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POINT OF VIEW

Pushing the boundaries of organs before it's too late: pre-emptive regeneration

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SUMMARY

Solid organ transplantation is marked by accelerated aging and inexorable fibrosis. It is crucial to promote strategies to attenuate, or to reverse, damage before organ failure. Hence, the objective of this article is to provide insight into strategies, which aim to regenerate or rejuvenate the transplanted organs. Cell therapy with mesenchymal stromal cells is currently under investigation because of their antifibrotic properties. Their ability to promote mitochondrial biogenesis, and to transfer mitochondria to wounded cells, is another approach to boost the organ regeneration. Other teams have investigated bioengineered organs, which consists of decellularization of the damaged organ followed by recellularization. Lastly, the development of CAR-T cell-based technologies may revolutionize the field of transplantation, as recent preclinical studies showed that CAR-T cells could efficiently clear senescent cells from an organ and reverse fibrosis. Ultimately, these cutting-edge strategies may bring the holy grail of a pre-emptive regenerated organ closer to reality.

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Introduction

The prognosis of solid-organ transplant (SOT) has largely improved over the past decades. Yet, one common culprit responsible for the decline of transplanted organ function is accelerated aging [1]. This chronic allograft dysfunction reaches almost every organ and, to date, no significant therapeutics has emerged to slow it down. The liver [2], heart [3], lungs [4], kidney [5], pancreas [6] and islets of Langerhans [7] may all suffer from a slow and inexorable fibrosis, which will impair the organ's functionality and progressively lead to graft failure. To illustrate this progressive decline in a kidney transplantation (KTx) setting, 50% of grafts may be lost because of chronic allograft nephropathy [1]. The mechanisms of allograft dysfunction are multiple and complex and include progressive factors that are both alloimmunity-dependent and independent [1,2,8,9]. Even though rejection, and infections, in part responsible for this accelerated aging, may be prevented or treated, the vasculopathy and fibrosis induced by these injuries currently result in an irreversible loss of function. There is a crucial need thus to define and promote strategies to attenuate, or even to reverse, the damage induced by this chronic allograft dysfunction. Innovative strategies have been recently proposed to boost organ regeneration, transforming this holy grail into a reality. Hence, the objective of this article is to provide insight into strategies, which aim to regenerate or rejuvenate the transplanted organs (Fig. 1).

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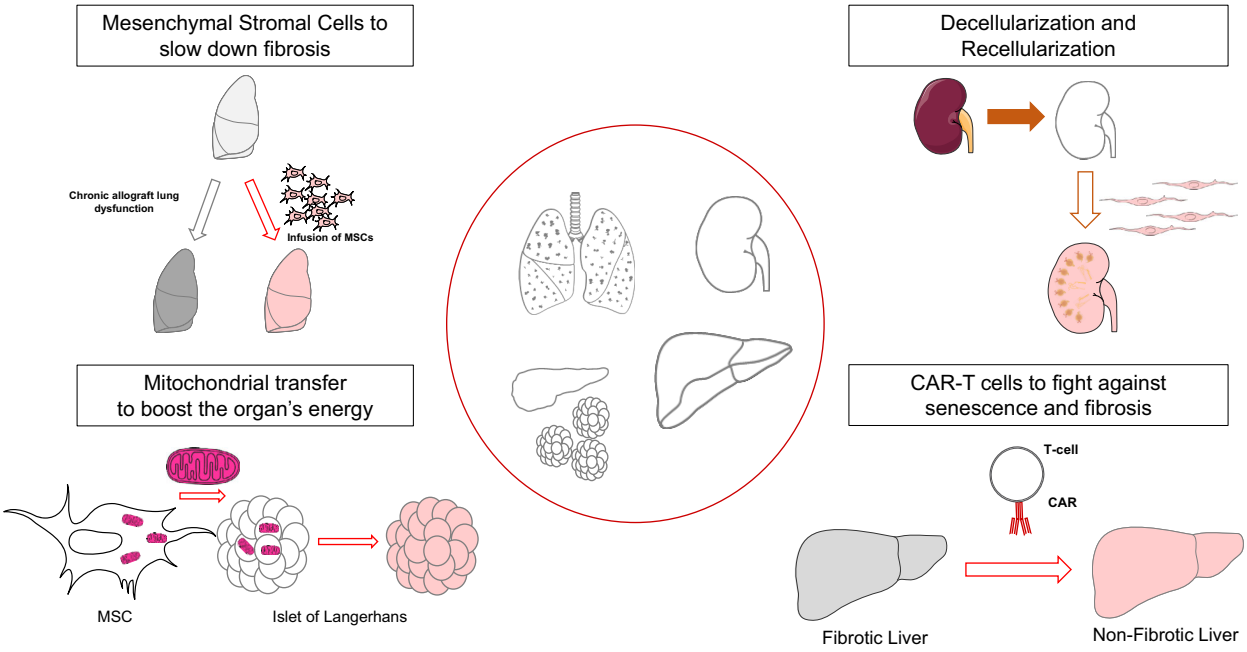

Figure 1 Pre-emptive organ regeneration in SOT: strategies and future therapeutic targets. Every transplanted organ suffers from an inexorable fibrosis over time after transplantation. MSCs, mitochondrial biogenesis, recellularized organs and CAR-T cells are few leading-edge strategies that may lead to organ regeneration. Created and modified with pictures from Smart Servier Medical Art (licensed under the Creative Commons Attribution 3.0 Unported License, https://smart.servier.com/).

Mesenchymal stromal cells to slow down fibrosis

Cell therapy in organ transplantation is an emerging field. Recent clinical trials have mainly focused on the early stage of transplantation, in order to decrease the burden of immunosuppressive agents or induce organ tolerance [10–13]. Mesenchymal stromal cells (MSCs) are currently the most evaluated cell type and the closest to being used in routine clinical practice. MSCs were first described as fibroblast-like cells organized in colonies, with the property of adhering to plastic *in vitro* [14]. These cells may be isolated and cultured from various tissues: bone marrow, fat, placenta and may be transplanted either from an allogenic or from an autologous source. Several phase I/II clinical trials have been performed, most particularly in the kidney field, and several conclusions can be drawn. First, the timing of cell infusions is critical. Perico *et al.* reported the first two patients treated with autologous MCs (1.7×10^6 cells and 2.0×10^6 cells per kg, respectively), at day 7 post-KTx, with no change in the immunosuppressive regimen. Both of these patients presented a transient acute kidney injury after cell infusion. Kidney biopsies from one patient revealed a nonspecific inflammatory infiltrate, related to engraftment syndrome from the MSCs [11], which resolved after bolus steroid therapy. The two subsequent recipients were infused with MSCs before KTx (2.0×10^6 cells/kg), without any consequent inflammatory infiltrates in the kidney graft [15]. Two recent trials, the TRITON study [13] and the NEPTUNE study [16] tested another approach, i.e. a delayed infusion of MSCs after several weeks post-KTx. Concerning the TRITON study, 57 recipients were randomly assigned to be treated or not with 1.5×10^6 per/kg body weight autologous MSCs at 6 and 7 weeks post-KTx. All the recipients had a baseline maintenance immunosuppressive regimen consisting of tacrolimus, everolimus and low-dose prednisone. Tacrolimus was discontinued in the MSCs-treated group after infusion. Up to five years post-KTx, there was no difference regarding graft loss, rejection or serious adverse events between the two groups, highlighting the safety of this MSC-based strategy. In contrast to the TRITON study, the NEPTUNE study evaluated the safety of allogenic MSCs, which potentially involve the risk of third-party alloimmunization. Ten recipients received two doses of 1.5×10^6/kg allogeneic MSCs 6 months after transplantation in the Neptune study, followed by a low-dose of tacrolimus in combination with everolimus and prednisone. They used a matching strategy that prevented repeated mismatches, and at one-year post-

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transplantation no *de novo* DSA targeting the kidney graft or MSCs were detected and all the recipients had a stable graft function. The safety of third-party allogenic MSCs was also recently provided by an open-labeled phase I and II study [12]. Ten kidney transplant recipients from deceased donors received allogenic MSCs (around 2×10^6 cells/kg) once a day 3 ± 2 days post-transplantation. On the contrary to the NEPTUNE study, no specific HLA matching strategy was implemented. As a direct consequence or not, four recipients developed *de novo* DSA targeting the MSCs. Among them, one recipient developed a shared *de novo* DSA targeting both the kidney graft and the MSCs. Yet, there were no significant differences in rejection or graft loss, compared with the control group.

These recent short-term clinical trials with allogenic and autologous MSCs confirm the safety and the possible implementation of MSCs in routine KTx albeit direct evidence of long-term advantages of MSC therapy is the next step. Notably, MSCs may be of particular interest for long-term outcomes such as the evolution of fibrosis (Fig. 2). Fibrosis is characterized by excessive accumulation of the extracellular matrix (ECM), which leads to the destruction of tissue architecture, and ultimately to SOT dysfunction. Fibrosis mainly evolves in the case of persistent allograft injury and chronic inflammation, induced by calcineurin inhibitors, infections or subclinical inflammation [17]. Neutrophils, macrophages and T cells are recruited in injury and secrete mediators promoting differentiation of myofibroblasts, which are collagen-producing cells generated from epithelial or endothelial-mesenchymal transition [18]. Among these mediators, Transforming Growth Factor-β (TGF-β) is considered as the master regulator of fibrosis, as it promotes myofibroblast differentiation [19]. MSCs improve fibrosis by decreasing leukocyte infiltration and the expression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [20]. MSCs' secretome is also a major actor against fibrosis and includes growth factors, extracellular vesicles (EVs) or microRNAs (miRNAs). For instance, Hepatocyte Growth Factor is secreted by MSCs and has been demonstrated to play a central role in the paracrine inhibition of TGF-β in mouse models [21,22]. Also, EVs are vesicles made of lipid bilayers, secreted by MSCs, which possess the ability to carry and deliver to surrounding cells small molecules such as proteins, miRNAs, nucleic acids and others [23]. These EVs inhibit and downregulate the local expression of TGF-β, acting as a cargo for miRNAs, which regulates fibrosis, such as mi-RNA29, mi-RNA30, mi-RNA210-3p or let-7 family [24–26]. Collectively, this fundamental data suggests a benefit of MSCs for SOT fibrosis; however, the clinical translation remains to be proven. Indeed, the NEPTUNE study did not find any significant improvement of fibrosis, measured with Red Sirius staining, between M6 and M12 kidney biopsies after MSC treatment. The TRITON study also showed a similar quantitative progression of the fibrosis score between Control and MSC treated recipients, up to 6 months post-KTx. However, these studies were not designed to assess long-term outcomes.

In another transplantation setting, the lung may be an appropriate model to study the effect of MSCs on SOT fibrosis, as lung diseases often involve a progressive and irreversible fibrosis and chronic allograft dysfunction resulting in lung retransplantation [4]. Preclinical studies indeed suggest a potential benefit of MSC administration for lung fibrosis [27,28]. Two safety-studies evaluated MSCs in the treatment of refractory chronic allograft lung disease and proved the therapy to be safe and feasible [29,30] without severe adverse events. However, for now, no significant benefit has been assessed for lung function.

To enhance the effect of MSCs on SOT, the route of administration may be critical. Currently, all clinical studies have been performed with MSCs that were intravenously infused in recipients. However, MSCs tend to migrate to the lungs after intravenous injection, and then quickly vanish [31]. Recent porcine studies evaluated the benefit of MSC treatment of organs during *ex vivo* normothermic machine perfusion. On the contrary to the intravenous injection, MSCs infused in the perfusion machine reached glomeruli in porcine kidneys and persisted [32] up to 14 days post-transplantation [33]. Even if no clear benefit could be seen concerning fibrosis, only short-term outcomes were evaluated. Overall, there is a lack of studies dealing with the impact of MSCs on long-term outcomes. Consequently, clinical trials evaluating the impact of MSC treatment on profibrotic conditions such as chronic active antibody-mediated rejection [34] or subclinical inflammation [35] may be of interest in the next few years.

Targeting mitochondrial: how to boost the cell's energy

Mitochondria are organelles that generate adenosine triphosphate to sustain the cell's basal activity, as well as cellular repair and regeneration. Organ transplantation is inherently associated with ischemia-reperfusion

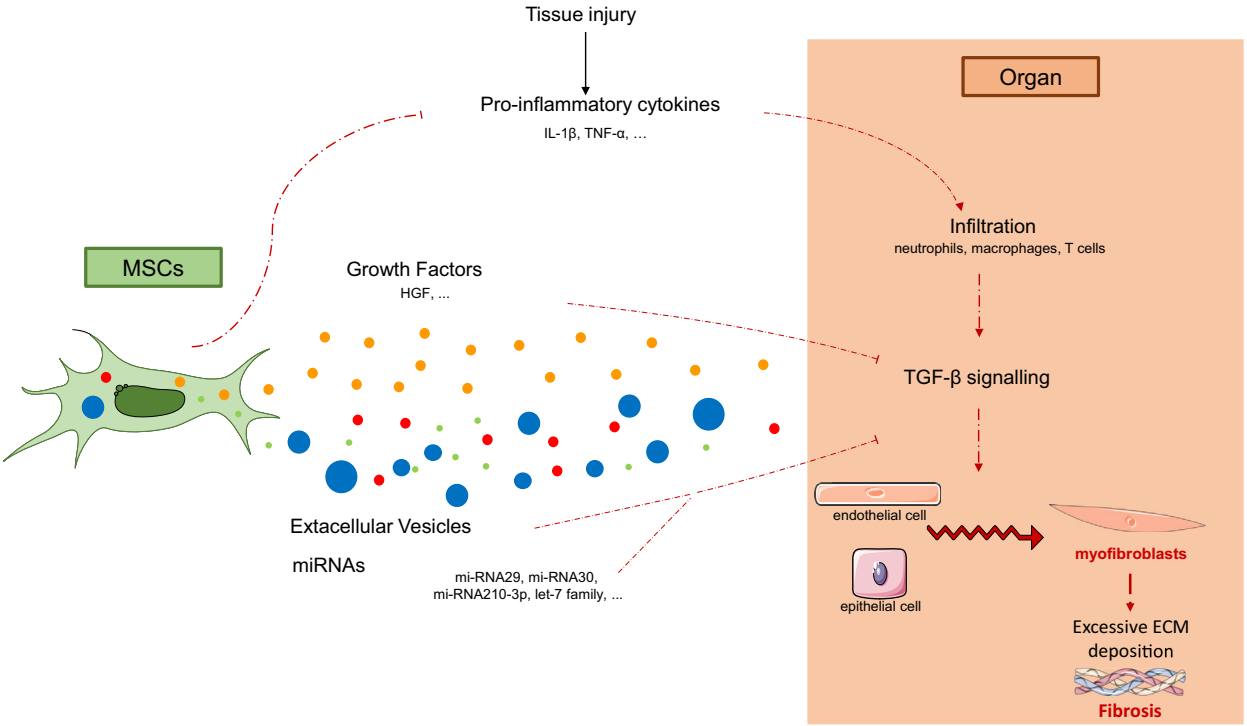

Figure 2 Mediators of fibrosis in SOT and related targets of MSCs. MSCs improve fibrosis by decreasing leukocyte infiltration and the expression of proinflammatory cytokines. MSCs' secretome is also a major actor against fibrosis and includes growth factors, EVs or miRNAs, which inhibit or downregulate the expression of TGF-β. ECM, extracellular matrix; HGF, hepatocyte growth factor; IL-1β, interleukin 1β; miRNAs, microRNAs; MSCs, mesenchymal stromal cells; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α. Created and modified with pictures from Smart Servier Medical Art (licensed under the Creative Commons Attribution 3.0 Unported License, https://smart.servier.com/).

injury, which is responsible for delayed graft function and/or chronic allograft dysfunction [36]. Ischemia-reperfusion injury is hypothesized to be mediated via mitochondrial function [37]. Mitochondria generate reactive oxygen species (ROS) during ischemia-reperfusion injury. This induces the formation of mitochondrial permeability transition pores, which can lead to mitochondrial swelling, dysfunction and cell death. It is even more detrimental for high energy-demanding organs, such as the heart or the kidney, which possess the highest mitochondrial content in the entire body [38]. Melis *et al.* [39] presented a porcine preclinical model of KTx-induced ischemia-reperfusion. Ischemia-reperfusion was associated with tubular damage and ROS production in the control group, whereas pigs treated with *N*1-guanyl-1,7-diaminoheptane (GC7), an inhibitor of eukaryotic initiation factor 5A hypusination, protected injured kidneys from apoptosis, mitochondrial dysfunction and ROS production. *In vitro* studies showed that inhibition of GC-7 prevented mitochondrial dysfunction and induced a reversible metabolic shift and ultrastructural modifications in mitochondria. These studies highlight the importance of mitochondrial preservation in the context of ischemia-reperfusion injuries. Furthermore, treated pigs exhibited less fibrosis at 3-months post-KTx in favor of long-term protection.

In addition to the mechanisms of mitochondrial preservation, mitochondria possess several pathways in the context of injury to maintain the mitochondrial homeostasis which requires a balance between mitochondrial biogenesis, fusion, fission or mitophagy [40]. Mitochondrial biogenesis produces new mitochondria in the cells in the event of increased energy demands. Mitochondrial biogenesis is a finely regulated mechanism, involving a range of transcriptional co-activators and co-repressors, and especially peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) [40]. These pathways may be stimulated by several mechanisms. EVs secreted by MSCs for instance can release their cargo in the cytoplasm of endangered cells where it can reach and target mitochondrial metabolic pathways to promote biogenesis. For example, the delivery of miR-30, miR-200a-3p,

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miR-214 or miR-122 by EVs to injured organs such as the kidney, the heart or the liver is known to promote mitochondrial biogenesis and protect from ischemic or hypoxic-induced injuries [23]. Mitochondrial biogenesis may also be stimulated by pharmacological agents such as FDA-approved formoterol [41], Sirt-1 agonists like SRT1720 [42], or the FDA-approved 5-HT_{1F} receptor agonist, lasmiditan [43]. All these pharmacological approaches may be translated to clinical trials in transplantation to evaluate their benefits on ischemia-reperfusion induced injuries.

In addition to the previously mentioned mechanisms, Rustom *et al.* [44] reported a novel way to increase mitochondria by the generation of nanotubular highways between two cells, which allows cell–cell transportation of mitochondria. MSCs display this ability to transfer their mitochondria to endangered cells [45]. Mitochondria-depleted cells could be rescued and replenished when co-cultured with MSCs. MSCs directed cytoplasmic extensions to mitochondria-depleted cells, which allowed for the active transfer of mitochondria [46]. This was confirmed in several organs, such as the lungs [47] or pancreatic islets [48]. The mechanism seems selective, as the release of danger-signaling organelles engulfed by MSCs, triggers mitochondrial biogenesis and the capacity of MSCs to donate their mitochondria to injured cells [49]. It may also be of particular interest in the context of transplantation, as mitochondrial transfer from MSCs to T cells is associated with immunomodulation properties. A defective Th17 pathway in a SOT recipient is presumed to be tolerogenic [50] and mitochondrial transfer from MSCs to Th17 cells has been demonstrated to result in a reduced production of pro-inflammatory cytokines and to induce a Treg phenotype [51].

In addition to this ability to transfer their mitochondria to another cell, MSCs induce mitochondrial trafficking among injured cells. Perico *et al.* [52] recently reported that intravenous injection of MSCs after a cisplatin challenge in mice could normalize the mitochondrial shape and density in injured tubular epithelial cells compared with the control group. *In vitro*, co-culture of MSCs with renal epithelial cells induced mitochondrial transfer among injured cells through cytoplasmic extensions. Although, to date, no clinical evidence has been provided on the benefit of MSC treatment on mitochondrial dysfunction in transplantation; converging preclinical data targeting mitochondria suggests promising results for the future of transplantation.

Decellularization and recellularization of organs

For almost a decade now, scientists have tried to come as close as possible to a functional bioengineered organ, using the decellularization and recellularization process. The concept is to perfuse the organ with saponifying agents that lyse the cells of a human sized organ, leaving behind the scaffold, composed of a functional ECM. The organ specific scaffold bears all the growth factors and proteins involved in cell migration, organization and proliferation and thus makes a perfect host environment [52]. Ott *et al.* [53] reported the first successful solid organ decellularization in 2008. Hearts from rats were successfully decellularized after a perfusion of Sodium-Dodecyl-Sulfate (SDS) and Triton X-100, producing heart scaffolds where all the main components of the ECM remained intact after decellularization. Subsequently, these hearts were recellularized with a mix of cell types and showed contractility and pacing electric activity in a few areas. They extended their experience to rat lungs [54], showing improved gas exchange after the orthotopic transplantation of recellularized lungs, compared with controls, and with rat kidneys [55], proving the feasibility of the orthotopic transplantation of a recellularized rat kidney with a blood-perfused vasculature and an immature urine production.

Considering almost a decade has passed between the first attempts to decellularize and recellularize solid organs and now, a few statements must be made. Decellularization methods are now well established. The sequential combination of SDS and Triton X-100 seems to be one of the most efficient detergents and one of the least damaging to the ECM structure [56]. The main challenge remains the recellularization process. From Ott *et al.*'s [57] first attempts, no major breakthroughs have emerged, and there are multiple challenges to address: lack of studies on human organs, uncomplete coverage of the whole ECM and early thrombosis in case of transplantation attempts [58]. Maybe the key lies within focusing on recellularizing only one compartment of an organ at a time. Accordingly, Leuning *et al.* [59] recently proposed one of the first successful attempts to recellularize the endothelium of a kidney scaffold. Using a novel simultaneous arteriovenous delivery system, they reported a complete re-endothelialization of the kidney vasculature using human inducible pluripotent stem cells–derived endothelial cells. Contrary to non-re-endothelialized human scaffolds, recellularized scaffolds could be fully perfused with whole blood, limiting early thrombosis.

Given the challenges that remain, the understanding of the ECM's ultrastructure and the impact of the decellularization process may be primordial. Mayorca-Guiliani *et al.* [60] recently reported a new methodology named ISDoT (*in situ* decellularization of tissues) to achieve complete decellularization of any organ *in situ* while preserving the ECM's architecture. The concept relies on a vascular-flow-directed decellularization system, which distributes decellularization reagents through the cardiovascular system to any organ of choice, preventing the vascular system from collapsing. Imaging of ISDot-processed organs confirmed the integrity of even the smallest blood vessels. When compared with standard decellularization methods, the ISDot method showed less damage of the ECM's integrity. Proteomic analyses extensively characterized all subclasses of the ECM's components at an unmet level. Ultimately, even if it currently sounds like science-fiction, a deeper understanding of the ECM composition and architecture may bring the concept of a transplantable bioengineered organ closer to reality. For now, it remains science-fiction, yet we could imagine that instead of retransplantation from a second or third donor in the case of graft failure, the transplanted organ could be rejuvenated with fresh cells after *in situ* decellularization and recellularization.

CAR-T cells to reverse fibrosis

To end with a salute to cancer-designed therapeutics, chimeric-antigen receptors (CAR) T-Cells have extensively widened the possibilities for the treatment of cancer. This tailored therapy enables T cells to overcome mechanisms by which tumors escape from immune surveillance [61]. Genetically engineered T cells recognize a defined antigen without the need for presentation by the Human Leukocyte Antigen (HLA) system. T cells are first obtained from the patient by leukapheresis, and then processed *in vitro* with a viral vector to introduce the CAR. The fusion protein CAR consists of several domains, which associates an antibody fragment, capable of antigen recognition, and a T-cell activation domain. CART cells have drastically changed the prognosis of several cancers, especially in hematological malignancies [61]. Indeed, clinical trials revealed that patients suffering from refractory or relapsing lymphoid malignancies such as lymphoma [62], leukemia [63] or myeloma [64], could achieve a complete and sustained remission after treatment. Yet, even though these therapeutics were first designed to treat cancer, several teams have shifted their use to noncancer related antigens, which may be of interest in the field of transplantation [65]. For instance, MacDonald *et al.* [66] provided evidence that regulatory T cells engineered with CAR targeting the HLA A2 antigen was feasible and efficient. They showed that HLA A2 CAR-Treg interacted specifically with the A2 antigen, with minimal cytotoxic effects, and a preserved Treg phenotype and function.

In the specific context of organ regeneration, two teams have recently revolutionized the field of CAR-T based technologies. First, Aghajanian *et al.* [67] engineered T Cells with CAR targeting the Fibroblast Activation Protein (FAP), which is strongly expressed in cardiac fibroblasts in fibrotic hearts. In a mouse model of heart fibrosis, the administration of FAP-CAR T cells significantly reduced the myocardial fibrosis, compared with the control group. Secondly, Amor *et al.* [68] presented their data on the use of CAR-T cells as senolytics. There is a strong interrelationship between fibrosis and senescence in SOT. Senescent cells accumulate with aging in the transplanted organ and behave in a senescence-associated secretory phenotype. They secrete proinflammatory chemokines and cytokines, which accelerate graft fibrosis. There is a global interest in the development of senolytic agents and evidence exists that they may promote the survival of organs after transplantation [69]. Amor *et al.* [70] investigated whether CAR-T based technologies could serve as senolytic agents. They first identified the urokinase-type plasminogen activator receptor (uPAR) as a target of interest, being markedly upregulated in senescent cells. uPAR CAR T cells could efficiently target senescent cells *in vitro*, and achieve *in vivo* an effective clearance of senescent cells in a mouse model of hepatic senescent cells. Furthermore, in a mouse model of liver fibrosis, the administration of uPAR CAR T cells produced a significant reduction of liver fibrosis. These results may be game-changing; however, the translational prospects of these therapeutics should be balanced by the costs of the method and above all the potential side effects and risks induced by CAR-T cells. CAR-T-cell therapy-related Cytokine release syndrome and encephalopathy syndrome are frequent and sometimes have life-threatening adverse effects [71]. These side effects can be accepted in a risk/benefit ratio of a lethal malignant disease; however, in the context of SOT a safer alternative for graft failure is often proposed. Fortunately, the technology is constantly evolving and the development of inducible suicide mechanisms are currently explored to avoid the progression of potentially lethal adverse effects [72,73].

Conclusions

Cellular therapy with MSCs, mitochondrial biogenesis, rejuvenation with fresh cells after decellularization and recellularization, and CAR T-cell therapy are potential avenues to reverse or attenuate damage and fibrosis in transplanted organs. Each of the approaches described herein show beneficial effects in preclinical studies and need to be confirmed in pivotal clinical trials, which will ultimately bring pre-emptive regenerated organs closer to reality.

Authorship

MM and JKC: wrote the paper and made the critical revisions.

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Conflict of interest

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