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TOPIC HIGHLIGHT

Giuseppe Orlando, MD, PhD, MCF, *Series Editor*

How regenerative medicine and tissue engineering may complement the available armamentarium in gastroenterology?

Marco Carbone, Jan Lerut, James Neuberger

Marco Carbone, Liver Unit, Addenbrookes Hospital, Cambridge CB2 0QQ, United Kingdom
Jan Lerut, Starzl Abdominal Transplant Unit, University Hospitals St. Luc, Université Catholique de Louvain, B-1200 Bruxelles, Belgium
James Neuberger, Organ Donation and Transplantation, NHS Blood and Transplant, Fox Den Road, Bristol BS34 8RR, United Kingdom
Author contributions: Carbone M, Lerut J and Neuberger J participated in the writing of the paper.
Correspondence to: Jan Lerut, Professor, Starzl Abdominal Transplant Unit, University Hospitals St. Luc, Université Catholique de Louvain, B-1200 Bruxelles, Belgium. jan.lerut@uclouvain.be
Telephone: +32-2-7641401 Fax: +32-2-7649039
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Abstract

The increasing shortage of donors and the adverse effects of immunosuppression have restricted the impact of solid organ transplantation. Despite the initial promising developments in xenotransplantation, roadblocks still need to be overcome and this form of organ support remains a long way from clinical practice. While hepatocyte transplantation may be effectively correct metabolic defects, it is far less effective in restoring liver function than liver transplantation. Tissue engineering, using extracellular matrix scaffolds with an intact but decellularized vascular network that is repopulated with autologous or allogeneic stem cells and/or adult cells, holds great promise for the treatment of failure of organs within gastrointestinal tract, such as end-stage liver disease, pancreatic insufficiency, bowel failure and type 1 diabetes. Particularly in the liver field, where there is a significant mortality of patients awaiting transplant, human bioengineering may offer a source of readily available organs for transplantation. The use of autologous cells will mitigate the need for long term immunosuppression thus removing a major hurdle in transplantation.

Key words: Regenerative medicine; Tissue engineering; Organ transplantation; Cellular transplantation; Xenotransplantation

Peer reviewers: Giuseppe Orlando, MD, PhD, MCF, Nuffield Department of Surgery, University of Oxford, Radcliffe Hospital, Headley Way, Headington, Oxford OX3 9DU, United Kingdom; Masaki Nagaya, MD, PhD, Islet Transplantation and Cell Biology, Joslin Diabetes Center, One Joslin Place, Joslin Diabetes Center, Boston, MA 02215, United States

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INTRODUCTION

Failure of abdominal organs is a significant cause of morbidity and mortality (Table 1). Patients with organ failure may benefit from non surgical therapies, such as insulin for endocrine pancreatic failure, parenteral nutrition for bowel failure and renal replacement therapy for renal failure. Until now, solid organ transplantation, and less convincingly cellular transplantation, represent the only way to provide a definitive treatment for organ fail-

ure, although the long-term impact is limited.

SOLID ORGAN TRANSPLANTATION

Over the last half a century, there have been major advances in the field of transplantation because of improved surgical techniques, anaesthesia, immunosuppression and peri-operative care. All these elements substantially improved patient and graft survival. Five-years patient and graft survival rates of 73% and 68.4% can nowadays be achieved in liver transplantation (LT), but still relatively poor long-term results are obtained for intestinal and pancreas transplantation with five-year patient and graft survival rates of 47% and 38%, and 82% and 52%, respectively[1].

The major limitation to the widespread use of transplantation is the scarcity of organs. The gap between available organs and potential recipients increases every year, giving rise to serious ethical and practical dilemmas of equity and utility when allocating the organs. In March 2011, the United Network for Organ Sharing reported a solid organ transplant waiting list of 110 600; 16 133 patients were listed for liver, 1389 for pancreas and 262 for intestine transplants. The absolute numbers of transplants in 2010, in contrast, was 6291, 351 and 151 for liver, pancreas and intestine, respectively. These figures clearly illustrates that many patients will never benefit from transplantation and die on the waiting list[1]. The problem is even greater than these figures suggest as not all those who might benefit from transplantation are listed. In the United Kingdom, patients with chronic liver disease are listed only when estimated likelihood of death without transplant is greater than likelihood of death following transplantation. This estimation of survival is calculated based on validated models using objective laboratory data incorporated in scores such as developed by the Model for End-Stage Liver Disease (MELD) or United Kingdom End-stage Liver Disease (UKELD). The UKELD score includes, besides the MELD parameters, serum creatinine, bilirubin and INR, also serum sodium. The latter score seems to have an improved predictive accuracy. A UKELD score of over 49 predicts a > 9% 1-year mortality without liver transplant; this is the minimum criteria for entering the waiting list in the United Kingdom[2]. In the United States, survival benefit starts when MELD score exceeds 17, unless the patient has other co-morbid factors, such as liver cancer, affecting prognosis or unacceptable quality of life because of liver disease. In the United Kingdom, candidates for LT must also have a greater than 50% probability of surviving for 5 years post transplant with a quality of life acceptable to the patient[3].

Although not used as a direct criterion for selection or allocation, development of the concept of transplant survival benefit, i.e. the extra years of life attributable to transplant, might facilitate more effective use of scarce organs and restrict access to those whose lives will be extended minimally or not at all. However, it has proved very difficult to develop robust modelling on which to base such a benefits based approach to liver allocation.

Table 1 Deaths in United Kingdom[1] and United Kingdom Transplant Activity[2]

	Overall deaths	New registrations	Waiting list at 31/3/10	Re-movals	Trans-plants	3 yr post transplant survival
Liver	7503	962	371	123	679	85%
Pancreas	5223[3]	300[4]	335	22	200	74%
Small bowel	4[5]	NA	NA	NA	21	NA

[1]United Kingdom deaths from United Kingdom Mortality Statistics, London National Statistical Office, 2010; [2]United Kingdom Transplant Activity from National Health Service Blood and Transplant Activity Report 2010, Bristol; [3]Deaths for diabetes; [4]Including 243 kidney and pancreas transplantations; [5]Deaths in 2005. Note: Overall deaths include all causes and all ages so many of those who died would not be suitable for transplantation. NA: Not available.

In case of diabetes mellitus, numbers on waiting lists also underestimate the need for transplantation as this therapeutic modality is primarily applied to patients in need for a combined pancreas-kidney transplantation. Transplantation for diabetes alone is restricted, in many centers, to some patients, with hypoglycemia unawareness and brittle diabetes. The adverse consequences of immunosuppression, such as the increased risk of some infections and malignancies need to be balanced against the potential benefits of improved glycemic control so it is not clear which patients with diabetes mellitus might benefit from transplantation.

In part because of cultural and logistic issues, deceased donor rates vary considerably between countries, ranging from 2 per million population in Greece to 35 per million population in Spain[4]. The success of public health initiatives, leading to better awareness for vascular diseases such as hypertension and reduction in fatal road accidents, resulted in a fall in the number of "traditional ideal" (young post-traumatic) organ donors and an increase in the use of "high risk" donors, such as older and obese donors and donors after circulatory arrest[5]. The increasing donor risk profile partly negates the benefits made by better surgery and peri-transplant care.

To increase the number of available organs, several technical advances and policies have been adopted. Using extended criteria donors (ECD) and donation after circulatory death (DCD) [also referred to as non-heart beating donors (NHBD)] and implementing split LT (SLT), domino or living donor LT can contribute to enlarging the donor pool. All these techniques and policies are however not free from additional risk and therefore their use raises medical and ethical concerns as welfare of both the living donor and recipient may be compromised. Indeed all these allografts from deceased donors carry an increased risk of primary non-function, early or delayed dysfunction and possibly a greater risk trans-

mission of infection and cancers[6]. It has been clearly shown that ECD organs, defined as organs originating from donors dying from cerebrovascular catastrophe, DCD, longer ischemia time, older age and steatosis compromise outcome. Severe allograft steatosis, defined as > 60% fatty infiltration, is associated with a greater risk of primary graft dysfunction and lower patient and graft survival[7]. Grafts with more than 30% steatosis have been reported to be safe in low-risk recipients but associated with more risk in recipients with MELD scores greater than 30[8]. Clinical estimation of the degree of fatty infiltration correlates poorly with histologic assessment.

Guarrera *et al*[9] have undertaken a pilot study designed to evaluate the safety and feasibility of liver preservation with hypothermic machine perfusion (HMP), a technique widely used in kidney transplantation[10] although its potential has been shown in animal models of LT. Compared to standard cold preservation in human LT, HMP appears safe, may improve graft function and is reported to be associated with a reduction in preservation injury (PI). This strategy is likely to be most beneficial in older, steatotic and DCD grafts, which are most susceptible to PI.

Models investigating the interaction between donor and recipient risk profiles have been developed to predict the likelihood of graft and patient survival after LT. Feng *et al*[11] recently identified, in a large donor cohort study, nine factors (age, height, DCD donors, split liver grafts, black race, cause of death from cerebrovascular accident, regional sharing and cold ischemia time) which were associated with graft failure. As a corollary, a donor risk index predicting the effect of these variables on graft survival was developed[11]. It is clear that it will become more and more important to match donor risk score with recipient risk score in order to assure an acceptable outcome for the recipient.

SLT has been developed as a strategy to increase the number of liver grafts by creating 2 grafts from 1 donated liver. The bipartition of a liver is especially important in the small group of pediatric patients for whom size-matched whole liver allografts are scarce. Indeed the use of split grafts has been associated with a reduction in the risk of death on the pediatric waiting list; although some centers have reported an increased risk of graft failure, the split procedure for adult-pediatric pair is now accepted as a valuable technical variant in pediatric LT[11,12]. Donor selection for splitting, technical and logistic expertise to decrease total ischemia time are all important factors for a successful outcome of the procedure. This technique is much less successful in the adult-adult split constellation.

In order to expand further the donor pool, organs from DCD donors are increasingly used in liver and pancreas transplantation, especially in the United Kingdom and the Netherlands. Liver and pancreas grafts are usually restricted to those originating from controlled donors - those donors in Maastricht category Ⅲ (awaiting cardiac arrest)[13]. However in Europe, legal constraints do not allow use of NHBD in all countries[14]. Although, DCD LT can have good outcome, their use is associated with a significantly higher risk of graft failure[13-16], severe biliary complications and higher costs[17-19]. However, increasing understanding of the pathophysiology of the events surrounding DCD and better selection and timing may improve outcome in the future.

Several reports described successful islet isolation and transplantation from DCD donors[20-24]. These donors could provide an important resource for islet transplantation if used under strict criteria and in multiple transplantation, particularly in countries where heart-beating donors are not readily available.

The use of DCD organs has not been deemed suitable to intestinal recipients because of concerns about organ quality.

With respect to transplantation of organs of the gastrointestinal system, living donation is essentially confined to LT. Better understanding of the anatomy and increasing surgical skills has allowed living donor liver transplants (LDLT) to become a routine procedure in some, especially Asian, centres. LDLT has been widely adopted in Asia because of the very low rates of deceased organ donation and because of the very high incidence of liver cancer. LDLT accounts for over 95% liver transplants in Asia. In 2008, a Chinese series of 234 right-liver living donor liver transplants showed 1-, 3-, and 5-year overall survival rates of 93.2%, 85.7%, and 82.4%, respectively, comparable with deceased donor liver transplant outcomes[25]. Good outcomes have been shown even when using grafts with a graft-to-recipient weight ratio (GRWR) < 0.8%, with a rate of small-for-size syndrome similar to those receiving graft with a GRWR > 0.8%, provided the recipient is receiving the graft from a young donor[26].

In the Western world LDLT is practised much less frequently because of the greater availability of deceased donors but also because of major concerns with donor mortality, especially when transplanting the right lobe which is associated with an estimated risk of donor death of 0.08% and a morbidity around 20%. The reported morbidity and mortality data underestimate the real risk. There have been anecdotal reports of donors requiring a transplant for hepatic failure. The outcome of LDLT is good with a 1-, 5- and 10-years graft survival of 81%, 70% and 68%, respectively[27-30]. The survival rates after LDLT are better than full size deceased donor LT in children but somewhat lower in adults[29].

However, not all liver transplant candidates have suitable donors. Altruistic (non-directed) liver donation is done very rarely although the number of altruistic kidney donations, while still small, is increasing slowly.

Living donor bowel transplantation has been reported as an additional resource for patients with intestinal failure with total parenteral nutrition-related life-

threatening complications[31]. However the very limited data from the Intestinal Transplant Registry do not demonstrate a clear advantage of living donor intestine donation over deceased donor intestine transplant[32-34]. The early outcomes of combined intestinal and LT using living donors are promising and the elimination of the high mortality on the cadaver waiting list (30%) for this category of patients represents a substantial advantage[35].

Limitations of solid organ transplantation

Transplantation is associated with a significant improvement in both quality and quantity of life for most organ recipients but does not reach normal values[36]. Patient and graft survival is limited by many factors including recurrent disease, immune mediated graft damage, technical problems and long-term infectious, malignant and cardiovascular consequences of immunosuppression. It is noteworthy that recurrent hepatitis C virus (HCV) allograft infection is almost universal and associated with a worse outcome compared with most other indications, yet HCV related cirrhosis is one of the leading indication for LT in the Western world[37].

Although operational tolerance is found in a small proportion of highly selected liver allograft recipients, most patients will require life-long immunosuppression. Attempts to induce tolerance strategies which are successful in the laboratory, have yet to be reliably achieved in man[38].

Transmission of donor-related disease, especially some infections and cancers, can be mitigated but not abolished[39].

XENOTRANSPLANTATION

Research in xenotransplantation has grown in the last decades[40,41]. The use of knock-out pigs with multiple gene modifications reduced the frequency of hyperacute rejection which was a major problem in earlier models[42,43]. Many physiological restraints, as evidenced by a systemic inflammatory response involving the innate immune system, by platelet, leucocyte and complement activation, by coagulation dysfunction associated with coagulation-anticoagulation incompatibilities of primates and pigs, remain. The transplantation of porcine organs has been carried out in non-human primates with better outcomes with pig hearts or kidneys compared with pig livers, the main problems being in the latter a coagulation dysfunction with thrombocytopenia leading to spontaneous bleeding[44]. However, pig livers may provide sufficient function to maintain short term support and might so be used in patients with acute liver failure, either until the native liver recovers or as a bridge to liver allograft. Only one clinical pig-to-human LT has been reported so far by a surgical team in Los Angeles headed by Leonard Makowka, in a patient with fulminant hepatitis. The patient underwent preoperative plasmapheresis to remove circulating xenoantibodies and the porcine liver graft was placed in a heterotopic position. The pig liver was rejected in few hours and the patient died before a human liver became available[45].

Significant roadblocks, such as the immunologic hurdle of cross species transplantation and transmission of infections, particularly endogenous retroviruses, need to be overcome before pig organ xenotransplantation can become a clinical reality. Furthermore, the physiological impact of xenotransplantation remains unclear as shown by the immunogenicity and uncertain physiological functioning of pig proteins in the maintenance of homeostasis.

Progress is being made in this difficult field of transplantation as shown by the report of encouraging outcome of pig hepatocyte xenotransplantation with the benefit of the lack of an acute humoral xenograft rejection, the immediate restoration of the liver function and the resistance to specific human viruses[46]. Are equally encouraging the results of xenotransplantation of encapsulated pig islet cells with the aim to prevent antibody or T-cell contact with islets, allowing though insulin to reach the systemic circulation[47]. However, this xenotransplantation remains illegal at this time.

CELLULAR TRANSPLANTS

Cellular transplants are being used in a very limited number of indications, such as use of pancreatic islets for diabetes mellitus[48] and hepatocytes for some metabolic liver diseases and parenchymal liver diseases[49]. The Collaborative Islet Transplant Registry reports that the majority of islet transplant procedures are performed in the islet transplantation alone setting, with Islet-after-Kidney-Transplantation representing only a small fraction of all islet transplants performed[50].

Islet transplantation is limited to a highly selected group of patients with type 1 diabetes (T1DM) and aims to achieve adequate glycemic control and removal of need for exogenous insulin[48] (Table 2). Islet transplantation is associated with short-term benefits of insulin independence, normal or near normal HbA1C levels, sustained marked decrease in severe hypoglycemic episodes and a return of hypoglycemia awareness. However the long-term efficacy is disappointing. In 2006, an international, multicenter trial reported on 36 subjects with T1DM; insulin independence with adequate glycemic control at 1 year after the final transplantation was achieved in only 44% of patients and 31% remained insulin-independent at 2 years[51]. Follow-up of a larger cohort of 65 patients showed that insulin independence was achieved in 69% at 1 year, 37% at 2 years and only 7.5% at 5 years[52].

A successful islet transplantation can effectively reverse and stabilize the risk of secondary diabetic complications. The Vancouver group compared islet recipients to best medical therapy and found that islet transplanta-

Table 2 Conditions where human cell transplants have been used clinically

	Indications	Ref.
Islet transplantation	Patients with type 1 diabetes with severe glycaemic lability, recurrent hypoglycemia, and a reduced hypoglycaemia awareness	Shapiro *et al*[84] Shapiro *et al*[51] Ryan *et al*[52] Matsumoto *et al*[54] Strom *et al*[57] Vantyghem *et al*[85]
Hepatocyte transplantation	Inherited metabolic disorders: Familial hypercholesterolemia Urea cycle deficit α1 antitrypsin-deficiency Glycogen storage dz 1a Infantile refsum's dz Factor Ⅶ deficiency Crigler-Najar type 1 syndrome Progressive familial intrahepatic cholestasis	Grossman *et al*[86] Strom *et al*[87] Horlsen *et al*[88] Mitry *et al*[89] Fox *et al*[90] Muraca *et al*[91] Sokal *et al*[92] Dhawn *et al*[93] Hughes *et al*[62] Ambrosino *et al*[94]
	Chronic liver failure (Child A-C)	Sterling *et al*[61] Mito *et al*[60] Strom *et al*[95] Strom *et al*[57]
	Acute liver failure (as bridge to transplant)	Sterling *et al*[61] Habibullah *et al*[96] Bilir *et al*[97]

tion had a better impact upon risk of secondary complication[53].

In contrast to the time consuming and expensive islet isolation from donor pancreas, islet transplantation is minimally invasive and carries a low morbidity and mortality compared to whole pancreas transplantation because the islets can be infused percutaneously into the hepatic portal vein. However, its use is limited because of the shortage of high quality donor pancreases, the high cost of the isolation procedure, the maintenance of a specialised human islet isolation laboratory, the need for life-long immunosuppression and the need of multiple organs to obtain enough islets. The ability to achieve single-donor islet transplantation would provide many more islet grafts and also increase the number of recipients with T1DM. Although Matsumoto *et al*[54] recently published a protocol describing successful single-donor islet transplantation, further studies are needed to confirm the benefit of this protocol. Recipients of IT are also exposed to a wide range of human leukocyte antigens from multiple donors due to repeated islet infusions, which are still, in many units, matched for ABO blood group only. The development of antibodies may be an important issue in end stage diabetic patients also requiring a kidney transplant, as their appearance possibly limits the chance to find a compatible kidney[55]. It may therefore be difficult to resolve the competing requirements of islet and renal transplantations.

The research in hepatocyte transplantation (HT) has shown encouraging results in particular in the treatment of some inherited metabolic disorders, and have raised expectations for a new therapeutic approach as a possible alternative to LT (Table 2)[56]. About 30 patients had HT for liver-based inborn errors of metabolism; the main indication was urea cycle defects. About 5% of newly formed, exogenous-derived, cells natively expressing the gene involved in the disease, suffice to significantly alleviate the consequences of many congenital liver metabolic diseases[57]. The procedure seems to be safe and results are encouraging; however, as seen in the field of islet transplantation, cell function often declines within 1 year ending finally up in LT.

A new approach of gene targeting technology has been recently proposed with the potential of combining human induced pluripotent stem cells with genetic correction to generate clinically relevant cells for autologous cell-based therapies for the treatment of some inherited metabolic disorders[58].

HT in patients with chronic liver failure has been associated with dismal results[59-63]. This might be related, at least in part, to the presence of fibrosis, which may impair cell engraftment in the liver[62]. HT has also been applied in patients with acute and acute-on-chronic liver failure, in the former with the aim to support the liver function as a bridge to LT. More than 40 patients with acute liver failure have been treated worldwide; a part from an improvement in bilirubin levels and hepatic encephalopathy, there was no significant impact on outcome[59]. Two large clinical trials assessed the effectiveness of bio-artificial liver devices including both a biological component and an artificial scaffold, and both failed to have a beneficial effect on survival[63,64].

HT is also limited by the availability of livers for cell isolation. The recipient's cirrhotic liver as a source for donor hepatocytes, would be an easily available option, but it is uncertain whether these cells behave *in vivo* as they do in culture.

Establishing banks of cryopreserved hepatocytes is challenging, because isolated liver cells are very sensitive to damage. Furthermore, cryopreserved hepatocytes engraft less efficiently than freshly-isolated cells. Another major issue is their time-limited efficacy, which may limit the use of HT as a bridge to LT. In contrast, the use of stem/precursor cells is associated with relatively easy procurement and as they proliferate well *in vitro,* such cells may provide sufficient cell mass available for transplantation. A further issue in this field is related to the mechanism of parenchymal integration and repopulation of exogenous hepatocytes[65]. "Making space" is a prerequisite in order to provide an initial regenerative stimuli; partial hepatectomy, partial embolization and irradiation of the liver all represent effective regenerative stimuli in animal models, but their efficacy in humans requires further investigation. Moreover, the exogenous cells should have a proliferative advantage with respect to autologous liver parenchymal cells; in order to proliferate some authors suggest that this problem may be overcome with the use of foetal hepatic progenitors cells that exhibit an intrinsic biological advantage[66].

REGENERATIVE MEDICINE AND TISSUE ENGINEERING

The manufacturing of bioengineered organs in the laboratory starting off from autologous differentiated cells and/or stem cells is undoubtedly ground-breaking and exciting for the transplant community. As there will always be more potential recipients than donors, many researchers are working in the field of artificial tissue engineering (TE) and regenerative medicine (RM).

In 2006, Atala *et al*[67] of the Wake Forest Institute for Regenerative Medicine in Wiston-Salem, North Carolina, implanted bladders engineered *ex vivo* from the seeding of autologous cells onto artificial supporting scaffolds; in 2008 the same group implanted a trachea manufactured from human components[68]. However, solid organs with lots of blood vessels, such as liver, pancreas and bowel are harder to grow.

Progresses in the development of clinically feasible liver TE approaches, has been hampered mainly by insufficient cell-to-cell contact of the engrafted hepatocytes. Ohashi *et al* developed a method of cell sheet technology to engineer a uniformly continuous sheet of hepatic tissue using isolated primary hepatocytes cultured on temperature-responsive surfaces. Sheets of hepatic tissue transplanted into the subcutaneous space resulted in efficient engraftment to the surrounding cells, with the formation of two-dimensional hepatic tissues that stably persisted for longer than 200 d and showed several characteristics of liver-specific functionality.

Decellularization-recellularization technology has done steps forward in order to manufacture liver organoids. Uygun *et al*[69] decellularized rat livers and repopulated them with rat primary hepatocytes, showing hepatic function. Atala's group implemented the technique by bioengineering livers with human cells[70,71].

Extracellular matrix (ECM) scaffolds might provide the desired natural environment to enhance current cell-based approaches aimed at producing large quantities of functional pancreatic endocrine cells[72,73]. However, compared to islet transplantation, whole organ transplantation using ECM scaffolds is clearly more invasive, requiring revascularization and possibly even exocrine drainage.

Primary hurdles to intestinal bioengineering are the functional regeneration of diverse motility patterns and the complexity of intestinal anatomy.

The implantation of organoid units of intestine has been successful in rats and pigs; however, this technology is time consuming and expensive, as several centimetres of bowel are needed to obtain a sufficient number of organoid units able to repopulate just a few centimetres of engineered intestine. Moreover, organoid units cannot be cultured and grown easily in vitro[74,75]. Decellularization-recellularization technology has been used to engineer less complex gut structures such as the esophagus. A 10-cm segment of porcine jejunum was decellularized and repopulated with autologous cells. After maturation, the construct was implanted in the arm of a patient suffering from a major esophago-tracheal defect and retrieved after 7 d; the construct showed patent vessels and viable cells, showing that sustained the implantation[76].

The attraction of a failing organ being replaced by a bioengineered organ generated from a decellularized scaffold and seeded with autologous stem cells is obvious but not without limitations. It will take time to isolate and grow such organs or sufficient cells to provide adequate function so this approach would not be an option for those with acute liver failure where liver replacement is required within some days and liver assist devices have yet to demonstrate an effective role. Similar time constraints may also preclude the use of such organs in patients with primary liver cancer. The use of autologous stem cells, able to differentiate into the kind of required cells and with the potential to expand without limitation, may resolve the issue of rejection and long-term immunosuppression requirement, even though very little is understood regarding the host immune response to bio-engineered constructs.

However, organs derived from autologous stem cells may be subject to the same risk of damage from virus or immune mechanisms that result in the failure of the native organ. Recurrent HCV allograft infection remains a major cause of graft failure despite major advances in antiviral treatments and recurrent autoimmune diseases such as diabetes or autoimmune hepatitis, contribute to graft loss despite associated immunosuppression. Without genetic modification, autologous stem cell derived organs or cells will not correct the disease resulting from metabolic conditions that result in cirrhosis and so leading to organ failure, such as is seen in Wilson's disease or tyrosinosis. Organs or cells derived from allogeneic stem cells would provide an alternative to deceased donor organs and so mitigate against the organ shortage and allow greater access to life-saving care. The application of bone marrow-derived stem cells has shown good results in small groups of patients however in the absence of control groups[71,72,77,78].

In addition to supplementing or replacing the traditional abdominal organs from living or deceased donors, RM could be of help in other patient groups such as those with inflammatory bowel disease and selected small bowel disease refractory to treatment. Non-responding patients frequently require surgery to control symptoms, although such interventions will not necessarily resolve their disease. An approach based on new findings of RM could drive major changes in management and treatment of such diseases. RM therapy can become effective either in repairing damaged intestinal tissue and correcting immunological underlying disorders. Therefore, RM should not only be considered as a potential therapy for patients with inflammatory disease refractory to standard medical and biological therapy but, hopefully, as a curative treatment that allows achievement of long-lasting

remission.

RM could also have a great impact in the area of gastrointestinal motility disorders, particularly those associated with the aganglionic gut or Hirschsprung's disease and other congenital or acquired enteric nervous system disorders, and may obviate the need for surgical therapies that, although life-saving, are associated with an unsatisfactory long-term prognosis for many.

RM and TE have the enormous potential to help not only those patients who would otherwise be candidates for liver, pancreas or bowel transplantation but also those who would not, under current restrictions, be eligible for listing. Despite promising pre-clinical results[69,73-75,79-83], many critical aspects of cell therapy and TE need to be further addressed, including long-term safety, tolerability and efficacy in the clinical setting, and last but not least the development of an European Medicines Agency/Food And Drug Administration approved product, before they become protagonists of a new scientific era. Doubts whether such aspirations can be fulfilled in the near future remain, despite the significant advances already made, uncertain. Lessons from other technologies, such as gene therapy, suggest that expectations must be managed carefully.

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