for the beneficial
3 effect. An important study by Ninomiya and colleagues challenged the assumption that the promotion of
4 regeneration would be beneficial in the rat 90% hepatectomy model. They hypothesized that the rapid
5 regenerative response of small remnant liver is actually responsible for the poor outcome seen. They
6 administered dHGF which promoted the rate of liver regeneration in the 70% hepatectomy model. However,
7 in the 90% hepatectomy this had no benefit upon the survival rates. Instead they sought to delay the
8 regenerative response through the administration of either NS-398 (an ERK1/2 inhibitor) or PD98059 (a
9 selective MEK inhibitor). Deceleration of the regenerative response by NS-398 or PD98059 treatment
10 resulted in a significant and exciting improvement in day 7 survival (approximately 70%) compared to the
11 vehicle treated group (10%)¹⁴. Interestingly the lobular spatial integrity was better preserved in animals that
12 had their regenerative response lowered. Presumably this enables the portal blood flow and resulting
13 physiological function to be maintained during the regenerative phase.

14
15 The rat has further been widely used to study liver regeneration when the regenerative capacity of mature
16 hepatocytes is compromised. Here hepatocyte proliferation is inhibited by the chemical 2-
17 Acetaminofluorene (AAF). This can be combined with either partial hepatectomy or the hepatotoxin carbon
18 tetrachloride to prompt liver regeneration^{15, 16}. In a classic paper from the Thorgeirsson laboratory
19 [3H]thymidine was administered to the AAF treated rats at 6 days following partial hepatectomy. The
20 [3H]thymidine labelled the only epithelial cells that were proliferating in the liver at this time, the
21 oval/HPCs. When the rats were subsequently sacrificed from 9 to 13 days the [3H]thymidine was then
22 identified in hepatocytes. Whilst not definitive proof that the oval cells/HPCs were the source of hepatocytes
23 in the rat, this was suggestive of a product-precursor relationship¹⁷. Later work in the rat AAF/partial
24 hepatectomy model confirmed that cell proliferation was limited to the oval cells/HPCs and that hepatocytes
25 were senescent (i.e. unable to proliferate) and p21 positive, making them an unlikely source of
26 regeneration¹⁸. However, a recent report has challenged the concept that oval cells/HPCs contribute to liver
27 parenchyma regeneration in the AAF/PH model in the rat and following careful observations suggested that
28 the replication of mature hepatocytes mainly contributes regenerate the liver, even in these circumstances
29 where there is a challenge to hepatocyte regeneration¹⁹; presumably these hepatocytes, if able to regenerate
30 hepatocytes, would have initially escaped the effects of AAF. Such results emphasize the need for reliable
31 lineage tracing systems to make the claims of regenerative potential of various cell populations secure and
32 the technology to achieve this is not well developed in the rat compared to mouse²⁰.

34 **The mouse as a model of liver regeneration.**

35 More recently the mouse has been used as a model of liver regeneration, this has facilitated the use of the
36 many mouse transgenic strains that enable the understanding of the role of various genes that control or

1 modulate liver regeneration²¹. The transgenic mice may have a permanent over or under-expression of a
2 normal or mutated gene, a refinement of this is that the gene may be “conditionally” deleted from a
3 particular cell type in the liver at a set time- for example before a partial hepatectomy. The physiology of
4 regeneration in the mouse following partial hepatectomy is similar to the rat although following partial
5 hepatectomy the peak of regeneration, as measured by BrdU incorporation into hepatocytes in DNS
6 synthesis (S phase), occurs slightly later, at 24-48 hours²².

7 The mouse has been used to model liver regeneration in the setting of chronic liver damage where “normal
8 liver regeneration” is impaired. The most frequently used model of iterative liver damage is the chronic
9 carbon tetrachloride (CCl₄) model of liver injury (which has also been used in the rat). Following CCl₄
10 administration there is predictable parenchymal necrosis most prominently surrounding the central veins
11 peaking at 24 hours which is then followed by liver regeneration. After repeated dosing of CCl₄ liver fibrosis
12 begins to develop, with the activation of stellate cells into scar forming myofibroblasts, the deposition of
13 excess liver scar tissue, and ultimately the development of nodular liver cirrhosis. The collagen scar
14 becomes increasingly crosslinked over time, making degradation of the scar more difficult, and further
15 inhibiting regeneration²³. Upon cessation of CCl₄ administration, there is regeneration of the liver
16 parenchyma which is combined with scar degradation and resolution of the inflammation. In this setting the
17 importance of macrophages in collagen scar regression^{24, 25}, has been shown to be critical for effective liver
18 regeneration²⁶.

19 There are several dietary models of liver injury in mouse commonly used to model liver disease including
20 the 1,4-dihydro-2,4,6-trimethyl-pyridine-3,5-dicarboxylate (DDC) diet²⁷, which induces biliary injury and
21 regeneration. Following the diet there is a proliferation of primitive ductules with poorly defined lumens that
22 spread from the portal tract into the hepatic lobule. This “ductular reaction” is associated with significant
23 fibrosis^{28, 29} and thus is a model of biliary injury and fibrosis. Mice subject to the DDC diet respond poorly
24 to partial hepatectomy²⁷. Another diet model which is commonly used in mice is the modified CDE diet
25 which was developed by the Yeoh Group³⁰ which induces hepatocellular injury with a degree of steatosis
26 and a secondary “ductular response” where oval/ductular cells spread from the portal tract^{28, 31, 32}. These
27 ductular reactions are important for biliary regeneration following injury and if their proliferative response is
28 impaired following biliary injury then there is an increase in hepatic necrosis³³. Whether the ductular
29 reactions contain bipotential hepatic progenitor cells capable of regenerating hepatocytes as well as biliary
30 cells is a controversial area. In mouse, in the absence of significant hepatocyte senescence, then hepatocyte
31 self-replication seems to provide practically all hepatocyte regeneration with little or no contribution from
32 hepatic progenitor cells^{34, 35}. However, in the context of severe liver injury and hepatocyte replication failure
33 then hepatic progenitor cells may have hepatocyte regenerating capacity³⁶- the degree and importance of this
34 axis in severe liver injury needs further study and this may require the development of models where
35 hepatocyte replication can be inhibited to model the severe human liver disease.

1 **Zebrafish**

2 Zebrafish have recently been developed to model many diseases and understand pathophysiological
3 processes (**see figure 1**)³⁷. Their small size and optically translucency brings the advantages of low cost,
4 rapid analysis. Because they grow in water zebrafish have been used as model system for in vivo chemical
5 screening. To date, their use has shown that many of the biological processes and signalling pathways seen
6 in the mouse and rat are recapitulated in zebrafish.

7 There are a number of ways of provoking liver regeneration in the zebrafish including surgical partial
8 hepatectomy, drug induced liver injury and nitroreductase-mediated hepatocyte ablation³⁸. The zebrafish has
9 a trilobar structure and the one-third partial hepatectomy model has been established in the zebrafish by
10 removal of one lobe³⁹. Clearly this is currently a more limited resection than performed in rodents. These
11 studies have established signals such as Wnt⁴⁰, BMP and FGF as important for liver regeneration in
12 zebrafish⁴¹. Interestingly zebrafish exhibit cellular plasticity in that bile ducts can convert to hepatocytes
13 following large-scale hepatocyte loss. Two independent reports found, using hepatocyte ablation and lineage
14 tracing, that following extensive hepatocyte loss the biliary cells are able to regenerate the hepatocytes⁴²⁻⁴⁴.
15 Interestingly, in an ethanol induced model of liver fibrosis Huang et al. found that Wnt and Notch has
16 opposing roles in directing HPCs in their regeneration of hepatocytes. Low levels of Notch stimulation
17 stimulated HPC proliferation and hepatocyte differentiation, high levels of Notch suppressed this pathway.
18 Wnt ligands were found to suppress Notch signalling via Numb a protein inhibitor of Notch⁴⁵. Importantly
19 this helps to validate the zebrafish model in the liver regeneration setting, as the same opposing signals Wnt
20 and Notch acting via the node Numb signals have previously been shown to control the behaviour of HPCs
21 in mouse and are differentially expressed in hepatocellular versus biliary injury in human liver³¹. Zebrafish
22 are an ideal model for “forward genetic” due to their small size and ability to screen large numbers of
23 organisms following exposure to a chemical mutagen. Phenotypes can be screened and the actual gene/s
24 responsible then mapped, an approach that has already yielded results in the setting of liver development⁴⁶.
25 This exciting new model system looks set to make important inroads especially into the area of screening
26 compounds and drugs for their effects upon liver regeneration. However we should still express some
27 caution and there is an important need to show that the signals and targets identified translate through into
28 mammalian systems including human liver regeneration.

30 **The cellular sources and mechanisms controlling epithelial regeneration in various model systems**

31 As discussed above, mouse models of liver injury recent lineage tracing experiments have failed to show
32 convincing regeneration from non-hepatocyte sources^{34, 47} unless there is significant liver injury and
33 hepatocyte proliferation is strongly inhibited³⁶. Furthermore, there is evidence that hepatocytes can undergo
34 a ductular change and at least partly contribute to the ductular cell population^{48, 49}. However as outlined
35 above, in zebrafish there is strong evidence using lineage tracing systems that following significant liver
36 injury ductular cells/HPCs can give rise to significant hepatocyte regeneration⁴²⁻⁴⁴. In the rat there is some

1 circumstantial evidence suggestion oval cells/HPCs can regenerate hepatocytes when hepatocyte
2 regeneration is compromised;¹⁷ however, opposing data does exist suggesting that this regenerative pathway
3 is not significant¹⁹. A common theme is the need for reliable lineage tracing systems to provide proof of the
4 regenerative lineages in the models commonly studied, a further issue is the whether the liver injury systems
5 reliably recapitulate the severity of liver injury seen in human disease. Morphological studies have recently
6 claimed that hepatic progenitor cells regenerate hepatocyte “buds” in areas of liver parenchyma has been
7 obliterated⁵⁰. However, performing lineage tracing experiments in humans is not possible and caution is
8 required in interpreting such studies. The nearest approach to lineage tracing in the human liver is the use of
9 mitochondrial DNA mutation analysis to show that regenerative nodules and adjacent ducts can be clonal⁵¹.
10 Although an important observation and a technical “tour de force” this does not conclusively prove the any
11 precursor- product relationship. Understanding the cellular contributions to hepatocyte and biliary
12 regeneration may seem an academic exercise and remote from clinical practice, however, defining the
13 regenerative cells in clinically relevant models of liver injury and regeneration will considerably aid the
14 development of strategies to promote liver regeneration, either through cell therapy or through the
15 stimulation of endogenous repair and regeneration.

16 17 **Bile acids and liver regeneration**

18 Bile acids (BAs) have recently been recognised as important for liver regeneration. Ueda et al. showed that
19 liver regeneration is impaired in rats in the absence of intestinal bile⁵². Following this report, it was shown
20 that increased bile acid levels accelerate regeneration, whilst low levels of BAs impair regeneration as does
21 absence of the BA receptor Farnesoid X Receptor (FXR)⁵³. BAs are rapidly increased following partial
22 hepatectomy and signal via the receptors FXR and G-protein-coupled BA receptor 1 (GPBAR1). FXR
23 signalling reduces liver injury and promotes liver regeneration following CCl4 induced liver injury⁵⁴. The
24 potential clinical application of these basic studies was indicated by Otato et al. who analysed liver
25 regeneration in patients following major hepatectomy and found that patients who had external biliary
26 drainage had lower levels of liver regeneration than those patients without external biliary drainage⁵⁵. This
27 was a retrospective study and there may be confounding factors that could explain the striking results
28 however further studies into this area are warranted. This interesting study highlights a general point, that
29 prospective trials are warranted in the clinical setting where there is strong animal data indicative of efficacy
30 and where there is an acceptable risk/benefit ration from a new intervention.

31 32 **2. The gap between animal models and the clinical experience of liver regeneration**

33 Both animal models and clinical studies are informative but there still remains a gap that is poorly bridged
34 between these two disciplines (see table 1). Ideally to maximise the development of understanding liver
35 regeneration and develop new techniques to enhance liver regeneration observations made using in vivo
36 models should inform human studies and the human studies should feed-back to refine the in vivo models.

1 At one end of this “bridge” animal models have been highly informative regarding the drivers of liver
2 regeneration, the timing of response and cellular sources of the regenerative cells in the liver. Through the
3 use of modern cell and molecular biology techniques, combined with modern transgenic mouse and now
4 zebrafish technology, the signalling and cellular mechanisms underpinning liver regeneration are rapidly
5 being described.

6 At the other side of the bridge human clinical studies of liver regeneration have often followed patients who
7 have has a heterogeneous collection of liver insults. The studies have primarily looked at clinical outcome
8 and sought to define associations of poor outcome and pre-operative markers of poor outcome. The study
9 techniques have often revolved around whole organ imaging and serum analysis.

10 To date there is still a marked gap between these areas of study with the signalling mechanisms rarely
11 translating to clinical trials or indeed human observational studies, and thereby rarely having any clinical
12 impact. Likewise, the human observational studies providing little data to support further discriminatory *in*
13 *vivo* studies. Non-invasive measurements during liver regeneration should be particularly insightful in the
14 future. Modern functional imaging techniques, such as MRI spectroscopy⁵⁶⁻⁵⁸, where distinct metabolic
15 signatures are seen in patients regenerating liver should impact on. Likewise proteomic analysis of patients’
16 blood during regeneration seems an obvious future development; studies in mice have already shown a
17 distinct proteomic signature in plasma following hepatectomy and during liver regeneration that were
18 strongly associated to metabolims.⁵⁹ Another non-invasive method analysis is the 13C-breath tests which
19 can measure hepatic mitochondrial, microsomal, and cytosolic function.⁶⁰ The 13C-phenylalanine breath test
20 has been used in a rat model of 70% hepatectomy and showed good discrimination between 70%
21 hepatectomy rats and controls at 24 hours post-surgery, indicating possible future clinical utility⁶¹. The
22 above techniques all show promise and may help to build strong links between the *in vivo* models and
23 human studies and indeed allow further refinement of the current *in vivo* models.

24 25 **3. Regeneration in the “abnormal liver”**

26 In the clinic the regeneration of normal liver is relevant- for example when a well relative donates part of
27 their liver to a recipient with liver disease- so called living donor liver transplantation”. Here, the donor will
28 have been specifically screened to exclude significant liver disease⁶². However, in the majority of clinical
29 scenarios the abnormal- damaged liver is the one required to perform the feat of regeneration. The
30 challenges to regeneration are very different across the different clinical scenarios and some of these are
31 detailed below:

32 33 **Severe acute liver damage resulting in fulminant liver failure**

34 Common causes of acute liver failure include viruses such as hepatitis A, B and E, drugs such as
35 acetaminophen, and auto-immune liver disease⁶³. By definition the liver was previously normal and the
36 damage acute- often with widespread hepatocyte apoptosis and necrosis. Following moderate liver injury

1 and necrosis, there is proportional hepatocyte proliferation until homeostasis is achieved. However, with
2 increasing injury a threshold is reached beyond which the remaining liver fails to regenerate adequately.
3 This has been clearly shown by Bhushan et al. in mice that, compared to a moderate dose of acetaminophen
4 (300mg/kg), a higher dose (600mg/kg) actually resulted in poorer liver regeneration in the non-necrotic parts
5 of the liver⁶⁴. There have been attempts to stimulate liver regeneration following acute liver injury in
6 humans. In mice, using the acetaminophen model of liver injury, loss of β -Catenin activation prevents liver
7 regeneration. In patients with APAP mediated liver injury the degree of β -Catenin activation correlated with
8 the degree of liver regeneration, indicating that β -Catenin activation could be a possible therapeutic strategy
9 in patients with acute liver injury⁶⁵.

10
11 In the setting of acute liver injury, the innate immune system is critical for coordinating and stimulating
12 regeneration, as well as for maintaining immunity⁵. In particular macrophages are important for the
13 phagocytosis of the necrotic tissue and the stimulation of liver regeneration. Following acetaminophen
14 induced liver injury macrophages are rapidly recruited to the areas of liver necrosis⁶⁶. Mice deficient in
15 CSF-1 have reduced numbers of tissue macrophages and an impairment in liver regeneration, which can be
16 overcome by the addition of exogenous CSF-1⁶⁷. As well as helping to co-ordinate a liver regeneration
17 response the hepatic macrophages are important in controlling sepsis, a major complication of acute liver
18 failure that is associated with clinical deterioration, systemic inflammatory response and multi-organ failure.
19 Hepatic macrophages, so-called Kupffer Cells (**Figure 2**), are a major filter of portal blood and are
20 particularly important when the gut barrier function is compromised in liver failure. CSF-1 levels are related
21 to prognosis in acetaminophen induced fulminant liver failure and in experimental models of acute liver
22 injury the exogenous administration of CSF-1 has been used to boost immunity and hepatic macrophage
23 phagocytic function⁶⁸.

26 **Fatty liver**

27 Given the increasing levels of fatty liver in the West it is not surprising that the impaired regeneration of
28 fatty livers is an increasingly important clinical question. Many fatty liver grafts have to be discarded as
29 above a threshold the liver often fails upon transplant. Obese patients regenerate their livers more slowly
30 than non-obese controls⁶⁹. Dietary induced hepatic steatosis reduces liver regeneration following 70%
31 hepatectomy in rats⁷⁰. In the setting of liver regeneration of fatty liver, growth arrest and DNA damage-
32 inducible 34 (Gadd34) inhibition was shown to be important, and Gadd344 overexpression through gene
33 therapy increased liver regeneration in mice with fatty liver⁷¹.

34
35 Clinically, fatty livers are the subject of potential hepatectomy when the resection of colorectal metastasis is
36 being contemplated in patients with fatty liver due to NAFLD or as a secondary response to chemotherapy.

1 Interestingly in a recent analysis of patients who had undergone liver resection for colorectal liver
2 metastases, in over 5800 patients that had not received pre-operative chemotherapy, and 4000 patients that
3 had received pre-operative chemotherapy, the presence of hepatic steatosis did not worsen their 90 day or 5
4 year overall survival rates^{72,73}.

6 In transplantation the presence of macroscopic hepatic steatosis can have serious consequences and increase
7 the risk of primary graft non function⁷⁴, the more severe the steatosis then the greater the risk of hepatic
8 dysfunction. The assessment of steatosis can be made by the surgeon at the time of organ procurement but
9 has an inbuilt subjective element. A pathologist can quantify hepatic steatosis on biopsy but this is not
10 always convenient and of course is a limited sample area. Imaging methods are therefore being trialled as a
11 way of objectively quantifying hepatic steatosis. A CT assessment of the donor liver was tested in 109
12 consecutive cadaveric donors and the liver/spleen attenuation ratio determined (used in liver donor imaging).
13 All graft had a biopsy and blinded pathological assessment. The CT scan was able to predict significant
14 steatosis (defined as >30%) with a sensitivity of 79% and a specificity of 97%⁷⁵ indicating its potential
15 future utility.

17 **Small liver grafts, ischaemia-reperfusion injury and RAGE**

18 The transplantation of small grafts relative to the recipient can result in so-called “small for size syndrome”-
19 SFSS. There are several putative pathological mechanisms thought to be causative including intrahepatic
20 vascular shear stress. SFSS results in a combination of injury in the liver and poor regeneration of the
21 graft⁷⁶. The clinical presentation often includes liver failure, coagulopathy, ascites, cholestasis and
22 encephalopathy. Whilst the mainstay of management is prevention by careful volume analysis, surgical
23 techniques are developing to reduce the incidence of this serious but thankfully rare condition⁷⁷.

25 Ischaemia –reperfusion injury is a major issue affecting transplanted livers. Reactive oxygen species
26 increase in the graft during removal of the graft and cold storage due to anaerobic metabolism. Receptor for
27 advanced glycation endproducts (RAGE) is markedly increased in mice subject to hepatic ischaemia-
28 reperfusion injury. Two important studies have shown that RAGE is a potential therapeutic target. In a
29 murine model of ischaemia-reperfusion, blockage of RAGE signalling reduced liver injury and increased
30 regeneration⁷⁸. In a murine model of hepatectomy, RAGE was increased in 85% hepatectomy mice,
31 compared to 70% hepatectomy in mice. RAGE was expressed in dendritic cells mononuclear phagocyte -
32 derived dendritic cells. Blockade of RAGE reduced hepatocyte death, increased regeneration and increased
33 survival in the 85% hepatectomy mice from 30% to 90%⁷⁹. In patients with acetaminophen induced acute
34 liver failure, increased circulating levels of soluble receptor for advanced glycation end products (sRAGE)
35 was found to be associated with liver transplant or death rather than spontaneous recovery⁸⁰. Future therapy
36 may be indicated by a study in mice which reduced hepatic IR injury by prior treatment with the drug

1 Losartan which increased PPARG signalling and reduced RAGE activation⁸¹. Clearly the early detection and
2 quantification of the livers metabolic status to predict post-transplant ischaemia-reperfusion may allow
3 therapeutic trials and interventions. It has been suggested, that ex vivo spectroscopy of the organ could be
4 performed to gain a real time assessment of the metabolic status of the graft and facilitate possible pre-
5 conditioning interventions⁵⁶. Whilst this is an interesting future technique it is technology and operator
6 intense and at present time this may limit widespread uptake.

8 **The issue of abnormal and excessive extracellular matrix**

9 There is activation of the quiescent stellate cells (pericytes into activated scar forming myofibroblasts
10 leading to excessive deposition of extracellular matrix (**Figure 2B**). This aberrant scar formation in the liver
11 has been shown to inhibit hepatocyte proliferation²³. The collagen scar also needs to be remodelled for the
12 formation of a ductular response²⁶. The relationship between the “ductular reaction”, which occurs during
13 times of impaired regeneration, and fibrosis is complex as a florid ductular response is also commonly
14 associated with a rapid fibrotic response²⁹. Following cessation of injury the scar tissue can be degraded, the
15 hepatic macrophages are thought to be key cellular mediators of this action²⁴, secreting matrix
16 metalloproteinases that can degrade scar tissue^{24, 82}. If liver injury and scarring progresses, then eventually
17 bridging fibrosis and the appearance of regenerative nodules occur. In this setting vascular abnormalities
18 develop and the blood flow to the liver switches from being predominantly from the portal vein to
19 predominantly from the hepatic artery-i.e. becomes “arterialised”. The livers epithelial cells- hepatocytes
20 and biliary epithelial cells- become increasingly senescent (unable to divide)⁸³. In this setting removal of the
21 injurious agent is the key goal to promote endogenous liver repair but there is evidence that the addition of
22 additional ex vivo cell therapies such as macrophages may promote the endogenous repair by increasing the
23 resolution of liver fibrosis and promoting liver regeneration^{84, 85}.

26 **Clinical evidence of regeneration in the setting of cirrhosis and chronic liver injury**

27 Liver disease is often silent and patients may present to liver physicians with established cirrhosis. Often the
28 insult can be removed such as the treatment of hepatitis C, cessation of alcohol or treatment of an auto-
29 immune disease, however patients are on a precarious tightrope with very small changes leading to
30 decompensation and frequent hospital admissions with decompensated liver cirrhosis- likewise natural
31 history studies clearly show that some patients can re-compensate at this stage and not require further
32 inpatient hospital treatment. By providing a stimulus to the natural regenerative process this treatment is
33 targeted to a patient group who will benefit hugely from a successful strategy to improve liver regeneration.
34 Even in cirrhosis, if the injurious insult can be treated the liver can regenerate and remodel to some degree.
35 D’Ambrosio et al. showed in a paired biopsy study in patients with hepatitis C induced liver cirrhosis who
36 had been successfully treated for the hepatitis C infection that after 61 months from viral eradication (SVR),

1 cirrhosis regression was observed in 61%, and the collagen content decreased in 89%⁸⁶. Critically,
2 regression of cirrhosis can lead to a reduction in hard clinical endpoints (complications, death)⁸⁷. This
3 clearly indicated that even in the context of liver cirrhosis the natural history can be modified and patient
4 outcome improved. With the new, exciting and effective anti-viral treatments appearing for hepatitis C there
5 will be many patients with hepatitis C induced cirrhosis, who have been cleared of virus and have non-
6 progressive disease but who are still at high risk of clinical events and decompensation, who will benefit
7 greatly from treatments which improve liver regeneration and background liver function.

8
9 The requirement for regeneration is even greater when considering liver resection for HCC in the setting of a
10 patient with cirrhosis. Whilst regeneration can still occur in this setting, there is a risk of developing liver
11 decompensation which is often guided by clinical features such as the presence of portal hypertension.

12
13 The changes in liver function can be clinically measured using the MELD scoring system using objective
14 variables that are readily obtained namely; serum bilirubin, serum creatinine and INR. MELD has been
15 validated in outpatients with compensated cirrhosis and across a broad spectrum of liver disease. It is highly
16 accurate in predicting one week, three month and one year mortality. MELD independently predicts clinical
17 decompensation in patients with compensated cirrhosis. MELD score has been used by all the major
18 Western regulatory authorities involved in liver transplantation (UK Transplant, Eurotransplant and UNOS)
19 to help prioritise the allocation of liver transplants. This indicates that simple measurements can have
20 predictive power.

21 22 **4. Clinical measurement of liver regeneration**

23 Much of the literature around measurement of human liver regeneration relates primarily to liver resection
24 and liver cancer and to a lesser extent to acute liver failure. Consequently there has been a greater focus on
25 measures of volume replacement or recovery from very low levels of hepatic functionality as seen in acute
26 liver failure. The advent of new therapeutic strategies, and in particular their use in the setting of chronic
27 liver damage, will require additional measures of liver regeneration that more appropriately reflect the less
28 profound changes, albeit still clinically relevant, that may occur (Figure 3).

29 30 **Clinical symptoms and signs of liver dysfunction:**

31 For the patient with compensated liver cirrhosis it is unlikely that there will be any significant symptoms or
32 signs that can be used as a measure of regeneration. Symptoms that exist in this setting are often less precise
33 such as fatigue, sub-clinical hepatic encephalopathy or muscle weakness and can sometimes be multi-
34 factorial in origin. Nevertheless such parameters can be measured using validated questionnaires and may
35 provide clues to the effect of an intervention⁸⁸⁻⁹⁰. For patients with more advanced chronic liver disease

1 there will be more over features of liver dysfunction (hepatic encephalopathy, jaundice, ascites) that can
2 serve as a point from which to measure improvement in liver function (regeneration). This evaluation can
3 range from a categorical assessment (clearance of ascites/encephalopathy), to subjective semi-quantitative
4 scores (grades of ascites or encephalopathy) through to a more formal model such as the modified Child-
5 Pugh score (CPS). The CPS which encompasses both objective (bilirubin, albumin, INR) and subjective
6 (hepatic encephalopathy & ascites) assessments of liver dysfunction generates a numerical value to reflect
7 the state of liver dysfunction. This scoring system was originally validated as a prognostic tool to predict
8 mortality during surgery for patients with liver cirrhosis⁹¹, although it is now more commonly used to
9 determine their overall prognosis.

11 **Blood measures of liver regeneration:**

12 Simple measures of regeneration include measurement of the cancer neo-antigen alpha-fetoprotein (AFP) in
13 serum which is also released in response to ⁹²hepatocyte turnover. The rate of increase of serum AFP has
14 been shown to correlate with survival of patients with acute liver failure, although its utility in the setting of
15 chronic liver disease is less well established. Higher serum levels of miR-122, miR-21 and miR-221 have
16 been reported in patients spontaneously recovering from acute liver failure due to a range of aetiologies as
17 compared to patients that did not recover. Additionally, patients with elevated serum miR levels displayed
18 increased hepatocyte proliferation and down-regulation of hepatic miRNA target genes that impaired liver
19 **regeneration**. Recently the Acute Liver Failure Study Group [ALFSG] index was established and compared
20 with the long-standing King's College criteria (KCC) and Model for End Stage Liver Disease (MELD).
21 Hepatic coma grade, INR, serum bilirubin, serum phosphorus and serum M30 value accurately identified
22 patients that would require liver transplantation or die. The ALFSG index identified these patients with
23 85.6% sensitivity and 64.7% specificity. The ALFSG Index was superior (AUROC 0.822) to KCC (AUROC
24 0.654) or MELD (AUROC 0.704) (p=0.0002 and p=0.0010 respectively) in identifying patients that would
25 require **LT**.

26 Recognising the potential limitation of scoring systems such as CPS which include subjective assessments
27 the MELD score was developed in 2000 and consisted of bilirubin, creatinine and INR^{93, 94}. Its ability to
28 predict prognosis of patients with liver cirrhosis has been validated in many studies. Prognosis can be
29 deduced from the absolute value as well as a change in MELD over a defined time-period such as 3
30 months⁹⁵, which may be of particular relevance for the assessment of new therapies. Studies using MELD as
31 an outcome measure will need to determine the durability of any observed change as well as establishing if it
32 has the same clinical prognosis.

Comment [PN1]: PMID 24913549
R3P3

Comment [PN2]: PMID 22885329

1 Less often used tests for liver function include those that test the ability of the liver to metabolise
2 administered chemicals such as Erythromycin breath test (EBT)⁹⁶ and Caffeine elimination rate (Caff kelim)
3 and clearance (Caff CI)⁹². Other tests measure hepatic circulation using test compounds with flow-
4 dependent, high first-pass hepatic extraction such as galactose (Galactose elimination capacity; GEC)⁹⁷ and
5 cholate clearances (CA CI) and shunt (CA shunt)⁹⁸. Whilst attractive candidates to assess response to a
6 treatment the clinical validity of these parameters have yet to be established and thus the clinical
7 significance of any change remains uncertain.

9 **Imaging assessment of liver regeneration:**

10 In the setting of liver resection the rate at which liver volume recovers is commonly used as a measure of
11 regeneration, and can be undertaken with a range of imaging modalities including CT, MRI and SPECT
12 scanning (See table 2). The relevance of this relatively crude measure of regeneration is not entirely clear in
13 the setting of more subtle interventions and thus new approaches are needed. In the setting of chronic liver
14 disease imaging modalities are being increasingly used to determine the extent of liver fibrosis through
15 assessments of liver stiffness. Resolution of liver fibrosis is likely to be an important therapeutic target when
16 trying to promote liver regeneration. These include ultrasound based modalities (Fibroscan) which are
17 widely used in clinical practice⁹⁹ as well as CT and MRI approaches which may be superior in their ability
18 to provide a more global assessment of fibrosis and hence allow for identification of more subtle changes.
19 Dynamic contrast enhanced CT (DCE-CT) and MRI (DCE-MRI) imaging allow¹⁰⁰ for measurement of
20 hepatic perfusion, and in the setting of liver cirrhosis may be able estimate the extent of portal hypertension
21 which is commonly elevated in this setting. A commonly used measure is the hepatic perfusion index which
22 is calculated by estimating the slope of arterial perfusion divided by the sum of the slope of the arterial and
23 portal perfusions¹⁰¹. The ability to non-invasively measure portal hypertension would be an important
24 outcome although as yet this has not been achieved. It represents a clinically relevant parameter against
25 which the effectiveness of new therapies can be judged and is accepted as a licensing end-point by
26 regulatory authorities.

28 **The future:**

29 The most immediate developments will likely focus on non-invasive assessment of portal hypertension
30 which will represent a significant step forward. There is also a requirement however for the development
31 and validation of new non-invasive tests to inform on early signals of hepatocyte turnover and fibrosis re-
32 modelling.

5. The management and therapeutic targeting of liver regeneration

Regenerative mechanisms are present during liver injury, even after chronic damage, but in many cases they are insufficient to overcome the ongoing insults, necessitating additional measures to be explored (see table 3).

Reduction or removal of injurious process:

In many cases the optimal method to promote liver regeneration is to either stop or interfere with the injurious process. In many cases this involves stopping or reducing the aetiological factors such as alcohol cessation or losing weight, whilst in the case of viral hepatitis the use of new anti-viral medications has had a major impact on disease progression/resolution^{102, 103}. In many situations though there are either no effective treatments (non-alcoholic fatty liver disease¹⁰⁴/autoimmune liver disease¹⁰⁵) or patients continue to consume alcohol, and thus in these settings additional interventions to promote liver regeneration. Moreover, there are occasions where even after the injurious agent has been removed the residual liver damage is so advanced, and/or continues to progress such that adjunctive measures are needed.

In many forms of liver disease there is a superimposed immune-driven component to the ongoing liver damage, leading to strategies to modulate immune responses in this setting. This has taken the form of either pharmacological approaches to reduce lymphocyte ingress to the liver as well as cell therapy approaches to reduce the activity and ingress of inflammatory cells¹⁰⁶.

Encouraging endogenous liver regeneration:

In the setting of chronic liver disease hepatocyte proliferation is impaired, and the presence of liver fibrosis is recognised as a major factor inhibiting hepatocyte proliferation¹⁰⁷. Thus approaches to reduce liver fibrosis as discussed below may be effective in promoting liver regeneration. Other strategies include the use of pharmacological agents or cytokines to stimulate hepatocyte proliferation. Granulocyte colony stimulating factor (GCSF) has been used extensively in pre-clinical models where it has been demonstrated to stimulate proliferation of endogenous hepatocytes resulting in both less liver damage and less fibrosis¹⁰⁸. GCSF has also been demonstrated to increase both the proliferation and motility of hepatic progenitor cells¹⁰⁹, which may in turn also aid regeneration. However, there still remains uncertainty about the efficacy of GCSF in liver disease with the majority of clinical studies being small in nature and not powered to confirm efficacy¹¹⁰. A notable exception to this relates to a randomised controlled trial in acute on chronic liver failure, where GCSF administration was demonstrated to improve survival of patients. The mechanism of this effect was not established although the authors speculated that GCSF may improve neutrophil

1 function, which is commonly diminished in the setting of chronic liver disease. Thyroid hormone (T3) has
2 been demonstrated by several groups to be a strong inducer of liver cell proliferation in rats and mice³, and
3 recent studies have shown that the hepatocyte mitogenic response is mediated by PKA-dependent β -catenin
4 activation¹¹¹. Enthusiasm for T3 is reinforced by the observation that its administration helps inhibit/reverse
5 non-alcoholic fatty liver disease¹¹² and lowers the risk of hepatic tumour development¹¹³. An important
6 concern when the promotion endogenous regeneration is considered is the potential development of
7 hepatocellular carcinoma (HCC). This is pertinent in the context of cirrhosis when the risk of HCC is raised
8 and there is frequently activation of the ductular compartment. The cellular origin of the HCC is therefore a
9 consideration. Recent data from the Schwabe group convincingly found that in 2 mouse models of HCC that
10 the cancers arose from lineage traced hepatocytes rather than the biliary/progenitor compartment¹¹⁴. The
11 important study, reviewed above, by Ninomiya et al. in the 90% hepatectomy model raises the idea that
12 when there is a small liver remnant and significant liver volume gain is required, then controlling the rate of
13 liver regeneration and thus minimising architectural and sinusoidal disorganisation is a valuable concept that
14 may be worth translating toward the clinic¹⁴.

16 **Degradation of fibrosis:**

17 There has been extensive investigation of potential effective anti-fibrotic agents in pre-clinical models of
18 liver disease, predominantly in the carbon tetrachloride (CCl₄) model. Resolution of liver fibrosis is known
19 to be more difficult in its more advanced stages, and thus there are uncertainties about the generalizability of
20 pre-clinical models such as CCl₄ to the clinical situation of liver cirrhosis. One of the other major challenges
21 in the translation of such agents into the clinical arena, is the lack of any satisfactory non-invasive methods
22 to quantify liver fibrosis, and hence the reliance on liver biopsy for measurement of fibrosis which poses
23 significant logistical issues. Nevertheless, several anti-fibrotic drugs have been studied in early phase
24 clinical trials¹¹⁵ with no compelling signal for efficacy seen with Colchicine¹¹⁶, IL10¹¹⁷, IFN γ ¹¹⁸ and a
25 possible signal with Losartan¹¹⁹.

27 Cell therapies have also been studied in pre-clinical models of liver fibrosis, with macrophages¹²⁰, bone
28 marrow¹²¹ and mesenchymal stromal cells¹²² all having demonstrated efficacy in models of CCl₄-induced
29 liver fibrosis (see **table 3**). Smaller scale clinical studies in patients with chronic liver disease have also
30 suggested reductions in liver fibrosis alongside improvements in liver synthetic function¹²³. These studies
31 have predominantly used haematopoietic stem cells or mononuclear preparations which have either been
32 harvested from bone marrow or mobilised into the circulation by the use of GCSF, which as indicated
33 earlier, may have additional beneficial effects on liver regeneration. Whilst these improvements in synthetic
34 function came from small non-powered studies there was little to suggest any significant safety concern,
35 with the exception of when cells were infused intra-portally¹²⁴. In that setting there was an increase in portal
36 hypertensive bleeding, which serves as a caution for such routes. Indeed, homing of stem cells to the liver is

1 enhanced after liver injury and given that trial data do not suggest superior efficacy with liver-directed
2 infusions logistically easier routes can be used. To determine the mechanisms by which these potential
3 effects are achieved in the clinical setting requires further investigation, as does their confirmation in larger
4 clinical trials. To date there are no adequately powered randomised trials of cell therapy that show a positive
5 effect¹²⁵.

6 7 8 **6. Conclusions**

9 Liver regeneration in the normal liver is well described in validated model systems such as the rat and
10 mouse partial hepatectomy models. The cellular and signalling mechanisms described using these models
11 have provided a general template for understanding liver regeneration and to plan therapeutic interventions.
12 New models such as the zebrafish are bringing the ability to rapidly screen compounds for their ability to
13 improve liver repair and regeneration following injury.

14 In the clinical setting, the deficiencies of regeneration usually impact when there is a grossly abnormal liver
15 architecture or when normal liver regeneration is severely impaired. Understanding the abnormal
16 regenerative responses, and how they differ from “normal healthy regeneration” will be critical to accurately
17 targeting new therapies. Such strategies may have several broad targets such as the excessive fibrosis,
18 abnormal ductular responses and the impaired innate immunity which is a feature of liver dysfunction.
19 Advances in imaging technology such as MRI combined with liver spectroscopy may provide more
20 complete picture of the liver volume and anatomy, liver blood flow data, measures of whole liver fibrosis
21 and whole liver signatures of metabolic function that could provide a “whole liver” picture of structure and
22 function to guide surgical resection and other therapeutic decisions. Overall, we conclude that where there is
23 good animal data of efficacy for a particular intervention, and there is an acceptable risk-benefit ration, then
24 the time is right to translate this knowledge and perform appropriate prospective clinical studies.

7. References

1. Michalopoulos, G.K. & DeFrances, M.C. Liver regeneration. *Science* **276**, 60-6 (1997).
2. Bird, T.G., Lorenzini, S. & Forbes, S.J. Activation of stem cells in hepatic diseases. *Cell Tissue Res* **331**, 283-300 (2008).
3. Forbes, S.J. et al. Retroviral gene transfer to the liver in vivo during tri-iodothyronine induced hyperplasia. *Gene Ther* **5**, 552-5 (1998).
4. Higgins, G.M. & Anderson, R.M. Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol Lab Med* **12**, 186-202 (1931).
5. Forbes, S.J. & Rosenthal, N. Preparing the ground for tissue regeneration: from mechanism to therapy. *Nat Med* **20**, 857-69 (2014).
6. Moolten, F.L. & Bucher, N.L. Regeneration of rat liver: transfer of humoral agent by cross circulation. *Science* **158**, 272-4 (1967).
7. Nakamura, R.M., Miyada, D.S. & Moyer, D.L. Effect of Liver Regeneration Following Partial Hepatectomy on the Uptake of Tritiated Thymidine in the Pituitary Gland of the Rat. *Nature* **199**, 707-8 (1963).
8. Michalopoulos, G.K. Liver regeneration. *J Cell Physiol* **213**, 286-300 (2007).
9. Martins, P.N., Theruvath, T.P. & Neuhaus, P. Rodent models of partial hepatectomies. *Liver Int* **28**, 3-11 (2008).
10. Demetris, A.J. et al. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* **30**, 986-93 (2006).
11. Ren, W. et al. Selective bowel decontamination improves the survival of 90% hepatectomy in rats. *J Surg Res* **195**, 454-64 (2015).
12. Capussotti, L. et al. Liver dysfunction and sepsis determine operative mortality after liver resection. *Br J Surg* **96**, 88-94 (2009).
13. Du, Z. et al. Octreotide prevents liver failure through upregulating 5'-methylthioadenosine in extended hepatectomized rats. *Liver Int* (2015).
14. Ninomiya, M. et al. Deceleration of regenerative response improves the outcome of rat with massive hepatectomy. *Am J Transplant* **10**, 1580-7 (2010).
15. Fujio, K., Evarts, R.P., Hu, Z., Marsden, E.R. & Thorgeirsson, S.S. Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. *Lab Invest* **70**, 511-6 (1994).
16. Ghoshal, A.K., Mullen, B., Medline, A. & Farber, E. Sequential analysis of hepatic carcinogenesis. Regeneration of liver after carbon tetrachloride-induced liver necrosis when hepatocyte proliferation is inhibited by 2-acetylaminofluorene. *Lab Invest* **48**, 224-30 (1983).
17. Evarts, R.P., Nagy, P., Marsden, E. & Thorgeirsson, S.S. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. *Carcinogenesis* **8**, 1737-40 (1987).
18. Trautwein, C. et al. 2-acetaminofluorene blocks cell cycle progression after hepatectomy by p21 induction and lack of cyclin E expression. *Oncogene* **18**, 6443-53 (1999).
19. Dusabineza, A.C. et al. Participation of liver progenitor cells in liver regeneration: lack of evidence in the AAF/PH rat model. *Lab Invest* **92**, 72-81 (2012).
20. Lemaigre, F.P. Determining the fate of hepatic cells by lineage tracing: Facts and pitfalls. *Hepatology* **61**, 2100-3 (2015).
21. Bockamp, E. et al. Conditional transgenic mouse models: from the basics to genome-wide sets of knockouts and current studies of tissue regeneration. *Regen Med* **3**, 217-35 (2008).
22. Nikfarjam, M., Malcontenti-Wilson, C., Fanartzis, M., Daruwalla, J. & Christophi, C. A model of partial hepatectomy in mice. *J Invest Surg* **17**, 291-4 (2004).
23. Issa, R. et al. Mutation in collagen-1 that confers resistance to the action of collagenase results in failure of recovery from CCl4-induced liver fibrosis, persistence of activated hepatic stellate cells, and diminished hepatocyte regeneration. *FASEB J* **17**, 47-9 (2003).
24. Duffield, J.S. et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* **115**, 56-65 (2005).

- 1 25. Ramachandran, P. et al. Differential Ly-6C expression identifies the recruited macrophage
2 phenotype, which orchestrates the regression of murine liver fibrosis. *Proc Natl Acad Sci U S A* **109**,
3 E3186-95 (2012).
- 4 26. Kallis, Y.N. et al. Remodelling of extracellular matrix is a requirement for the hepatic progenitor cell
5 response. *Gut* **60**, 525-533 (2011).
- 6 27. Preisegger, K.H. et al. Atypical ductular proliferation and its inhibition by transforming growth
7 factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic
8 liver disease. *Lab Invest* **79**, 103-9 (1999).
- 9 28. Hsieh, W.C. et al. Galectin-3 regulates hepatic progenitor cell expansion during liver injury. *Gut* **64**,
10 312-21 (2015).
- 11 29. Williams, M.J., Clouston, A.D. & Forbes, S.J. Links between hepatic fibrosis, ductular reaction, and
12 progenitor cell expansion. *Gastroenterology* **146**, 349-56 (2014).
- 13 30. Akhurst, B. et al. A modified choline-deficient, ethionine-supplemented diet protocol effectively
14 induces oval cells in mouse liver. *Hepatology* **34**, 519-22 (2001).
- 15 31. Boulter, L. et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell
16 fate in chronic liver disease. *Nat Med* **18**, 572-9 (2012).
- 17 32. Tsuchiya, A. et al. Polysialic acid/neural cell adhesion molecule modulates the formation of ductular
18 reactions in liver injury. *Hepatology* **60**, 1727-40 (2014).
- 19 33. Kim, K.H., Chen, C.C., Alpini, G. & Lau, L.F. CCN1 induces hepatic ductular reaction through
20 integrin alphavbeta(5)-mediated activation of NF-kappaB. *J Clin Invest* **125**, 1886-900 (2015).
- 21 34. Yanger, K. et al. Adult hepatocytes are generated by self-duplication rather than stem cell
22 differentiation. *Cell Stem Cell* **15**, 340-9 (2014).
- 23 35. Jors, S. et al. Lineage fate of ductular reactions in liver injury and carcinogenesis. *J Clin Invest* **125**,
24 2445-57 (2015).
- 25 36. Lu, W.Y. et al. Hepatic progenitor cells of biliary origin with liver repopulation capacity. *Nat Cell*
26 *Biol* **17**, 971-83 (2015).
- 27 37. Cox, A.G. & Goessling, W. The lure of zebrafish in liver research: regulation of hepatic growth in
28 development and regeneration. *Curr Opin Genet Dev* **32**, 153-161 (2015).
- 29 38. Curado, S., Stainier, D.Y. & Anderson, R.M. Nitroreductase-mediated cell/tissue ablation in
30 zebrafish: a spatially and temporally controlled ablation method with applications in developmental
31 and regeneration studies. *Nat Protoc* **3**, 948-54 (2008).
- 32 39. Sadler, K.C., Krahn, K.N., Gaur, N.A. & Ukomadu, C. Liver growth in the embryo and during liver
33 regeneration in zebrafish requires the cell cycle regulator, uhrf1. *Proc Natl Acad Sci U S A* **104**,
34 1570-5 (2007).
- 35 40. Goessling, W. et al. APC mutant zebrafish uncover a changing temporal requirement for wnt
36 signaling in liver development. *Dev Biol* **320**, 161-74 (2008).
- 37 41. Vliegenthart, A.D., Tucker, C.S., Del Pozo, J. & Dear, J.W. Zebrafish as model organisms for
38 studying drug-induced liver injury. *Br J Clin Pharmacol* **78**, 1217-27 (2014).
- 39 42. Choi, T.Y., Ninov, N., Stainier, D.Y. & Shin, D. Extensive conversion of hepatic biliary epithelial
40 cells to hepatocytes after near total loss of hepatocytes in zebrafish. *Gastroenterology* **146**, 776-88
41 (2014).
- 42 43. He, J., Lu, H., Zou, Q. & Luo, L. Regeneration of liver after extreme hepatocyte loss occurs mainly
43 via biliary transdifferentiation in zebrafish. *Gastroenterology* **146**, 789-800 e8 (2014).
- 44 44. Verfaillie, C.M. Biliary cells to the rescue of Prometheus. *Gastroenterology* **146**, 611-4 (2014).
- 45 45. Huang, M. et al. Antagonistic interaction between Wnt and Notch activity modulates the regenerative
46 capacity of a zebrafish fibrotic liver model. *Hepatology* **60**, 1753-66 (2014).
- 47 46. Jiang, F. et al. Analysis of mutants from a genetic screening reveals the control of intestine and liver
48 development by many common genes in zebrafish. *Biochem Biophys Res Commun* **460**, 838-44
49 (2015).
- 50 47. Schaub, J.R., Malato, Y., Gormond, C. & Willenbring, H. Evidence against a stem cell origin of new
51 hepatocytes in a common mouse model of chronic liver injury. *Cell Rep* **8**, 933-9 (2014).
- 52 48. Tarlow, B.D. et al. Bipotential adult liver progenitors are derived from chronically injured mature
53 hepatocytes. *Cell Stem Cell* **15**, 605-18 (2014).

- 1 49. Yanger, K. et al. Robust cellular reprogramming occurs spontaneously during liver regeneration.
2 *Genes Dev* **27**, 719-24 (2013).
- 3 50. Stueck, A.E. & Wanless, I.R. Hepatocyte buds derived from progenitor cells repopulate regions of
4 parenchymal extinction in human cirrhosis. *Hepatology* **61**, 1696-707 (2015).
- 5 51. Lin, W.R. et al. The histogenesis of regenerative nodules in human liver cirrhosis. *Hepatology* **51**,
6 1017-26 (2010).
- 7 52. Ueda, J., Chijiwa, K., Nakano, K., Zhao, G. & Tanaka, M. Lack of intestinal bile results in delayed
8 liver regeneration of normal rat liver after hepatectomy accompanied by impaired cyclin E-
9 associated kinase activity. *Surgery* **131**, 564-73 (2002).
- 10 53. Huang, W. et al. Nuclear receptor-dependent bile acid signaling is required for normal liver
11 regeneration. *Science* **312**, 233-6 (2006).
- 12 54. Meng, Z. et al. FXR regulates liver repair after CCl₄-induced toxic injury. *Mol Endocrinol* **24**, 886-
13 97 (2010).
- 14 55. Otao, R. et al. External biliary drainage and liver regeneration after major hepatectomy. *Br J Surg*
15 **99**, 1569-74 (2012).
- 16 56. Black, S.M., Whitson, B.A. & Velayutham, M. EPR spectroscopy as a predictive tool for the
17 assessment of marginal donor livers perfused on a normothermic ex vivo perfusion circuit. *Med*
18 *Hypotheses* **82**, 627-30 (2014).
- 19 57. Qi, J. et al. 31P MR spectroscopic imaging detects regenerative changes in human liver stimulated by
20 portal vein embolization. *J Magn Reson Imaging* **34**, 336-44 (2011).
- 21 58. Zakian, K.L. et al. Liver regeneration in humans is characterized by significant changes in cellular
22 phosphorus metabolism: assessment using proton-decoupled 31P-magnetic resonance spectroscopic
23 imaging. *Magn Reson Med* **54**, 264-71 (2005).
- 24 59. Kumar, S., Zou, Y., Bao, Q., Wang, M. & Dai, G. Proteomic analysis of immediate-early response
25 plasma proteins after 70% and 90% partial hepatectomy. *Hepatology* **43**, 876-89 (2013).
- 26 60. Afolabi, P., Wright, M., Wootton, S.A. & Jackson, A.A. Clinical utility of 13C-liver-function breath
27 tests for assessment of hepatic function. *Dig Dis Sci* **58**, 33-41 (2013).
- 28 61. Miura, Y., Washizawa, N., Urita, Y., Imai, T. & Kaneko, H. Evaluation of remnant liver function
29 using 13C-breath tests in a rat model of 70% partial hepatectomy. *Hepatogastroenterology* **59**, 311-6
30 (2012).
- 31 62. Lee, S.G. A complete treatment of adult living donor liver transplantation: a review of surgical
32 technique and current challenges to expand indication of patients. *Am J Transplant* **15**, 17-38 (2015).
- 33 63. Bernal, W., Lee, W.M., Wendon, J., Larsen, F.S. & Williams, R. Acute liver failure: A curable
34 disease by 2024? *J Hepatol* **62**, S112-S120 (2015).
- 35 64. Bhushan, B. et al. Pro-regenerative signaling after acetaminophen-induced acute liver injury in mice
36 identified using a novel incremental dose model. *Am J Pathol* **184**, 3013-25 (2014).
- 37 65. Apte, U. et al. Beta-catenin activation promotes liver regeneration after acetaminophen-induced
38 injury. *Am J Pathol* **175**, 1056-65 (2009).
- 39 66. Holt, M.P., Cheng, L. & Ju, C. Identification and characterization of infiltrating macrophages in
40 acetaminophen-induced liver injury. *J Leukoc Biol* **84**, 1410-21 (2008).
- 41 67. Amemiya, H., Kono, H. & Fujii, H. Liver regeneration is impaired in macrophage colony stimulating
42 factor deficient mice after partial hepatectomy: the role of M-CSF-induced macrophages. *J Surg Res*
43 **165**, 59-67 (2011).
- 44 68. Stutchfield, B.M. et al. CSF1 Restores Innate Immunity After Liver Injury in Mice and Serum Levels
45 Indicate Outcomes of Patients With Acute Liver Failure. *Gastroenterology* **149**, 1896-1909 e14
46 (2015).
- 47 69. Truant, S. et al. Volumetric gain of the liver after major hepatectomy in obese patients: a case-
48 matched study in 84 patients. *Ann Surg* **258**, 696-702; discussion 702-4 (2013).
- 49 70. Vetelainen, R., van Vliet, A.K. & van Gulik, T.M. Severe steatosis increases hepatocellular injury
50 and impairs liver regeneration in a rat model of partial hepatectomy. *Ann Surg* **245**, 44-50 (2007).
- 51 71. Inaba, Y. et al. Growth arrest and DNA damage-inducible 34 regulates liver regeneration in hepatic
52 steatosis in mice. *Hepatology* **61**, 1343-56 (2015).

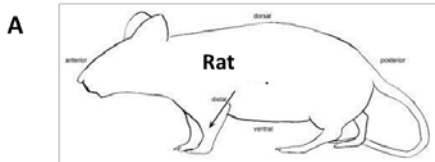
- 1 72. Parkin, E. et al. Equivalent survival in patients with and without steatosis undergoing resection for
2 colorectal liver metastases following pre-operative chemotherapy. *Eur J Surg Oncol* **40**, 1436-44
3 (2014).
- 4 73. Parkin, E. et al. The effect of hepatic steatosis on survival following resection of colorectal liver
5 metastases in patients without preoperative chemotherapy. *HPB (Oxford)* **15**, 463-72 (2013).
- 6 74. Ploeg, R.J. et al. Risk factors for primary dysfunction after liver transplantation--a multivariate
7 analysis. *Transplantation* **55**, 807-13 (1993).
- 8 75. Rogier, J. et al. Noninvasive assessment of macrovesicular liver steatosis in cadaveric donors based
9 on computed tomography liver-to-spleen attenuation ratio. *Liver Transpl* **21**, 690-5 (2015).
- 10 76. Dahm, F., Georgiev, P. & Clavien, P.A. Small-for-size syndrome after partial liver transplantation:
11 definition, mechanisms of disease and clinical implications. *Am J Transplant* **5**, 2605-10 (2005).
- 12 77. Eshkenazy, R. et al. Small for size liver remnant following resection: prevention and management.
13 *Hepatobiliary Surg Nutr* **3**, 303-12 (2014).
- 14 78. Zeng, S. et al. Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia
15 and reperfusion injury to the liver in mice. *Hepatology* **39**, 422-32 (2004).
- 16 79. Cataldegirmen, G. et al. RAGE limits regeneration after massive liver injury by coordinated
17 suppression of TNF-alpha and NF-kappaB. *J Exp Med* **201**, 473-84 (2005).
- 18 80. Basta, G., Del Turco, S., Navarra, T., Lee, W.M. & Acute Liver Failure Study, G. Circulating levels
19 of soluble receptor for advanced glycation end products and ligands of the receptor for advanced
20 glycation end products in patients with acute liver failure. *Liver Transpl* **21**, 847-54 (2015).
- 21 81. Koh, E.J., Yoon, S.J. & Lee, S.M. Losartan protects liver against ischaemia/reperfusion injury
22 through PPAR-gamma activation and receptor for advanced glycation end-products down-regulation.
23 *Br J Pharmacol* **169**, 1404-16 (2013).
- 24 82. Pellicoro, A. et al. Elastin accumulation is regulated at the level of degradation by macrophage
25 metalloelastase (MMP-12) during experimental liver fibrosis. *Hepatology* **55**, 1965-75 (2012).
- 26 83. Marshall, A. et al. Relation between hepatocyte G1 arrest, impaired hepatic regeneration, and fibrosis
27 in chronic hepatitis C virus infection. *Gastroenterology* **128**, 33-42 (2005).
- 28 84. Bird, T.G. et al. Bone marrow injection stimulates hepatic ductular reactions in the absence of injury
29 via macrophage-mediated TWEAK signaling. *Proc Natl Acad Sci U S A* **110**, 6542-7 (2013).
- 30 85. Thomas, J.A. et al. Macrophage therapy for murine liver fibrosis recruits host effector cells
31 improving fibrosis, regeneration, and function. *Hepatology* **53**, 2003-15 (2011).
- 32 86. D'Ambrosio, R. et al. A morphometric and immunohistochemical study to assess the benefit of a
33 sustained virological response in hepatitis C virus patients with cirrhosis. *Hepatology* **56**, 532-43
34 (2012).
- 35 87. Mallet, V. et al. Brief communication: the relationship of regression of cirrhosis to outcome in
36 chronic hepatitis C. *Ann Intern Med* **149**, 399-403 (2008).
- 37 88. Tandon, P. et al. Severe muscle depletion in patients on the liver transplant wait list: its prevalence
38 and independent prognostic value. *Liver Transpl* **18**, 1209-16 (2012).
- 39 89. Weissenborn, K., Ruckert, N., Hecker, H. & Manns, M.P. The number connection tests A and B:
40 interindividual variability and use for the assessment of early hepatic encephalopathy. *J Hepatol* **28**,
41 646-53 (1998).
- 42 90. Younossi, Z.M., Guyatt, G., Kiwi, M., Boparai, N. & King, D. Development of a disease specific
43 questionnaire to measure health related quality of life in patients with chronic liver disease. *Gut* **45**,
44 295-300 (1999).
- 45 91. Angermayr, B. et al. Child-Pugh versus MELD score in predicting survival in patients undergoing
46 transjugular intrahepatic portosystemic shunt. *Gut* **52**, 879-85 (2003).
- 47 92. Wahllander, A., Mohr, S. & Paumgartner, G. Assessment of hepatic function. Comparison of
48 caffeine clearance in serum and saliva during the day and at night. *J Hepatol* **10**, 129-37 (1990).
- 49 93. Kamath, P.S., Kim, W.R. & Advanced Liver Disease Study, G. The model for end-stage liver disease
50 (MELD). *Hepatology* **45**, 797-805 (2007).
- 51 94. Malinchoc, M. et al. A model to predict poor survival in patients undergoing transjugular
52 intrahepatic portosystemic shunts. *Hepatology* **31**, 864-71 (2000).

- 1 95. Merion, R.M. et al. Longitudinal assessment of mortality risk among candidates for liver
2 transplantation. *Liver Transpl* **9**, 12-8 (2003).
- 3 96. Watkins, P.B. et al. Erythromycin breath test as an assay of glucocorticoid-inducible liver
4 cytochromes P-450. Studies in rats and patients. *J Clin Invest* **83**, 688-97 (1989).
- 5 97. Keiding, S. Galactose clearance measurements and liver blood flow. *Gastroenterology* **94**, 477-81
6 (1988).
- 7 98. Everson, G.T. et al. Portal-systemic shunting in patients with fibrosis or cirrhosis due to chronic
8 hepatitis C: the minimal model for measuring cholate clearances and shunt. *Aliment Pharmacol Ther*
9 **26**, 401-10 (2007).
- 10 99. Cassinotto, C. et al. Non-invasive assessment of liver fibrosis with impulse elastography: comparison
11 of Supersonic Shear Imaging with ARFI and FibroScan(R). *J Hepatol* **61**, 550-7 (2014).
- 12 100. Pandharipande, P.V., Krinsky, G.A., Rusinek, H. & Lee, V.S. Perfusion imaging of the liver: current
13 challenges and future goals. *Radiology* **234**, 661-73 (2005).
- 14 101. Shikare, S.V., Bashir, K., Abraham, P. & Tilve, G.H. Hepatic perfusion index in portal hypertension
15 of cirrhotic and non-cirrhotic aetiologies. *Nucl Med Commun* **17**, 520-2 (1996).
- 16 102. Marcellin, P. et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. *N*
17 *Engl J Med* **359**, 2442-55 (2008).
- 18 103. Pearlman, B.L., Ehleben, C. & Perrys, M. The Combination of Simeprevir and Sofosbuvir is More
19 Effective Than That of Peginterferon, Ribavirin, and Sofosbuvir for Patients with Hepatitis C-related
20 Child's Class A Cirrhosis. *Gastroenterology* (2014).
- 21 104. Dowman, J.K., Armstrong, M.J., Tomlinson, J.W. & Newsome, P.N. Current therapeutic strategies
22 in non-alcoholic fatty liver disease. *Diabetes Obes Metab* **13**, 692-702 (2011).
- 23 105. Dyson, J.K. et al. Unmet clinical need in autoimmune liver diseases. *J Hepatol* **62**, 208-218 (2015).
- 24 106. Eksteen, B., Afford, S.C., Wigmore, S.J., Holt, A.P. & Adams, D.H. Immune-mediated liver injury.
25 *Semin Liver Dis* **27**, 351-66 (2007).
- 26 107. Iredale, J.P. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a
27 solid organ. *J Clin Invest* **117**, 539-48 (2007).
- 28 108. Yannaki, E. et al. G-CSF-primed hematopoietic stem cells or G-CSF per se accelerate recovery and
29 improve survival after liver injury, predominantly by promoting endogenous repair programs. *Exp*
30 *Hematol* **33**, 108-19 (2005).
- 31 109. Piscaglia, A.C., Shupe, T.D., Oh, S.H., Gasbarrini, A. & Petersen, B.E. Granulocyte-colony
32 stimulating factor promotes liver repair and induces oval cell migration and proliferation in rats.
33 *Gastroenterology* **133**, 619-31 (2007).
- 34 110. Garg, V. et al. Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival
35 of patients with acute-on-chronic liver failure. *Gastroenterology* **142**, 505-512 e1 (2012).
- 36 111. Fanti, M., Singh, S., Ledda-Columbano, G.M., Columbano, A. & Monga, S.P. Tri-iodothyronine
37 induces hepatocyte proliferation by protein kinase A-dependent beta-catenin activation in rodents.
38 *Hepatology* **59**, 2309-20 (2014).
- 39 112. Perra, A. et al. Thyroid hormone (T3) and TRbeta agonist GC-1 inhibit/reverse nonalcoholic fatty
40 liver in rats. *FASEB J* **22**, 2981-9 (2008).
- 41 113. Perra, A., Kowalik, M.A., Pibiri, M., Ledda-Columbano, G.M. & Columbano, A. Thyroid hormone
42 receptor ligands induce regression of rat preneoplastic liver lesions causing their reversion to a
43 differentiated phenotype. *Hepatology* **49**, 1287-96 (2009).
- 44 114. Mu, X. et al. Hepatocellular carcinoma originates from hepatocytes and not from the
45 progenitor/biliary compartment. *J Clin Invest* **125**, 3891-903 (2015).
- 46 115. Schuppan, D. & Kim, Y.O. Evolving therapies for liver fibrosis. *J Clin Invest* **123**, 1887-901 (2013).
- 47 116. Rambaldi, A. & Glud, C. Colchicine for alcoholic and non-alcoholic liver fibrosis and cirrhosis.
48 *Cochrane Database Syst Rev*, CD002148 (2005).
- 49 117. Nelson, D.R. et al. Long-term interleukin 10 therapy in chronic hepatitis C patients has a proviral and
50 anti-inflammatory effect. *Hepatology* **38**, 859-68 (2003).
- 51 118. Pockros, P.J. et al. Final results of a double-blind, placebo-controlled trial of the antifibrotic efficacy
52 of interferon-gamma1b in chronic hepatitis C patients with advanced fibrosis or cirrhosis.
53 *Hepatology* **45**, 569-78 (2007).

- 1 119. Colmenero, J. et al. Effects of losartan on hepatic expression of nonphagocytic NADPH oxidase and
2 fibrogenic genes in patients with chronic hepatitis C. *Am J Physiol Gastrointest Liver Physiol* **297**,
3 G726-34 (2009).
- 4 120. Thomas, J.A. et al. Macrophage therapy for murine liver fibrosis recruits host effector cells
5 improving fibrosis, regeneration and function. *Hepatology*.
- 6 121. Sakaida, I. et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice.
7 *Hepatology* **40**, 1304-1311 (2004).
- 8 122. Meier, R.P. et al. Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice.
9 *J Hepatol* **62**, 634-41 (2015).
- 10 123. Houlihan, D.D. & Newsome, P.N. Critical review of clinical trials of bone marrow stem cells in liver
11 disease. *Gastroenterology* **135**, 438-50 (2008).
- 12 124. Salama, H. et al. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage
13 chronic liver diseases. *Cell Transplant* **19**, 1475-86 (2010).
- 14 125. Moore, J.K., Stutchfield, B.M. & Forbes, S.J. Systematic review: the effects of autologous stem cell
15 therapy for patients with liver disease. *Aliment Pharmacol Ther* **39**, 673-85 (2014).
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Schemata of Normal and Abnormal liver regeneration

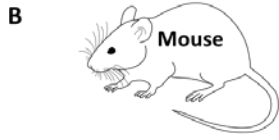


Classic model of partial hepatectomy

Well described models of acute liver injury

Reliable models of chronic injury and regeneration such as carbon tetrachloride

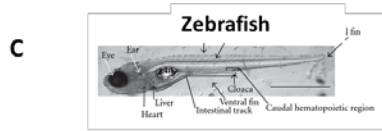
Hepatocyte proliferation readily inhibited



Well developed transgenic technology allows genetic dissection of regeneration signals and lineage tracing

Surgical models such as partial hepatectomy

Dietary models of biliary and hepatocellular injury



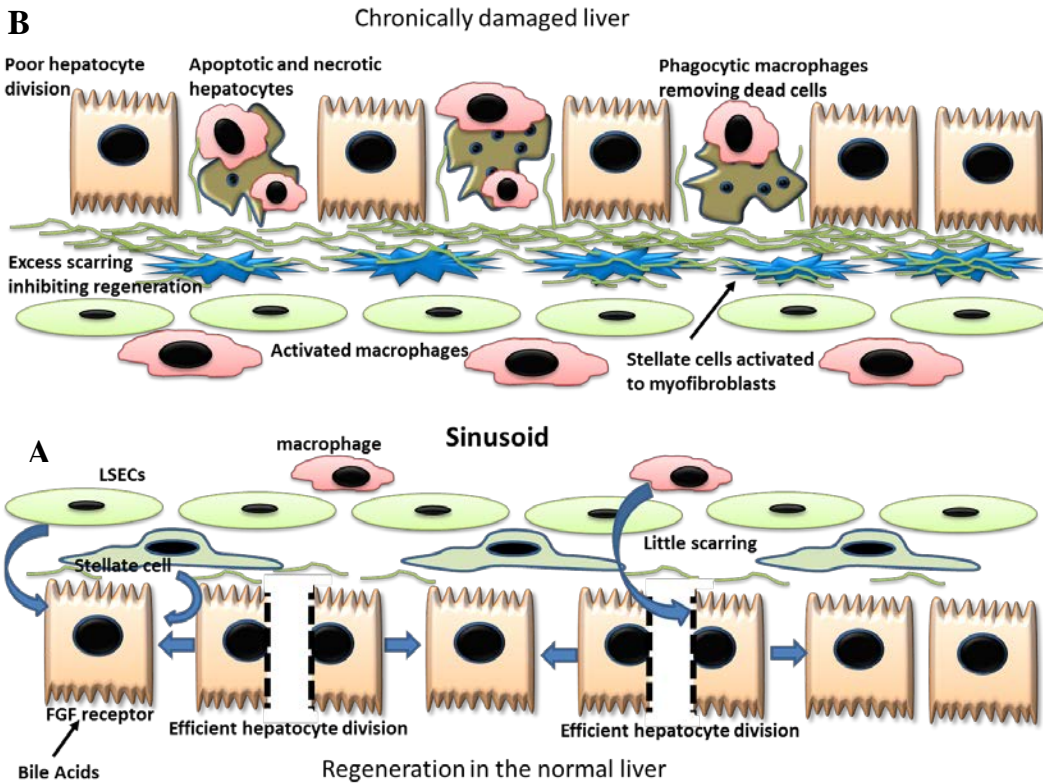
Good transgenic technology

Surgical, drug and genetic models of liver injury and regeneration

Rapid screening of chemical libraries

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Figure 1: Animal models of liver regeneration: A. the rat is the classic model for studying liver regeneration following 70% hepatectomy which results in rapid activation of regenerative signals peaking at 24hours. Hepatocyte proliferation can be inhibited efficiently by toxins, when additional injury such as partial hepatectomy is added then a widespread “oval cell response” is activated. B. The mouse has similar properties to the rat but with the additional advantage of good transgenic technologies. Hepatocyte proliferation is not readily inhibited using standard methodologies. C. The zebrafish is rapidly gaining favour because it is rapid to use, is cost effective, allows excellent imaging and permits chemical screening.



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3 **Figure 2: A:** Regeneration in the normal liver follows partial hepatectomy or moderate liver injury. In this
4 setting bile acids are rapidly upregulated, and serum factors are able to rapidly induce regeneration in the
5 liver. Non-parenchymal cells; macrophages, stellate cells and LSECs signal to hepatocytes to leave their
6 mitotically quiescent state and enter mitosis. Stellate cells are not activated to myofibroblasts and there is
7 little or no scar tissue. **B:** Regeneration in the abnormal chronically damaged liver is hampered by several
8 factors. Hepatocytes are increasingly senescent and unable to divide efficiently, the stellate cells are
9 activated to myofibroblasts and excessive scar tissue inhibits regeneration. Excessive cellular debris inhibits
10 efficient liver regeneration.

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Strategies to improve liver regeneration

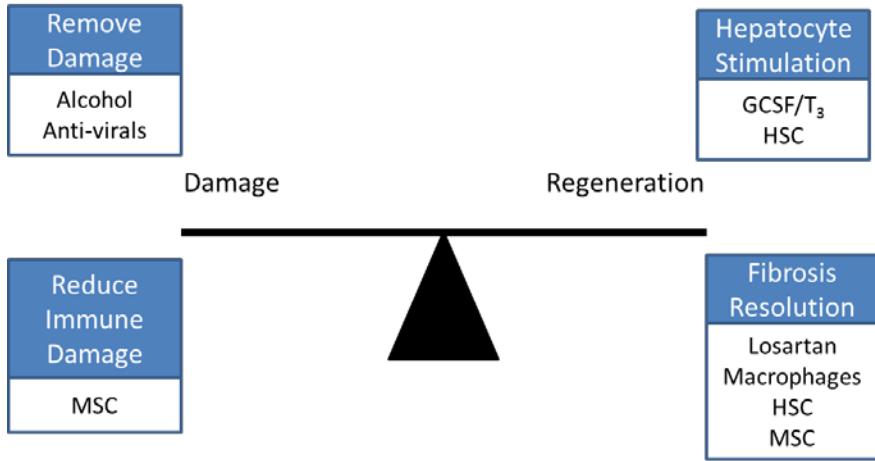


Figure 3: Strategies to improve liver regeneration include the removal of injurious agents, the promotion of fibrosis resolution and the direct stimulation of hepatocyte proliferating. The balance between these two sets of processes is key in determining the clinical outcome.

Animal Models	Clinical Studies
<p>Have helped to define many signals and cellular sources controlling liver regeneration</p> <p>Transgenic technology enables cell specific and temporally controlled gene deletion to define gene function during liver regeneration</p> <p>Lineage tracing studies help define the cells that actually regenerate the liver</p>	<p>Liver pathology studies show static pictures of cellular and extracellular architecture during regeneration</p> <p>Gene expression studies complement the pathological studies</p>
<p>Studies of injury and regeneration in animals are often relatively short lived and mild</p>	<p>Chronic injury can develop over decades in humans and produce very abnormal liver architecture</p>
<p>Animal models usually study one form of injury</p>	<p>Patients often have multiple factors affecting their liver physiology and regeneration</p>
<p>Imaging modalities are developing rapidly but are often not widely clinically applicable</p>	<p>Clinical studies rely heavily on serum markers and non-invasive markers of liver function</p>

1

2 **Table 1:** the differences between animal models of liver regeneration and clinical studies of liver
3 regeneration

Modality	Mode of action	Purpose
Elastography	Measurement of shear wave velocity after stimulus	Measure liver stiffness (fibrosis)
Computed tomography (CT)	2-D reconstruction of x-ray imaging	Information on liver structure and size
Magnetic resonance imaging (MRI)	2-D/3-D reconstruction based on radiofrequency wave detection	Information on liver structure and size
Dynamic contrast enhanced CT (DCE-CT) and MRI (DCE-MRI)	Measurement of changes in contrast enhancement over time in vascular beds/organs	Measurement of hepatic perfusion and guide to portal pressure
Single-photon emission computed tomography (SPECT)	Measurement of uptake of radioactive tracer by metabolically active cells	Visual measure of hepatic metabolic function

1

2 **Table 2:** non-invasive methods of assessing liver injury, structure, volume, and function

Cell type	Indication	Advantages	Disadvantages
Haematopoietic stem cells	Promote hepatocyte proliferation; Reduce liver fibrosis	Safe; Supportive pre-clinical and early phase clinical data	Autologous therapy; Cost; Still unproven
Macrophages	Reduce liver fibrosis	Supportive pre-clinical data	Autologous therapy; Cost; Possible off-target effects; Still unproven
Endothelial progenitor cells	Reduce liver fibrosis; Promote revascularisation	Supportive pre-clinical data	Autologous therapy; cost; Possible off-target effects; Still unproven
Regulatory T cells	Reduce immune-mediated damage	Supportive pre-clinical data; efficacy in renal transplant clinical studies	Autologous therapy; cost; still unproven
Mesenchymal stromal cells	Reduce immune-mediated damage; Reduce liver fibrosis	Allogeneic; Supportive pre-clinical data; Efficacy in non-liver transplant clinical studies; Safety profile encouraging.	Still unproven; Phenotypic characterisation of infused cells poorly defined

Table 3: different types of potential cell therapies for liver injury and regeneration