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Opinion

The translational approach to liver transplantation

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Liver transplantation has proved to be a successful life-saving treatment for end-stage liver diseases. Yet, despite the remarkable progress made since the first successful human liver transplant, many open questions still need to be addressed. The solution for a number of current issues requires the implementation of adequate research programs as part of the clinical agenda. However, the process of bringing basic science knowledge into clinical practice remains a challenge. The need to potentiate the development of innovative discoveries capable of improving human health has opened the doors to new possibilities for experts and programs. Salient among them is the opportunity to establish a multi-step process for transforming observations in the clinic, laboratories and the community into interventions that enhance people's lives through diagnostic, therapeutic, procedural, device and behavioral changes that have been designated as translational research and science [1,2]. This type of research has found transplantation to be a fertile ground for development; transplantation and translational science call for and encourage multidisciplinary collaborations to identify and support the adoption of more effective medical practices for the benefit of patients requiring novel and unique therapeutic approaches [1,2].

Over the last decades**,** liver transplantation has benefited from translational research programs. We will expand on two areas of research aimed at maximizing the use of scarce organ resources by minimizing the risks of losing transplanted organs: one concerns the reduction of graft rejection, while the other seeks to expand the pool of available organs utilizing novel preservation technologies.

The liver is the largest solid organ in the body**. I**n addition to its classical, widely described functions in metabolism, detoxification and nutrient storage, it has been recognized as a unique immunological organ characterized by a tolerogenic microenvironment. [3] Nonetheless, despite its well-known immunotolerant properties, hepatic allografts are susceptible to cellular and antibody-mediated rejection. The mechanisms involved in hepatic tolerance and rejection are as yet only partially understood; further insight is required not only into the interaction between hepatocytes, non-parenchymal cells and immune cell populations, but also into the underlying molecular signaling pathways that govern these phenomena. The cytokine IL-33 and its specific receptor (ST2) play important roles in organ transplantation [4,5]. Studies have shown that the IL-33/ST2 pathway takes part in the development of liver pathology [6–8], but its function in liver transplantation remains obscure. Our research group is currently focused on the study of the IL-33/ST2 axis during allograft rejection, fibrosis and liver injury in the transplant context as part of our translational research program [manuscript in preparation].

Early works on immunosuppressive drugs discovered and established the association between calcineurin inhibitors and other drugs such as the backbone regimes used for most organs including the liver [9]. However, given the adverse effects caused by the long-term use of immunosuppression and the lack of new drugs in the pipeline, cell therapies have been proposed in the translational field as a promising tool for controlling the alloimmune response in transplant patients. Several cell types with immunomodulatory properties have been evaluated in preclinical models and phase I clinical trials focused on CD4+ regulatory T-cells, with results confirming their safe and feasible usage (Tregs). Todo S. et al. investigated the adoptive transfer of *ex-vivo*-generated Treg-enriched products in 10 consecutive adult patients undergoing living-donor liver transplantation. The therapy proved to be safe, with seven recipients successfully stopping immunosuppression for nearly two years [10]. More recently, Sánchez-Fueyo et al. performed an open-label, dose-escalating phase I clinical trial, demonstrating that using *ex-vivo* expanded autologous polyclonal Tregs in adult liver transplant recipients was safe, did not increase the incidence of infections or cancer, and appeared to induce anti-donor-specific hyporesponsiveness [11].

Over the past few years, several researchers have been engineering chimeric antigen receptor (CAR)-Tregs to produce donor antigen-specific Tregs [12] Experiments in animal models have demonstrated that CAR-Tregs reduce alloimmune responses more effectively than polyclonal Tregs in skin humanized models and prevent xenogeneic GVHD [13,14]. The evolution from transferring polyclonal Tregs to the recipient to producing alloantigen-specific CAR-Tregs provides an example of a promising translational strategy for achieving tolerance; nonetheless, further studies are required to more conclusively define the applicability, safety and efficacy of using CAR-Tregs in human liver transplantation worldwide.

The second area of research aimed at deriving maximum benefit from available organs seeks to reduce the use of static cold preservation (SCP), the gold standard method employed since the 1960s, and expand the use of dynamic organ preservation. SCP involves organ flushing and cooling in the donor, followed by immersion at 0–4°C in the same preservation solution until engraftment. The infusion of preservation solution is intended to minimize cell metabolism and

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cell damage [15]. Notwithstanding the low temperature, however, substantial anaerobic activity persists, leading to ATP depletion, anaerobic activity by-product accumulation, and the generation of extensive reactive oxygen species. This, in turn, contributes to ischemia–reperfusion injury (IRI), which can occur once the blood supply is restored. The process described can cause primary graft dysfunction, with cold ischemia time and donor characteristics (extended donor criteria) being the principal determinants.

Some researchers continue studying novel SCP solutions. Rodriguez JV. et al. have developed a solution using N,N-bis-2-hydroxyethyl-2-aminoethanesulfonic acid–gluconate–polyethylene glycol (BGP). In a rat transplant model [16], this new component played a more promising role in preventing IRI than did HTK preservation solutions. Nonetheless, current strategies are moving towards the assessment of organ functionality and/or the performance of *ex-vivo* organ repairing prior to transplantation. Hypothermic perfusion (HMP) was the initial method utilized for perfusing organs, combining the positive effects of hypothermia with those of dynamic preservation [17]. Nowadays, two main strategies prevail in the clinical approach to liver allograft *ex-vivo* machine perfusion: hypothermic oxygenated perfusion (HOPE) and normothermic preservation (NMP). The first, developed by Dutkowski et al. as a HMP technique for delivering a hyperbaric oxygenated perfusate through the portal vein at low pressure [18], has demonstrated protective effects against liver IRI in experimental models [19,20]. Furthermore, in a multicenter randomized controlled trial, using HOPE resulted in diminished early allograft injury, and produced more favorable post-transplant outcomes than did SCP [21]. On the other hand, NMP has managed to maintain donor organs at body temperature while ensuring an adequate oxygen and nutrient supply [15]. Evidence from organs donated after brainstem death and organs donated after circulatory-death liver transplantation showed that NMP produced an improvement in the post-transplant survival of transplanted livers and enabled the assessment of organ viability during preservation [22–25]. These findings allowed for immediate translation to the clinical field as part of randomized control trials in large series (220 liver transplantations) [26]. Compared to the cold-storage sample, the NMP group exhibited a smaller amount of aspartate aminotransferase (AST), a reduction of early allograft dysfunction rates, and 50% lower organ discard [26]. These results demonstrated improved graft preservation and highlighted the feasibility, usability and safety of NMP [26]. In light of these findings, the Viability Testing and Transplantation of Marginal Livers (VITTAL) phase II clinical trial (ClinicalTrials.gov number NCT02740608) in the UK explored the benefits of NMP. Designed to assess the degree of organ improvement and define the best timing for engraftment by measuring lactate clearance, the VITTAL trial confirmed that NMP was an objective strategy for determining viability [23].

The final "game changer" for the future of NMP in liver transplantation was the translational research model developed by Clavien PA et al., which succeeded in preserving, treating and reconditioning discarded human livers for up to seven days before engraftment [27]. The novelty of this "Liver4Life" perfusion machine lay in the integration of multiple core physiological functions including the automated management of glucose levels and oxygenation; parenteral nutrition and the delivery of ursodeoxycholic acid into the portal vein line of our perfusion machine; waste-product removal; and hematocrit control. The Clavien model introduced a perfusion technology developed by a multidisciplinary group of surgeons, biologists and engineers, which enabled the translational science to maintain injured human livers in a functional status *ex vivo*. Evolving applications of this technology in injured and non-injured livers are expected to boost the capacity of liver transplant teams by eventually transforming liver transplantation into a "scheduled procedure." Such a breakthrough would increase liver transplant applicability and enhance the quality of life of patients.

Declaration of interest

None

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