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Special article

# Strategies for liver support: from stem cells to xenotransplantation

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Summary of an EASL MONOTHEMATIC CONFERENCE held in Venice, September 25 and 26, 2003. Additional invited speakers were: N. Fausto (USA), D. Tosh (UK), E.B. Petersen (USA), M. Alison (UK), D.A. Shafritz (USA), M. Strazzabosco (Italy), D. Adams (UK), M. Pinzani (Italy), S. Forbes (UK), H. Gilgenkrantz (France), E. Cozzi (Italy), U. Baccarani (Italy), M. Muraca (Italy), I. Fox (USA), J. Gerlach (USA), R.A. Chamuleau (The Netherlands), F.S. Larsen (Denmark), and A. Bader (Germany).

## 1. Introduction

The continuing shortage of donor organs has been a major roadblock in orthotopic liver transplantation (OLT). This has led to the consideration of several potentially viable alternatives, including bioartificial and nonbiological liver assist devices, transplantation of mature hepatocytes or of stem/progenitor cells, and potential of transplanting xenogeneic organs and cells. Numerous investigators throughout the world are engaged in these investigations and the pace of discovery has begun to accelerate in recent years. To obtain an overview of progress in these areas, EASL sponsored a Monothematic Conference, which was held in Venice on 25th-26th September 2003.

This conference was characterized by the enthusiastic participation of many leading investigators from various parts of the world. The present paper is a summary of the Monothematic Conference, including the related discussion and highlights some of the controversies in the areas of stem cells and transplantation. Since any such summary obviously cannot do full justice to the presentations and discussions at the conference, we apologize in advance for inadvertent omissions or lack of suitable emphasis on specific points.

The conference was divided into several major sessions dealing with the biology of stem cells and nonparenchymal liver cells; basic aspects of hepatocyte transplantation; genetic and other manipulations of cells from the perspective of clinical applications; hepatocyte transplantation in people; and strategies for engineering bioartificial liver and nonbiological liver support devices. Moreover, several investigators presented ongoing research in relevant areas of stem cell biology.

## 2. General principles concerning modification of cells

Insights into mechanisms of cell engraftment and proliferation are critical for cell therapy. In animals, transplanted cells can repopulate the liver following injury or inhibition of proliferation in native cells with various manipulations [1–6]. Dr. S. Gupta provided an overview of mechanisms concerning transplanted cell engraftment and proliferation. Also, he reviewed suitable targets for liver cell therapy, which extend from metabolic disorders, e.g. Crigler-Najjar syndrome or familial hypercholesterolemia, to acquired diseases, e.g. liver failure or cirrhosis (Table 1).

### 2.1. Genetic manipulation of cells

One goal of genetic manipulation for ex vivo gene therapy is to replace deficient function, which will be helpful in many conditions (Table 1). In addition, genetic manipulation of either cells or native liver could benefit liver repopulation, if introduced genes would improve transplanted cell engraftment, proliferation or allograft rejection. However, manipulation of stem cells for gene therapy offers both challenges and opportunities in respect with the tropism of viral vectors, as well as promoter regulation during differentiation of stem/progenitor cells. A major benefit of working with stem cells for gene therapy

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**Table 1**
**Selected disorders amenable to liver-directed cell therapy**

| Liver disease present | Liver spared from disease |
|---|---|
| Congenital metabolic disease | Genetically transmitted metabolic disease |
| Alpha-1 antitrypsin deficiency | Crigler-Najjar syndrome |
| Erythropoietic protoporphyria | Familial hypercholesterolemia |
| Lipidoses, e.g. Gaucher's disease, Niemann-Pick disease | Hyperammonemia syndromes |
| Tyrosinemia, type 1 | Defects of carbohydrate metabolism |
| Wilson's disease | |
| Acquired diseases | Deficiency of circulating proteins |
| Chronic viral hepatitis, cirrhosis | Coagulation defects, e.g. Factor VIII or IX deficiency |
| Fulminant liver failure due to viral hepatitis, drugs, etc. | Hereditary angioedema |

will obviously concern their replication potential, which means that suitable cell subsets could be selected beforehand and expanded to desired masses in vivo.

Although ex vivo gene therapy was studied in familial hypercholesterolemia, following initial studies in Watanabe rabbits, therapeutic benefits of gene therapy were limited [7,8], likely due to inefficient gene transfer in cultured hepatocytes, loss of transduced cells in culture, and perhaps limited transplanted cell engraftment, which required further developments. Correction of many genetic diseases requires gene therapy vectors that integrate in cells and express transgenes permanently. This restricts the choice of vectors at the practical level to retroviruses, including lentivirus, adeno-associated virus, and SV40. The ideal vector will transduce nondividing cells, integrate into the host genome without insertional mutagenesis, be nonimmunogenic, have an adequate size to accommodate genes, and grow to high titers. Recently, much interest has begun to focus on lentiviral vectors, which efficiently transduce human hepatocytes [9,10]. However, more insights are needed to understand the nature of immune and cellular responses against lentivirus components.

### *2.2. Expanding the supply of cells with genetic manipulations*

Immortalization of hepatocytes represents another way to augment the availability of transplantable cells, e.g. by expressing the SV40 T antigen, which induces cell cycling, but has oncogenic properties. When SV40 T antigen was regulated in a conditional manner in adult hepatocytes, cells proliferated extensively, whereas suppression of SV40 T antigen expression decreased cell proliferation [11]. This approach produced immortalized hepatocytes capable of supporting metabolic function in liver failure.

On the other hand, use of fetal human hepatocytes represents a viable way to extend availability of donor organs. Fetal human hepatocytes are highly viable, cryopreserve well, exhibit stem/progenitor cell properties, and engraft and proliferate in animal models. Moreover, the cells can be transduced efficiently with viral vectors. When fetal or neonatal human hepatocytes were modified to express the catalytically active subunit of human telomerase reverse transcriptase (hTERT), cells showed superior maintenance of telomere length and showed extensive proliferation without oncogenesis [12]. These cells maintained liver-specific function and engrafted in intact animals, which raises further hopes for enhancing the availability of suitable cell types for clinical applications.

## 3. Potential of stem cells and mechanisms in liver regeneration

Identification of suitable additional sources of cells that could be transplanted in lieu of hepatocytes should be effective for cell therapy applications. In this pursuit, attention has been focused on embryonic stem (ES) cells and nonhepatic stem cells.

### *3.1. Embryonic stem cells*

In principle, ES cells can replicate indefinitely and provide an unlimited number of donor cells. Although ES cells can be differentiated to obtain hepatocyte-like cells, whether such cells are indeed authentic hepatocytes has been unclear due to experimental uncertainties. Dr. D. Tosh presented the utility of gene traps as an approach to overcome this difficulty. In their studies, founder mice were generated by a gene trap insertion into an ankyrin-repeat containing gene (Gtar) with integration of a reporter gene (lacZ) fused to a splice acceptor sequence into a transcription unit in ES cells. Gtar provided a suitable marker for eliciting early hepatocyte differentiation during mouse liver development. Subsequently, ES cells were cultered and embryoid bodies obtained.

Such efforts will provide valuable information to understand how ES cells will generate liver cells.

### *3.2. Extrahepatic stem cells*

Dr. Tosh discussed additional studies concerning differentiation of pancreatic cells into hepatocytes.

The pancreas and liver share an ontological relationship, since these organs originate from the embryonic foregut endoderm. AR42J are pancreatic cells from azaserine-treated

rats and exhibit exocrine and neuroendocrine properties, as indicated by amylase and neurofilament expression.

These cells produce insulin-secreting cells following exposure in culture to activin A and HGF and in additional studies showed potential for differentiation along hepatic lineages. Such relationships between pancreatic and hepatic lineages are in agreement with the induction of insulin expression in fetal human liver cells following expression of the homeobox domain gene, Pdx-1 [13].

In recent studies, connections have been established between hematopoietic stem cells and hepatocytes, although some uncertainties have emerged as well. Dr. B. Petersen reviewed this area in detail. A variety of models established that bone marrow cells can produce hepatocytes, including in rats [14], mice [15,16], and humans [17,18], although most investigators agree that differentiation of bone marrow cells into hepatocytes is rare and occurs over prolonged periods [19–21]. Also, mouse bone marrow cells appear to produce hepatocytes primarily through cell fusion, which raises multiple issues with the fidelity of this process and potential for deleterious genetic perturbations in fused cells [22,23].

Dr. M. Alison and his group presented their studies of bone marrow transplantation in mice subjected to toxic liver injury using carbon tetrachloride along with G-CSF administration to stimulate the bone marrow. However, bone marrow-derived hepatocytes were infrequent, although relatively more bone marrow-derived hepatocytes were observed after carbon chloride treatment.

Further studies of this process in hepatitis B virus transgenic mice with chronic liver disease [24] using transplantation of bone marrow cells expressing green fluorescence protein (GFP) in total body irradiated HBV transgenic mice led to GFP-postive hepatocytes after 3 months, although the fraction of these hepatocytes was $<1\%$. Similarly, only occasional bone marrow-derived hepatocytes were observed in animals treated with the pyrrolizidine alkaloid, retrorsine, to block proliferation in native hepatocytes. Again, fusion of bone marrow cells and native hepatocytes was observed in HBV transgenic mice, although it was unclear whether cell fusion resulted from the association of macrophages-hepatocytes, hepatocytes–hepatocytes, or hematopoietic stem cells and other bone marrow stem cells-hepatocytes. More recently, bone marrow-derived mononuclear cells have been identified as the most effective hepatocyte fusion partners [25,26]. To make the matter difficult, other investigators found that bone marrow cells can produce liver cells without any cell fusion [27,28]. Also, it is unclear whether cell fusion is a unique property of mouse cells or of some mouse models, since transplantation of human hematopoietic stem cells into NOD-SCID mice led to production of hepatocytes without cell fusion [29]. Remarkably, analysis of tissues from sex-mismatched liver transplants with subsequent development of fibrosis showed that a circulating population of bone marrow-derived cells can differentiate into myofibroblast-like cells in the damaged liver [30]. Similarly, bone marrow cells can generate liver sinusoidal endothelial cells without the requirement for cell fusion [31]. Therefore, some liver cell compartments may be more readily amenable to bone marrow-related reconstitution. Further studies are necessary to resolve several of these issues in stem cell plasticity. Clinical studies of bone marrow transplantation for reconstituting the liver will certainly be premature at this stage!

### 3.3. Intrahepatic stem/progenitor cells

The liver itself has long been studied to address mechanisms in organ regeneration, as well as for resident stem/progenitor cells. The general discussion in liver regeneration was spurred by an outstanding overview by Dr. N. Fausto. The rationale is that during normal liver growth, hepatocytes, as well as stem/progenitor cells, play specific roles in organogenesis. Subsequently, facultative liver stem cells remain in intrahepatic compartments, e.g. oval cells and cells in the canals of Hering, and in extrahepatic locations, e.g. hematopoietic and mesenchymal stem cells in bone marrow, as partly discussed above. Major paradigms of the regenerative process in the liver are provided by the responses induced by partial hepatectomy or acute necrotic injury. In these situations, hepatocytes constitute the major cell compartment responsible for liver regeneration (Fig. 1), since the generation of hepatocytes

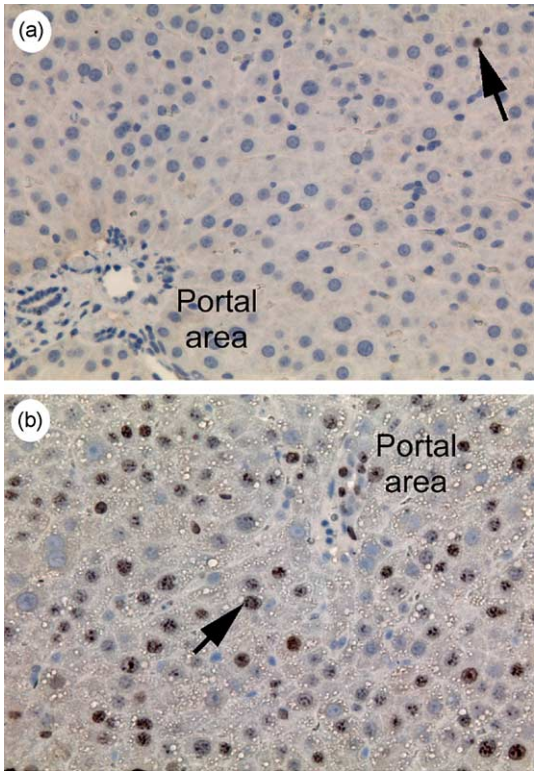


**Fig. 1. The pattern of hepatocellular proliferation in the liver demonstrated with Ki-67 antibody staining. (a) Shows normal rat liver with only rare cells with proliferation (arrows). In contrast, 24 h after two third partial hepatectomy in the rat leads to extensive cell proliferation as shown by Ki-67 stained nuclei in (b).**

from bone marrow cells is a very rare event in repopulation after injury [32]. There is no evidence for involvement of oval cells or extrahepatic stem/progenitor cells in liver regeneration following partial hepatectomy. Therefore, one view suggests that oval cells constitute a reserve compartment and these cells are activated most often either when hepatocytes are extensively depleted or when hepatocyte proliferation is inhibited. Dr. M. Strazzabosco elaborated on the role of oval cells in the canals of Hering and small interlobular bile ducts, which likely represent the equivalent of transit-amplifying stem cells found in other organs. Much effort has been devoted to understanding the nature and potential of hepatic oval cells. These cells become visible in conditions associated with chronic liver injury or carcinogenesis. Oval cells exhibit unique gene expression profiles, including genes expressed in hepatocytes, biliary cells, occasionally hematopoietic cells, and hepatic progenitor cells [33].

Moreover, hepatic oval cells exhibit the side-population phenotype defined by expression of ATPbinding cassette transporter ABCG2/BCRP1 [34]. The ductular reaction observed in various chronic and acute liver diseases may be representative of facultative stem cell-driven responses to hepatic injury [35]. One hypothesis is that perturbations in bile duct cells during liver damage are regulated by extracellular factors, e.g. neuroendocrine factors, adhesion molecules, cytokines or chemokines, angiogenic factors and other molecules. Oval cell activation or their persistence may be stimulated by integrin or cytokine production in infiltrating lymphocytes and mast cells and expression of specific signals in ductal plates may play further roles. Remarkably, the oval cell compartment appears to be liver-derived and oval cells do not arise from the bone marrow [36]. Recent studies demonstrated that oval cells can repopulate the liver and produce mature hepatocytes, which will be in agreement with their therapeutic potential, although more work is necessary to understand the role of oval cells in pathophysiological processes [37].

In humans, the kinetics of liver regeneration following partial hepatectomy is different, such that while the liver mass is restored within 7 days after two-thirds partial hepatectomy in rodents, this requires several weeks in people [38,39]. A variety of studies established that multiple signaling networks regulate and coordinate hepatocyte proliferation during liver regeneration. Nonparenchymal liver cells secrete some growth factors and cytokines needed for hepatocyte proliferation and the growth-promoting activity of nonparenchymal cells increases after partial hepatectomy or other types of liver injury.

Dr. M. Alison reviewed further issues concerning activation of stem/progenitor cells in the adult liver. While oval cells are activated in the adult human liver following various forms of injuries, more work is needed to understand their biological potential. For instance, one could potentially isolate and expand oval cells from the adult liver, e.g. from tissue explants following OLT. In

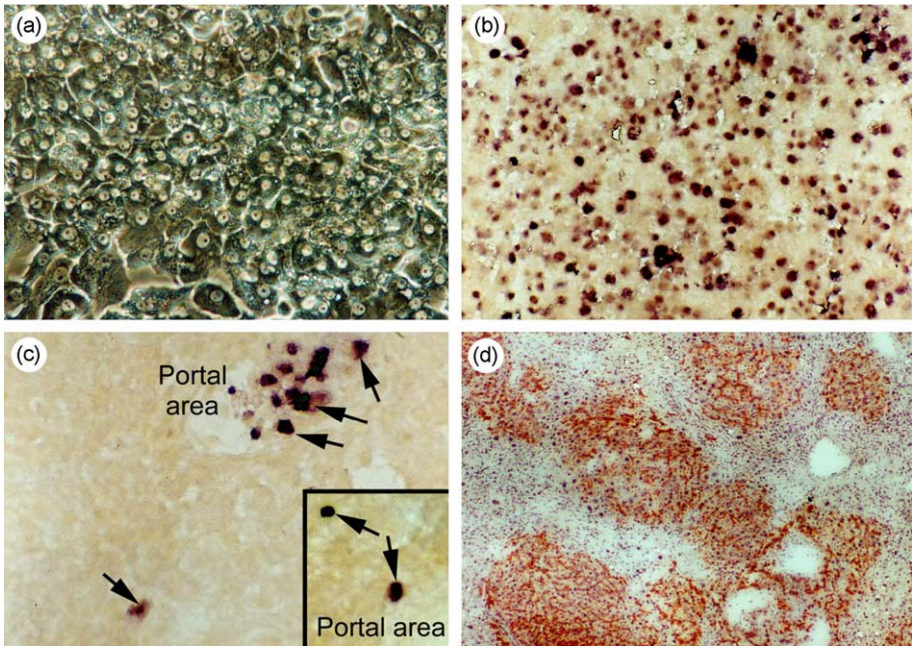


**Fig. 2. Transplanted cells in the rodent liver. (a) Shows primary fetal human liver epithelial cells in cell culture. These cells show extensive proliferation capacity while expressing genes observed in hepatocytes, biliary cells and additional cell types. (b–c) Use of a nonradioactive in situ hybridization probe to identify transplanted human cells in immunodeficient mice with b showing hybridization signals in fetal human liver. Panel c shows transplanted human cells within a portal vein radicle, as well as in a sinusoid (arrows) 2 h after transplantation. Inset shows survival of human cells in the mouse liver several weeks following transplantation. In general, it has been difficult to repopulate the mouse liver with human cells, whereas the rat liver can be readily repopulated with transplanted cells, as seen in d, where DPPIV-positive F344 cells (red color) are seen forming large foci following preconditioning of the recipient DPPIV- rat liver with retrorsine and partial hepatectomy.**

animal studies, progenitor liver cells have been isolated by fluorescence-activated cell sorting from adult GFP-transgenic mice [40]. On the other hand, hepatocytes themselves might constitute a large reservoir of stem-like cells, judging from their extensive replication (>80 divisions per cell) during serial transplantation in FAH mutant mice with tyrosinemia [41,42]. This property of mature hepatocytes suggests that differentiated cells obtained by manipulation of ES cells or other stem/progenitor cells will be capable of repopulating the liver and correcting diseases.

In contrast with the adult liver, the fetal liver represents a major source of stem/progenitor cells. Dr. D. Shafritz discussed aspects of their work with fetal rat liver cells, where the DPPIV-F344 rat is used for demonstrating transplanted cell engraftment and liver repopulation. Their studies of rat fetal liver epithelial cells isolated from ED14 gestation showed engraftment of cells capable of producing hepatocytes, biliary cells, as well as additional cell types, e.g. endothelial cells. However, phenotypic characteristics of transplanted fetal cells were altered in the recipient liver and the majority of repopulating cell clusters showed mixed phenotypes, with both hepatocytes and bile duct cells. Comparison of ED14 rat fetal liver cells with bone marrow cells isolated from mature rats showed that fetal liver cells repopulated the liver far more efficiently. Bone marrow cells produced only rare foci of hepatocytes in rats, despite retrorsine-partial hepatectomy preconditioning, which generates the stimulus for extensive liver repopulation with transplanted mature hepatocytes. Taken together with studies of fetal human liver cells by other groups, these findings suggest that fetal cells present a viable alternative to the use of adult hepatocytes (Fig. 2).

## 4. Immunological mechanisms involving hepatic dendritic and endothelial cells

The liver is an immunologically active organ and Dr. D. Adams reviewed this area. The liver is constantly exposed to gut antigens via the portal vein, to local pathogens via the biliary tree, and to systemic pathogens via the hepatic artery. Analysis of T cells in the liver following exposure to cytomegalovirus identified presence of specific CD8+T cell fractions, including long-lived viral memory cells that are sequestered in the liver [43,44]. The nature of the cytokine and costimulatory responses determines the outcome of T cell activation. The eventual outcome depends on the antigen-presenting cell and the local microenvironment. The liver differs from most other organs in its unique microanatomy (sinusoids, portal tracts) and cellular composition (hepatocytes, cholangiocytes, Kupffer cells, sinusoidal endothelial cells and dendritic cells). Dendritic cells in the human blood originate from bone marrow-derived myeloid or lymphoid precursor cells, which traffick in liver sinusoids [45] and may be found in liver parenchyma normally, while portal neolymphogenesis may be encountered during chronic inflammation [46,47]. The donor interstitial dendritic cells persist and self-replicate in recipients of liver allografts [48], which likely plays roles in tolerance.

Dendritic cells in the mouse liver are heterogeneous and these cells can be fractionated into several groups without the need for prior expansion [49]. Such insights into the function of liver dendritic cells is important in transplantation medicine because adoptive transfer of allogenic liver dendritic cells stimulates IL-10 secretion in lymph nodes and tolerance to allografts [50,51]. Dendritic cells may interact directly with other liver cell types, including Kupffer cells, endothelial cells, hepatocytes and cholangiocytes [52,53]. The local microenvironment and paracrine interactions may drive generation of regulatory cells in the liver [54–56]. Liver sinusoidal endothelial cells can regulate immune responses by sequestering activated T cells to facilitate Fas-dependent apoptosis during local inflammation [56,57] or antiviral responses [58]. In chronic rejection, liver sinusoidal endothelial cells strongly express CD40, Fas and FasL [59,60], with pathophysiological roles. Therefore, integration of this knowledge in antigen presentation will be critical in applications of allogeneic or xenogenic cell transplantation in people.

## 5. Role of hepatic stellate cells in tissue remodeling

Insights into the regulation of stromal development and maintenance will facilitate tissue engineering. Also, interactions among parenchymal cells and other cells are often modulated by stromal elements. Dr. M. Pinzani reviewed the biology of the hepatic stroma and the role of hepatic stellate cells in this process. Inflammatory, infectious and other processes can all damage the stroma and in response to these processes, cells capable of producing extracellular matrix components (ECM) are often recruited. The resultant deposition of ECM, predominantly of collagens I and III, induces stromal remodeling, where fibrillar ECM is degraded and replaced with other components, such as collagen IV and laminin. With the onset of liver disease, biliary reaction to aberrant mesenchymal expansion results from epithelial-mesenchymal interactions and aberrant bile duct proliferation is frequently associated with significant fibrosis with hepatic stellate cells playing a central role in generating myofibroblasts. The stroma regenerates after partial hepatectomy, in association with parenchymal cells, and activation of hepatic progenitor cells during liver injury is closely associated with mesenchymal cell activation. During hepatic remodeling, cytokine release from hepatic stellate cells, Kupffer cells or endothelial cells might regulate differentiation of oval cells into hepatocytes. Similarly, coordinate expression of c-Met and HGF in chronic liver disease may play regulatory roles in this process [61]. Therefore, pharmacological manipulation of

the hepatic stroma could particularly help regulate stromal composition for tissue engineering [62].

## 6. Challenges in transplanting cells in patients, liver support systems and xenografts

The ability to cryopreserve cells is critical for clinical programs so that cell preparations can be characterized beforehand and cells can be transplanted under better controlled settings. Transplantation of freshly isolated cells is beset with difficulties concerning the unpredictability of donor organ availability and eventual viability of cells. The general goal of cell cryopreservation is to obtain the highest viability of cells after thawing. In practice, the viability of cryopreserved adult human hepatocytes is generally less than 75%. The principles of hepatocyte cryopreservation are to minimize intracellular ice formation with the incorporation of cryoprotectants, such as dimethylsulfoxide, use of antioxidants, glutathione, and other substances aimed at minimizing ATP depletion, further additives, as well as controlled-rate freezing. The functional properties of cryopreserved cells need to be tested by assays for synthetic, metabolic, proliferation and engraftment function, for which appropriate strategies are being developed [63].

In recipients of allogeneic human hepatocytes, immunosuppressive regimens were similar to those utilized for OLT. However, the host immune response to transplanted cells might be different from that after OLT, with a preponderance of CD8+T cell response [64]. Therefore, further studies of suitable immunosuppression are necessary for optimizing cell transplantation protocols. However, the current experience with hepatocyte transplantation has been limited to approximately 50 patients worldwide, including studies of patients with acute liver failure, cirrhosis and metabolic conditions. In these studies, the most readily discernible therapeutic benefits were apparent in Crigler-Najjar syndrome and glycogen storage disease [65,66]. During the conference, Dr. I. Fox discussed their findings of transplanted hepatocyte function in one patient with Crigler-Najjar syndrome type-1, where deficient enzyme activity was partly restored, leading to decreased requirement for phototherapy to lower serum bilirubin levels, although the patient ultimately required OLT [65]. Dr. M. Muraca reported the case of an adult with glycogen storage disease type Ia, who experienced stable improvement in glucose control following liver cell transplantation [66]. In these studies, several relevant findings in animal studies have been extrapolated or verified, e.g. the cell number that can be transplanted safely, the hemodynamic consequences of cell transplantation, including cell translocation into lungs, as well as the need to establish correlations between the functional mass of transplanted cells and therapeutic benefits observed. Studies on laboratory animals indicate the potential usefulness of liver cell transplantation but despite potential advantages, widespread application of liver cell transplantation has been slow [67]. At present, more detailed studies of hepatocyte transplantation in groups of patients are necessary in the context of standardized cell preparations to compare results.

### 6.1. Tissue engineering and liver support systems

For applications of the bioartificial liver, the following requirements should be considered: performance, biotolerance, cell source, and logistics. It is crucial to recapitulate elements of the liver microanatomy and cell–cell interactions in designing bioartificial liver devices, which is, of course, easier said than done. Whether embryonic or fetal stem cells, bone marrow cells or other types of stem cells will eventually prove useful for this purpose is presently unclear. The proliferative capacity of primary hepatocytes is restricted and despite major efforts over the past many years, culture conditions that can lead to extensive proliferation of hepatocytes are lacking.

When one succeeds in inducing significant proliferation in immobilized cells in bioartificial devices, it will be necessary to demonstrate suitable patterns of liver gene expression, especially where stem/progenitor cells are utilized. Cryostorage of fully assembled bioreactors is currently under investigation with testing of xenobiotic and metabolic capacity [68]. A variety of indications exist for using liver support systems, especially to serve as a bridge for OLT [69], including fulminant hepatic failure, chronic liver failure, primary nonfunction of liver grafts, and liver failure after extensive liver resection. Available liver support devices require considerable development and testing before applications in humans. However, excellent large animal models of acute or chronic liver failure, where such devices can be adequately tested, are not available, and this area needs further development as well. Extracorporeal liver support systems most frequently use a hollow fibre cartridge containing immobilized hepatocytes with mass-exchange requiring either direct contact with perfused blood or through a semipermeable membrane separating hepatocytes from blood [70–73]. The support devices mostly utilize porcine hepatocytes or cell lines derived from hepatocellular carcinoma (HCC), which may exhibit only limited function compared with mature hepatocytes. Porcine cells are biologically different from human cells and could potentially harbor zoonotic agents. The porcine bioreactor (BELS) contains $1.8–4.0\times10^{10}$, up to 500 g, of pig hepatocytes. In one study, 8 patients listed for urgent liver transplantation were treated with a porcine bioreactor to support liver function without antibody reactivity to porcine endogenous retrovirus (PERV) [74–76]. A porcine radial flow bioreactor was used [77] to treat 7 patients, 6 of whom proceeded to OLT and similarly, 10 bioreactors produced with discarded human livers were used to treat 8 patients, 6 of whom underwent OLT [78,79]. The Amsterdam (AMC)-BAL is based on polysulfone housing, nonwoven polyester hollow oxygenation fiber, and extracapillary space. In 7 patients with acute liver failure, this

device showed significant promise with improved neurological and renal function and decreased serum bilirubin and ammonia levels in all patients [80]. Porcine hepatocytes with polysulfone membranes were applied 14 times in 12 patients along with plasma separation with or without charcoal and bilirubin adsorption. Of these, 3 patients died and 9 improved [81]. The BLSS (bioartificial liver support system) is based on porcine hepatocyte system and was found in a canine model of Dgalactosamine-induced liver failure to improve the duration of survival, 4 patients were reported with transient decreases in bilirubin and platelets count [82,83]. A hollow fibre cartridge with 50 g pig hepatocytes on collagen-coated microcarriers, incorporated into an extracorporeal circuit was applied [84,85]. HepatAssist in acute liver failure was used in 13 patients and results were compared to three patients not treated (2 of whom improved and 1 was transplanted), 6 patients had transient haemodynamic instability, 5 had bleeding complications, 2 died after OLT and 8 survived [86]. From a prospective, randomized, controlled trial of a BAL in treating acute liver failure, after exclusion of primary nonfunction in transplanted patients, survival at 30 day was 73% for BAL versus 59% for control [73]. The use of VitaGen ELAD—utilizing C3A hepatoma cells—was reported, with 8/12 in the ELAD group versus 3/7 in the control group listed for transplantation [87]. Dialysis methods undergoing testing are extracorporeal albumin dialysis (MARS), Prometheus, Ash, CVVHF and plasmapheresis. These devices remove ammonia and lactate efficiently with replacement of renal function, whereas the disadvantages are that exchanges are limited to watersoluble toxins, patients with acute liver failure tolerate hemodialysis poorly, and survival is not improved. Hemofiltration removes large molecules, is better tolerated than hemodialysis, provides renal replacement although only watersoluble toxins are exchanged, continuous treatment is usually required, and survival is not improved. Hemoperfusion removes a wide range of substances but shows poor biocompatibility with limited capacity of adsorption columns and no effect on survival. Most recently, albumin dialysis has been under investigation for removal of albuminbound substances. This has good biocompatibility and provides renal replacement. However, the adverse effects are similar to hemodialysis, e.g. hypotension and intracranial hypertension, and albumin is expensive. MARS has been used to support liver function in patients with liver failure in cirrhosis [88] or hepatorenal syndrome [89]. The Prometheus System is based on fractionated plasma separation and absorption, with removal of poorly water soluble (albumin-bound) toxins and removal of toxins through a dialyser for high flux hemodialysis [90].

A recent analysis of bioartificial liver assist devices [91] suggested that effect of artificial support system depends on the nature of liver failure. Although mortality in acute on chronic liver failure was reduced by 33%, the system had a significant effect on encephalopathy, no effect was observed on bridging to transplantation or survival in fulminant hepatic failure. The major complications encountered were bleeding, coagulopathy, decreased platelet count and allergic reactions. Therefore, further work is needed to identify the most effective configuration of a liver-assist device and the best cell type for repopulating the devices.

### 6.2. Potential of xenogeneic cell transplantation

Successful xenotransplantation of hepatocytes has been achieved in several animal models, including decreases in serum cholesterol in Watanabe rabbits following transplantation of healthy porcine hepatocytes [92]. Similarly, clinical parameters significantly improved in cirrhotic rats following transplantation of porcine hepatocytes [93]. Immunodeficient mice have been used as xenotransplantation models for developing in vivo models of hepatitis B or C virus infection. The data show that human hepatocytes can repopulate the mouse liver indicating no fundamental incompatibility between murine liver microenvironment and human hepatocytes [94,95].

The key issues in liver xenotransplantation are: immunology, physiology and zoonoses. Two major points for the immunological aspects are that the liver is the primary source of soluble complement factors. The immune response stimulated by xenotransplantation follows a different course, including hyperacute rejection (HAR) followed by acute vascular rejection, cellular rejection, and eventually chronic rejection. Transplantation of porcine renal and cardiac xenografts in primates usually results in hyperacute rejection. On the other hand, this does not occur when a normal pig liver is transplanted into a primate [96]. The liver is perhaps less susceptible to complement mediated injury. Also, several strategies (complement inhibitors, engineered animals) are being investigated to avoid hyperacute rejection. Ramirez [97] reported the absence of HAR and survival of up to 8 days in baboons following orthotopic human decay-accelerating factor (hDAF) liver xenograft.

Repeated exposure of patients to BAL leads to the production of antibodies against αGal epitope and other porcine antigens [98]. Formation of immune complexes following organ xenotransplantation has also been reported [99]. Regarding compatibility, data show physiological compatibilities between pig and primates, e.g. coagulation factor VII, as well as incompatibility, e.g. complement cascade, protein C and thrombomodulin. The mammalian liver produces over 2500 enzymes. Therefore, some incompatibilities between pigs and primates are to be expected. Nonetheless, Ramirez reported that two baboons survived liver xenografts for 4–8 days with normal oral intake, virtually normal coagulation tests and presence of porcine fibrinogen. Although over 60 zoonotic agents are known in pigs, specific pathogen free pigs are available, with the exception only of porcine endogenous retroviruses (PERV) [100–102]. However, PERV can infect human cells

in vitro and immunodeficient mice in vivo but there is no evidence that PERV is pathogenic to pigs themselves. Most PERV sequences in the pig genome are incomplete. After exposure to liver porcine tissue in 160 patients, 28 following treatment with BAL for 2–30 h, no evidence of PERV-related infection or disease was found. Similarly, Kuddus [103] used SS-PCR and PERV-specific RT assays and failed to detect PERV in patients exposed to BAL. Therefore, the risk of zoonotic transmission from porcine cells should be small.

## 7. Conclusions

This EASL Monothematic Conference helped to focus attention on recent progress in mechanisms regulating liver regeneration, the role of hepatic stem cells in liver regeneration, and the potential various types of extrahepatic stem cells in liver regeneration. The impact of cell transplantation and xenotransplantation in human disease was reviewed. Finally, progress in the areas of liver bioassist devices and other devices was discussed. The general agreement was that further work is needed to advance cell therapy and related interventions, especially in respect with the identification of suitable cell types for transplantation, development of effective strategies to foster cell engraftment and proliferation for liver repopulation, and high-density seeding of bioartificial devices to obtain effective hepatic function.

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