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Review

ORGAN THERAPEUTICS DURING *EX-SITU* DYNAMIC PRESERVATION. A LOOK INTO THE FUTURE

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Summary

Machine perfusion has transformed the field of organ preservation permitting longer preservation and open a new avenue for graft treatment. As we are entering an era of "precision medicine", the organ transplant field is becoming equipped with the tools necessary to personalize and optimize organs designed specifically to withstand injurious pathways that occur during transplantation. Here we highlight recent progress using different treatment strategies during *ex-situ* perfusion. In the future, customized graft therapy will create a reality where organs will be optimized, personalized, and likely be available on demand.

Key words: gene therapy, RNA interference, CRISPR-Associated Protein 9, gene editing, organ preservation, cell therapy, nanoparticles

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Abbreviations

AAV: adeno-associated virus; ASO: antisense oligonucleotides; CAR: chimeric antigen receptor; DCD: donation after circulatory death; EVs: extracellular vesicles; HCV: hepatitis C virus; HLA: human leukocyte antigens; ICAM-1: intercellular adhesion molecule 1; IRI: ischemia reperfusion injury; MAPc: multipotent adult progenitor cells; MHC: major histocompatibility complex; mRNA: messenger ribonucleic acid; MSCs: mesenchymal stromal cells; NPs: nanoparticles; PEG: polyethylene glycol; PLGA: poly lactic-co-glycolic acid; RNAi: ribonucleic acid interference; siRNA: small interfering ribonucleic acid; Treg: regulatory T cells

INTRODUCTION

The advent of dynamic organ preservation by means of machine perfusion promises to revolutionize organ transplantation practice, not only by facilitating the successful and uncomplicated transplantation of grafts procured from high-risk donors [1,2], but also by placing the field at the crossroad with precision and regenerative medicine[3].

Indeed, a wealth of evidence has consolidated the notion that machine perfusion preserves grafts at higher risk of post-transplant complications, or failure, better than conventional static cold storage, regardless of perfusion temperature [4-6]. Additionally, dynamic preservation strategies provide the means to objectively evaluate grafts quality before transplantation, allowing

for an informed decision when evaluating whether accepting a high-risk graft [7].

More importantly, by recirculating a perfusate to an isolated organ, *ex-situ* machine perfusion is the perfect platform for the selective delivery of therapeutics to the graft to ameliorate the sterile inflammation of ischemia-reperfusion injury (IRI) during organ transplantation [8], as well as to modulate the immune system, foster organ repair, and promote tissue regeneration. Indeed, ex-situ preservation offers a unique window of opportunity during which tailored treatments can be administered to the single graft, reducing off-target therapeutics delivery, dosage, side effects, and toxicity of therapeutic interventions. This is an unprecedented and clinically relevant innovation because organs that are considered too damaged for transplantation during *ex-situ* preservation are currently being turned down, fuelling the ever-widening gap between transplant need and organ donors offer. Therefore, effective therapeutic interventions during *ex-situ* organ preservation have the potential to expand the pool of transplantable organs by tapping into a source of grafts currently unutilized. If replicated for the preservation of other organs, recent progress in liver normothermic machine perfusion (NMP) allowing to preserve human grafts for multiple days up to one week [9], suggests not only that clinically meaningful organ repair and regeneration will be attained *ex-situ* in a near future, but also that response to treatment could be evaluated on the perfusion device.

In this fast-paced, novel niche of research in organ preservation and regeneration by means of machine perfusion, several proof-of-concepts have already been produced in pre-clinical settings, ranging from pharmacological interventions to genetic modulation and editing, nanotechnology, or cell-based therapy. This review compiles current evidence supporting the feasibility and preliminary results of therapeutic interventions during *ex-situ* dynamic organ preservation (Fig. 1) while providing an outlook on the future direction of this novel, exciting, pioneering age in organ preservation and transplantation.

PHARMACOLOGICAL INTERVENTIONS

Pharmacological agents of various classes can be added to the standard perfusate composition during *ex-situ* dynamic organ preservation, with the aim to interfere with the downstream signalling cascade of IRI and reduce inflammation, to ameliorate graft microcirculation, or to improve organ quality. Pharmacological interventions that tackle shared IRI damaging pathways can be applied to different organs, whereas others can be envisioned specifically for single tissue types, to improve organ specific conditions or pre-retrieval damage (i.e., hepatic steatosis and pulmonary oedema). The feasibility of some pharmacological interventions during *ex-situ* organ preservation has already been proven in pre-clinical models, whereas others show potentials for application in organ transplantation but have not been tested yet [10,11].

Anti-inflammatory agents can enhance the protective effect of *ex-situ* dynamic organ preservation by further curbing the inflammatory response during perfusion. Agonists of the adenosine receptor A2 mediate anti-inflammatory effects, the protective role of which is investigated in porcine models of *ex-situ* lung perfusion and transplantation, showing significant reduction of the inflammatory response and pulmonary oedema, with overall improved oxygenation after transplantation [10]. In a porcine donation after circulatory death (DCD) liver model, the addition of a combination of several anti-inflammatory drugs further lowered the perfusate levels of hepatic injury markers, as well as of tumour necrosis factor alpha and interleukin 6, while increasing the perfusate concentration of the anti-inflammatory interleukin 10 [12]. However, there was no significant improvement of readouts after transplantation during a 3 days follow-up [12]. To date, these approaches have not reached clinical application yet, but it is important to acknowledge that the dynamic of the inflammatory response during *ex-situ* preservation of organs is not completely understood, and it is unclear if further reduction of this response during perfusion is needed or beneficial. Indeed, the inflammatory response has a wide range of roles that reach beyond the mere defence from pathogens and injury and include promoting restoration of homeostatic control and tissue regeneration [13]. Therefore, fundamental gaps in our current understanding of the effect of dynamic organ preservation on inflammation should be addressed before moving to clinical application of anti-inflammatory therapies.

Damage to, or impairment of the microcirculation play an important role in IRI of transplantable organs, furthering ischemic injury and sustaining inflammation either because of imbalance between vasodilatation and constriction, or microthrombi formation [10]. Therefore, drugs with vasodilatory or thrombolytic effect may be advantageous during *ex-situ* organ perfusion. For instance, prostaglandin E1 [14] and prostacyclin [15] were investigated in rodent models of *ex-situ* liver perfusion and transplantation, both showing a significant reduction in the release of markers of hepatic injury, higher bile volume, and improved survival. In a porcine model of *ex-situ* lung perfusion, treatment with urokinase improved pulmonary vascular resistance and oxygenation, while reducing pulmonary oedema. Additionally, the infusion of tissue plasminogen activator during machine perfusion rescued and allowed the uncomplicated transplantation of human lungs that were initially declined because of pulmonary embolism [16], whereas its utilization during NMP in a rat model of DCD liver donation showed a significant reduction of the

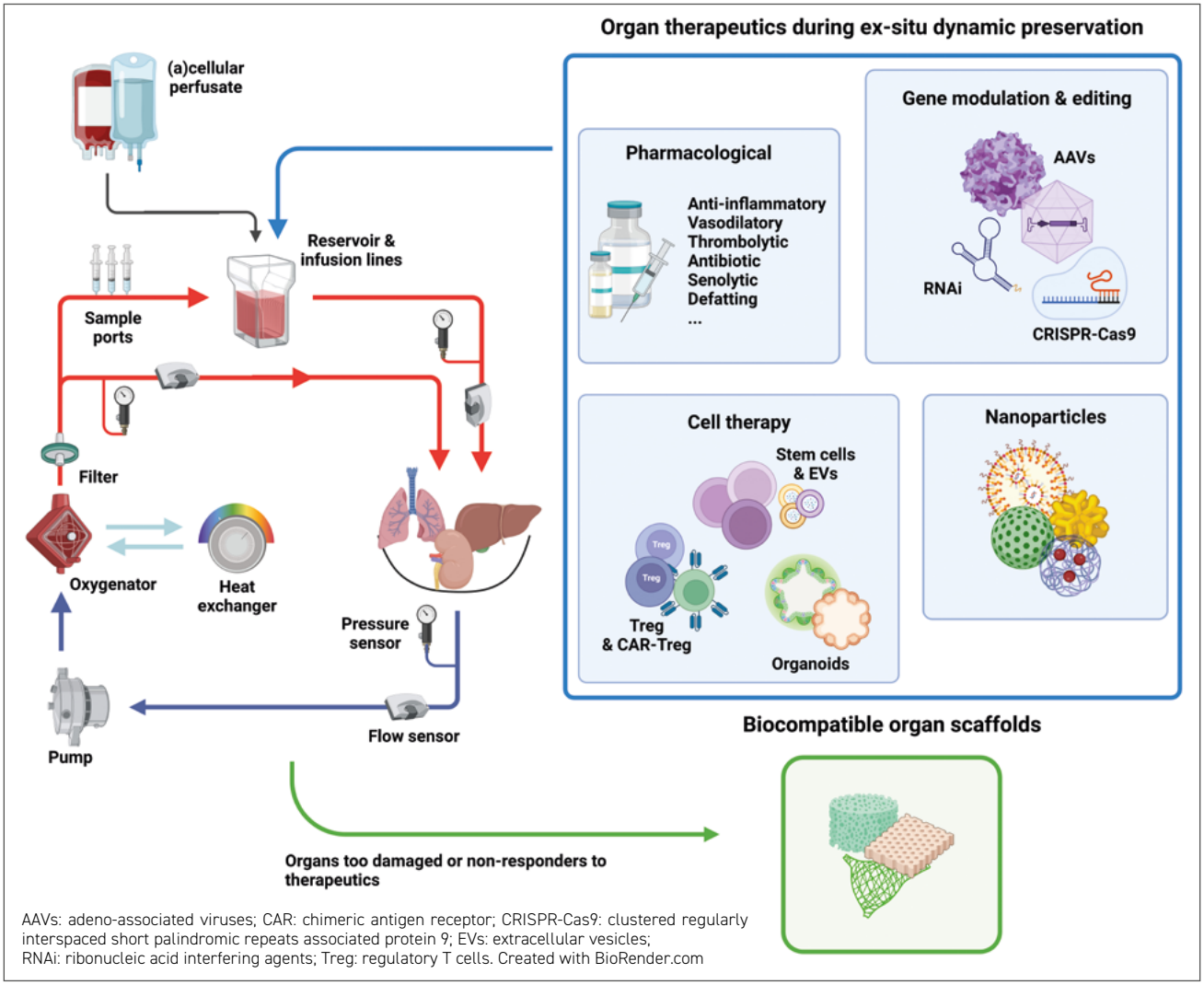

Figure 1. Overview of currently investigated therapeutic interventions during *ex-situ* dynamic organ preservation. The minimal set-up for *ex-situ*, dynamic organ preservation is composed by tubing lines and cannulas connecting the organ vasculature to at least one pump unit (either peristaltic or centrifugal), one oxygenator, filter(s), and a reservoir. An (a) cellular perfusate is recirculated trough the organ placed on a receptacle, preserving, and maintaining sterility. A heat exchanger connected to the oxygenator allows for hypothermic (4-10°C) or normothermic (37°C) *ex-situ* preservation, as well as controlled thermic transition from cold to warm perfusion during preservation in some commercially available devices. Pressure and flow sensors along the perfusion lines allow for hemodynamic monitoring, whereas sample ports or infusion lines allow sampling of the perfusate for graft quality evaluation, as well as targeted delivery of organ therapeutics. Pharmacological agents are used to tackle injurious pathways occurring during organ transplantation or to treat organ specific conditions, (i.e., hepatic steatosis). Gene modulation and editing is a recently developed approach in initial phase of exploration, whereby specific pathologic pathways are temporarily suppressed with transient inhibition of messenger RNA and corresponding protein levels. Nanoparticles are used to deliver agents for gene modulation and editing, as well as immunosuppressive drug directly to the graft, thereby avoiding systemic adverse effects. Additionally, nanoparticles interact directly with immune effector cells and exert direct antioxidant effects. Different stem cells populations and their paracrine secretory products can be used to reduce inflammation, modulate the immune response, and promote regeneration, with many proof-of-concepts already available. Recently, wild type or chimeric antigen receptor carrying regulatory T cells are being investigated to modulate allorecognition and foster pro-tolerant changes from the start of the transplantation process. Organoids are also gaining traction to promote organ repair and regeneration during *ex-situ* organ preservation. Finally, *ex-situ* dynamic preservation can facilitate the creation of biocompatible scaffolds from human organs that are too damaged or do not respond to therapeutic interventions. The repopulation of these scaffold with stem cells or cells derived from individual patients has the potential to provide in the future personalized organs for transplantation, available on demand.

histological damage to the peribiliary vascular plexus and biliary mural stroma [17].

The transmission of infectious pathogens from donor to recipient is a concrete threat to the outcomes after transplantation of all solid organs, as well as a potential reason for declining a graft for transplant. Targeted antimicrobial drugs delivered during *ex-situ* dynamic preservation have therefore the potential to treat microbial infections before implantation, thereby rescuing organs and expanding the donor pool. This may be particularly true in the case of long-term, *ex-situ* preservation for multiple days, during which repeated treatment and evaluation of response to therapy can be theoretically performed. Nevertheless, since devices for *ex-situ* preservation for multiple days will likely include a dialysis unit [9], pharmacokinetic studies, in close cooperation with microbiologists, are required to define the best approach for *ex-situ* antimicrobial therapy. To date, antimicrobial agents have been used to treat multi-resistant bacterial, and fungal infections of human lungs during *ex-situ* NMP, showing significant reduction of bacterial load in bronchoalveolar lavage fluid already after 6 and 12 hours of perfusion, and complete microorganism eradication in 4 of 18 perfused lungs, which were then transplanted without infectious complications [10]. This proof-of-concept provides the rationale to envision the treatment of multi-resistant microbiological infections before implantation also during *ex-situ* preservation of shorter duration (i.e., less than a day), an approach that can be replicated for all transplantable organs.

Drugs targeting senescent cells, also referred to as senolytics, have been recently proposed as potentially beneficial to treat the consequences of IRI. Senescent cells present a unique profile of cell-cycle arrest and resistance to apoptosis, associated with pro-inflammatory secretory profile, that has been found associated with age-related organs dysfunction. Additionally, IRI during organ transplantation induces cellular senescence. Senolytics revert apoptosis resistance and the pro-inflammatory secretory profile of senescent cells, and their utilization in the setting of organ transplantation is being investigated in recent years in preclinical models. Significant reversal of cells senescence pre-transplantation can be envisioned during *ex-situ* organ preservation and may be particularly advantageous for the reconditioning of grafts procured from elderly donors. Therefore, the role of senolytics therapy during *ex-situ* organ preservation should be investigated in future research.

Excessive lipid deposition in the liver parenchyma, or hepatic steatosis, is a condition frequently encountered in potential organ donors, especially in western countries due to the current obesity pandemic. Steatotic liver grafts are more vulnerable to IRI and, although mild to moderate steatotic livers (up to 60% of parenchymal involvement) are currently utilized for transplantation, an increased incidence of post-transplant complications is a toll that often is paid with their utilization. In contrast, severely steatotic livers (> 60% of parenchymal involvement) are usually not considered for transplant since they have been historically associated with unacceptably high rates of primary graft non-function [18]. Therefore, reducing the hepatic lipid content with pharmacological interventions during *ex-situ* preservation is an approach that has gained considerable traction. Although the identification of effective interventions would likely require first a complete understanding of adipogenesis and lipolysis in the context of *ex-situ,* isolated liver perfusion, defatting cocktails have already been tested in pre-clinical models of liver NMP, with promising results. Liver NMP alone has been shown to reduce hepatic steatosis at histology when utilized for 48 hours in a porcine model [19], but the addition to the perfusate of multiple defatting agents during rat liver NMP led to a significant reduction in hepatocellular lipid content already after 3 hours of perfusion [20]. Similarly, defatting agents administered during NMP of steatotic, discarded human livers decreased hepatocytes triglyceride content and macrovesicular steatosis at histology within 6 hours of perfusion [21]. Whether the reduction in hepatic lipid content achieved with this *ex-situ* defatting strategy translates in increased tolerance to IRI and effectively reduces of post-transplant complications remains to be evaluated. Unfortunately, some of the compounds utilized in these pre-clinical studies are not approved for clinical applications and research effort are now focusing on identifying valid alternatives. Additionally, with the advent of prolonged liver NMP for multiple days, it remains to be determined if defatting cocktails are necessary, since it seems reasonable to assume that clinically relevant reduction of hepatic steatosis can be achieved with prolonged perfusion alone.

GENE THERAPY STRATEGIES IN ORGAN TRANSPLANTATION

Among many approaches to ameliorate immune activation of the graft [22], gene therapy is very appealing because it can specifically target pathways [23,24], by treating the donor or the graft. Genetic manipulation of donor organs may render grafts more resistant to IRI, reduce immunogenicity and the requirement for systemic immunosuppression, thus promoting long-term graft survival [25]. The combination of gene therapy/modulation during *ex-situ* organ preservation is relatively new but very promising because it offers a controlled environment and avoid systemic therapy [10].

There is also a need to optimize grafts by gene therapy strategies as a result of the organ shortage. This shortage pressured the transplant community to use high-risk grafts and even explore possibilities of using organs of other species [26,27]. These scenarios highlight the potential for gene therapy and/or modulation strategies in transplantation to prevent organ ischemia, prevent rejection, induce tolerance, and expand organ supply.

Gene therapy delivery strategies

The main limitation of gene therapy is gene delivery [28]. The therapeutic efficiency of gene therapy is based on the efficacy of its delivery approach. *Ex-situ* organ preservation seems to improve the delivery of genetic therapies. There are numerous strategies to deliver gene therapies including viruses (e.g., adenovirus, lentivirus, and adeno-associated virus (AAV)) as well as nonviral vectors (e.g., extracellular vesicles, nanoparticles, cell-penetrating peptides, cationic lipids, conjugates, and polymers). Though viral vectors tend to exhibit greater transduction efficiency compared to nonviral vectors, concerns about viral gene therapy include mutagenesis at the site of gene insertion [29], which may cause uncontrolled transgene expression. Additional concerns include tissue tropism, gene size intended for delivery, as well as potential of viral infection triggering rejection [30], though this risk is minimal.

The use of AAVs, in particular, is a promising strategy for therapeutic gene delivery. For example, AAV was used for genetic load delivery during *ex-situ* dynamic preservation prior to implantation in a rodent liver transplant model, with preliminary results demonstrating that AAVs can be used to deliver a variety of gene-editing technologies (e.g. CRISPR/CAS) during *ex-situ* preservation [31]. Clinically, AAVs are promising because of their sustained duration of effect, with several clinical trials ongoing to treat a variety of human diseases [32]. Though neutralizing antibodies exist against several AAV serotypes in humans, their prevalence in serum is low, making AAV an appealing delivery method for gene therapy [33]. On the contrary, engineered adenoviruses efficiently transduce human cells in the lab, but wild-type variants can also infect people. Indeed, nearly 60% of some populations are seropositive for recombinant adenoviruses, with some individuals exhibiting adenoviral-deactivating antibodies [34]. The development of non-immunogenic gene delivery vehicles for durable host genome integration is an area of exciting exploration.

Gene therapy and gene silencing strategies in organ transplantation

Several gene therapy strategies have been explored in cases of allotransplantation as well. For example, studies have used adenoviral vectors encoding human interleukin-10 during *ex-situ* preservation of donor lungs in both discarded human and porcine models to inhibit pro-inflammatory cytokine secretion and promote improvement in lung function prior to transplantation [35,36]. Gene therapy strategies in allotransplantation can be potentially used to correct genetic deficiencies, inborn errors of metabolism, or clotting disorders that are associated with an increased risk of graft loss [37].

Gene silencing strategies have also been implemented in organ transplantation to modulate gene expression at the messenger RNA (mRNA) and protein level. Specifically, RNA interference (RNAi) is a powerful, clinically established therapeutic technology which enables repression of disease-associated or overexpressed genes, by knocking down the level of target mRNA and thus subsequent protein. The first ever RNAi drug to treat polyneuropathy caused by hereditary transthyretin amyloidosis received FDA approval in 2018, and several clinical trials using RNAi drugs to treat a variety of human diseases are ongoing [38]. RNAi therapies, specifically in the form of small interfering RNAs (siRNAs) can be chemically modified for enhanced stability, specificity, and potency, with a robust duration of effect for up to 6 months following a single systemic injection [39,40]. Delivering RNAi therapeutics during the transplantation process is an attractive method of organ protection for their ability to directly treat a procured graft during *ex-situ* preservation without the need for systemic therapy, their high specificity with minimal off-target effects, and their ability to be administered without the need for viral transfection agents [41]. The latter eliminates concerns of immunogenicity associated with the transfection agent itself and is an important consideration in the context of transplantation.

The application of RNAi-based therapies has recently been investigated to modulate alloimmune responses before and after transplant, to reduce graft injury and induce donor-specific tolerance. In addition to administering RNAi therapeutics during *ex-situ* machine perfusion, groups have demonstrated the feasibility of delivering siRNA in the preservation solution itself. In one such study, a cocktail of unmodified siRNA targeting TNF alpha, Fas, and complement C3 was administered to the heart in a syngeneic model of mouse heart transplantation as part of the preservation solution. After 48 hours, siRNA-treated hearts were transplanted into syngeneic recipients and demonstrated sustained beating for > 100 days (whereas controls lost function within 8 days), improved histology, and diminished neutrophil and lymphocyte accumulation [42]. This was one of the first studies to demonstrate that delivery of siRNA in the preservation solution is feasible and can effectively repress target mRNA expression to protect cardiac function and prolong graft survival against IRI [42]. Other groups have since tested the delivery of a siRNA cocktail (targeting complement C3, RelB, and Fas) in a similar mouse model of syngeneic kidney transplantation, highlighting the feasibility and clinical potential of delivering siRNA-based therapies during the preservation period of donor organs [43].

The first use of an antisense oligonucleotides (ASO) as a gene modulatory agent in organ transplantation was in 2017 when an ASO targeting miRNA-122 (Miravirsen) was delivered in a porcine model of *ex-situ* liver machine perfusion [44]. miRNA-122 was selected as a target for ASO-mediated knockdown for its high expression in hepatocytes and because its presence allows for hepatitis C virus (HCV)

replication. *In vitro* data confirms ASO-mediated repression of HCV replication during machine perfusion as a proof-of-concept, although it is unlikely that Miravirsen will be implemented in the clinic given high efficacy of current HCV antiviral regimens. Several other groups have since investigated the use of gene modulation strategies during the liver transplantation process to target components necessary for viral replication and genes implicated in IRI, such as those involved in inflammation, oxidative stress, and cell death. Numerous RNAi strategies have been tested experimentally in several transplantable organ animal models involving the liver, kidneys, heart, and lungs, and thoroughly reviewed elsewhere [10,45]. However, the utilization of RNAi during machine perfusion is very new and the available literature is very limited. Gillooly et al. first demonstrated the feasibility of delivering siRNA during *ex-situ* machine perfusion [46]. This group delivered unmodified siRNA targeting the apoptotic Fas receptor during *ex-situ* dynamic liver preservation under both hypothermic and normothermic conditions, and also tested it in a rat liver transplant model [47]. It must be acknowledged, however, that in cases where gene therapy is applied in the cold, the metabolic function of an organ may limit uptake, requiring higher doses or longer perfusion periods. The use of machine perfusion at physiologic conditions may therefore serve as a more effective platform for both gene therapy and RNAi-based drug delivery. In another study, Cui et al found that nanoparticles (NPs) (poly(amine-co-ester)) loaded with siRNA targeting major histocompatibility complex (MHC) II molecules and delivered via *ex-situ* perfusion decreased endothelial cell MHC II expression for up to 6 weeks, accompanied by decreased graft T cell infiltration and activation [48]. Thus, NPs may serve as a platform for RNAi-based drug delivery during *ex-situ* dynamic preservation to reduce allograft transplant injury and promote organ function and survival, at least in the short-term post-transplant period.

Though RNAi therapeutics have been investigated experimentally as a way to protect grafts against virus replication, rejection, and IRI, their implementation in the clinical setting, particularly during organ transplantation, has not yet occurred. RNAi-based therapeutics are dosed based upon weight. Thus, large quantities are likely required to reach therapeutic effects in both *ex-situ* and *in vivo* models. The specificity and duration of effect of RNAi-based therapeutics, as determined by chemical conjugate, backbone, and delivery strategy, is nevertheless exciting as it eliminates concerns for major off-target effects and permanent gene modulation, especially when the targets of IRI, for example, are involved in maintenance of homeostasis with numerous overlapping cellular signalling pathways. Transient repression of gene and protein expression, therefore, may sufficiently regulate immune responses while preventing potential toxicity and adverse effects of prolonged homeostatic signalling repression. The transient nature of mRNA silencing seen with RNAi therapeutics on the order of weeks to months is appealing during the transplantation process, where graft function within the first year following transplantation determines long-term success.

NANOTECHNOLOGY

The application of NPs in transplantation represents a new strategy to mitigate the inevitable damaging effects of IRI that lead to graft dysfunction, to prevent adverse side effects of immunosuppressive therapy, and to rehabilitate high-risk grafts [49].

The attraction of NPs is attributed in large part to their unique physiochemical properties, such as their small size, stability, and ability for tailoring with various functionalities [50]. By modulating properties such as composition, stability, responsivity and surface properties, NPs can be tailored to prolong circulation lifetimes, protect payloads from environmental and cross biological barriers of systemic, microenvironmental, and cellular milieu, and selectively enhance accumulation at specific sites of interest [50].

The NPs for clinical use are composed by natural/organic materials, such as biodegradable polymers, lipids utilized to encapsulate hydrophobic drugs in liposomes and micelle constructs, or inorganic materials (such as gold, iron oxide, quantum dots, etc.). Each class has numerous broad advantages and limitations regarding cargo, biocompatibility, and delivery system. Polymer-based NPs are ideal candidates for drug delivery because they are biodegradable, biocompatible, biomimetic, and stable during storage. NPs are an attractive carrier for immunosuppressive drugs and delivery in a targeted manner to induce transplant tolerance while avoiding systemic toxicity. Lipid-based NPs are most common class of FDA-approved nanocarriers [51] widely used for the delivery of nucleic acids or siRNA, and offer many advantages including formulation simplicity, biocompatibility, high bioavailability, and a range of physicochemical properties that can be controlled to modulate their biological characteristics [52]. In particular, liposomes, biocompatible spherical vesicles having at least one lipid bilayer, are used to encapsulate hydrophobic and hydrophilic drugs and often include surface modifications to extend their circulation and enhance delivery [52]. Liposomes can deliver immunosuppressive drugs, such as cyclosporine or tacrolimus [53], and RNAi [45] to the allograft. Inorganic materials (i.e., gold, iron) have been used to synthesize nanoparticles for drug delivery and imaging applications. Due to the properties of the material itself, these NPs have unique physical, electrical, magnetic, and optical properties for applications such as diagnostics, imaging, and photothermal therapies. Although they have good biocompatibility and stability, many inorganic NPs are limited in their clinical application by low solubility and toxicity. At the moment, iron

oxide NPs are the most studied FDA-approved, inorganic NPs [51].

In transplantation, the research is focusing on the possible use of NPs in both the recipient or the graft, during *ex-situ* preservation, as carriers of immunosuppressive agents or compounds for graft repair and/or protection against reperfusion injury (Tab. I), avoiding many of the limitations associated with drug systemic administration in recipients [49,54]. In fact, NPs can be delivered systemically to improve drug release kinetics, avoiding drug-induced toxicity, or in organ targeted delivery to localize the drugs into selected organs, in particular in combination with machine perfusion, plausibly allowing to recover high-risk organs that are more vulnerable to IRI. The use of appropriately modified NPs able to recognize the target organ and carry therapeutic agents has advantages over systemic therapy, such as the use of lower dosages, reduced systemic side effects, localized and controlled drug delivery and improved convenience and patient compliance [50].

The potential of systemically administered immunosuppressive NPs is to provide a sustained drug release, to modulate rejection avoiding systemic drug-induced toxicity. The intrinsic properties of NPs such as composition, size and surface charge, significantly influence their interaction with immune cells, including macrophages, antigen presenting cells, B cells or T cells, and exhibit an array of immunosuppressive effects [55]. Direct effects of carbon nanomaterials include the upregulation of transforming growth factor-β, interleukin-10, and decreased B cell activity [56]. Metal-oxide nanoparticles can directly affect adaptive immune cells, and nanoparticles of cerium oxide are powerful antioxidant agent with therapeutic properties in experimental liver disease and transplantation [57-60].

In an *in vivo* model of liver transplantation, PEG-NPs loaded with tacrolimus administered systemically were associated with longer retention time in plasma and prolonged graft survival, as compared to standard drug formulations [61]. Similar findings were also noted for cyclosporine, using poly lactic-co-glycolic acid (PLGA) based NPs in the liver [53]. Coating NPs with PEG on the surface is an optimal strategy to improve NP stability, prolong blood circulation half-life and reduce interactions with biological tissues and fluids [62]. Graft treatment before transplantation by delivery of therapeutics directly into donor graft is a strategy to reduce local injury, inflammation, allopresentation, and the harmful side effects associated with their systemic counterparts.

Table I. Overview of studies investigating the feasibility and efficacy of nanoparticles therapy during *ex-situ* dynamic organ preservation.

Study	Species	Organ	Transplant model	Injury	Type and duration of machine perfusion	Nanoparticle type	Effect
Tietjen et al. (2017) [84]	Human	Kidney (discarded)	No	CIT 13-26 hours	NMP, 4-8 hours	anti-CD31 polymeric NPs	↑ intravascular accumulation
Zhang et al. (2022) [85]	Rabbit	Kidney	No	WIT 35 minutes	HMP, 4 hours	amphiphilic chitosan derivatives micelle	↓ oxidative stress ↑ antioxidant defences antimicrobial activity ↓histological lesions (oedema, congestion) No effect on apoptosis
Del Turco et al. (2022) [93]	Human	Liver (discarded)	No	CIT 9-14 hours	NMP, 4 hours	Cerium oxide NPs	↑ antioxidant defences (glutathione, SOD and catalase assay) ↓ tissue mtDNA4977 deletion Rescue of mitochondrial phenotype, ↓lipid droplet peroxidation and lipofuscin granules No effect on perfusate concentration of pro-inflammatory cytokines

CIT: cold ischemia time; HMP: hypothermic machine perfusion; NMP: normothermic machine perfusion; NPs: nanoparticles WIT: warm ischemia time

The delivery of immunosuppressant-loaded NPs targeted to recognize specific receptors or antigens on dendritic and endothelial cells (ECs), involved in alloimmune response during reperfusion and graft rejection, represents an attractive approach to reduce side effects of systemic therapy in transplantation. The study of Nadig et al. has demonstrated that micelle NPs containing rapamycin (inhibits effector T-cells and protects the endothelium) targeted for ECs with the amino acid sequence Arg-Gly-Asp confer local immunosuppressive effects, and reduce inflammation and ECs oxidative stress, without systemic side effects [63]. This study demonstrates that targeted NPs containing immunosuppressant may positively alter the alloimmune response, counteract inflammatory processes, and may be applied to the pre-transplant preservation phase.

IRI is a multifactorial process involving oxidative stress, inflammation, immune activation and cellular death, all affecting allograft function [8]. Therefore, drug-loaded NPs delivery to the graft prior to transplantation by means of *ex-situ*, hypo- or normothermic, dynamic preservation could represent a strategy to alleviate the detrimental effects of IRI and render organs more resistant to reperfusion injury after transplantation. ECs express MHC molecules and are the first encountered by recipient lymphocytes upon graft reperfusion [64]. Therefore, ECs are the primary targets of IRI and preformed donor antibodies [65]. As such, the delivery by NMP of drugs that act directly on ECs is an attractive target for transplant therapeutics [66]. The conjunction of anti-CD31 antibodies to polymeric NPs surface enhanced the targeting of NPs to ECs of human kidney grafts during NMP [67], highlighting therapeutic potential for targeted nanomedicines delivered during *ex-situ* dynamic organ preservation. Additionally, PLGA-NPs conjugated with antibodies targeting the intercellular adhesion molecule 1 have been used to reduce graft immunogenicity [68], and similar approaches could be used to target MHC-II molecules on allograft ECs.

The delivery during hypothermic machine perfusion of NPs micelles containing an activator of mitochondrial acetaldehyde dehydrogenase 2, a key enzyme involved in protection against tissue injuries of various origin, ischemia included, reduced the ischemic damage and improved the function of high-risk, controlled, DCD kidneys donation [69].

Oxidative stress plays a key role in IRI, and antioxidant treatments include increasing endogenous antioxidants, supplementation of exogenous antioxidants, and strategies to reducing oxidative stress. Antioxidant molecules have poor water solubility, short biological half-life, and are subject to non-specific removal by the vascular endothelial and the mononuclear phagocytosis system, affecting their use in clinical applications [70]. Encapsulations of antioxidants into NPs could represent a potential solution to these problems. It has been demonstrated that the use of antioxidant alpha-tocopherol during hypothermic dynamic preservation in a DCD rodent model [71] improves liver graft preservation, limiting mitochondrial oxidative stress and inflammation. The use of cerium oxide nanoparticles, already known as antioxidant and anti-inflammatory agents [72,73], and NPs containing carnosic acid, a natural antioxidant, counteracted hepatic IRI by scavenging reactive oxygen species and ameliorating the pro-inflammatory response in animal models of hepatic ischemic injury [57,74], suggesting their future use as a prophylactic agent for the treatment of IRI during liver transplantation. Similar results were obtained in murine models of IRI with PEGylated bilirubin NPs [75]. Recently, we demonstrated that cerium oxide NPs are internalized by liver cells during NMP of human discarded livers, confirming that NMP is an optimal platform for NPs delivery. The administration of cerium oxide nanoparticles decreased oxidative stress, upregulating graft antioxidant defenses such as glutathione levels, superoxide dismutase, and catalase activity [60]. The coexistence of both Ce^{3+}/Ce^{4+} ions on their surface enables these NPs to buffer reactive oxygen species without being consumed, providing long-term antioxidant effects compared to the shorter half-life of classic antioxidants [76]. Therefore, these NPs could represent an antioxidant strategy aimed at protecting the liver graft against IRI, and be a tool to improve graft quality during NMP.

Another effective approach for attenuation of oxidative stress during IRI may be the delivery of antioxidative genes to increase the levels of antioxidant enzyme expression. Mice pretreated with NPs containing gene plasmid for superoxide dismutase and catalase provided elevated antioxidative enzyme activity as the result of the gene delivery in the liver and protection against hepatic IRI [77].

Although the use of NPs during preclinical studies of *ex-situ* NMP have demonstrated a great potential to expand their use *in vivo*, some shortcomings, such as potential toxicity, off-target accumulation, long-term effects, and final fate of NPs in recipients, require future research efforts to reach their successful translation to the clinical practice.

CELL THERAPY

Cell therapy harnesses the biological properties of specific cell populations to treat human diseases of various aetiologies. In the setting of solid organ transplantation, cell therapy has been historically attempted with mesenchymal stromal cells (MSCs) to modulate the immune response of the recipient, induce a pro-tolerant state, and reduce the need of immunosuppressive agents. More recently, regulatory T cells (Treg) have also gained traction as putative therapy to modulate allorecognition and promote tolerance.

MSCs are a population of non-hematopoietic, undifferentiated cells with self-renewing properties present virtually

in every adult tissue. MSCs differentiate *in vitro* into different cellular lineages [78], are relatively easy to expand in culture, and off-the-shelf products are already available. MSCs downregulate both innate and adaptive immunity [79], blunt inflammatory processes [80], and promote regeneration of damaged tissues [81]. As such, MSCs tackle all major pathophysiologic events occurring during IRI [8] of all transplantable organs. Although several animal studies have demonstrated that MSCs ameliorate cardiac, intestinal, hepatic, and renal IRI [82], the clinical translation of these approaches failed to deliver favourable results after organ transplantation. This may be partly explained by the fact that systemically infused MSCs are short-lived because they are sequestered in the lungs, where they are phagocytized by resident monocytes [83]. *Ex-situ* dynamic organ preservation has reignited the interest on cell therapy to improve post-transplant results because the recirculation of a perfusate deprived of leukocytes ensure cells delivery directly to the targeted organ.

Numerous animal and human preclinical studies have already shown promising results for lung [84,85], liver [86-92], and kidney [93-95] MSCs therapy, as well as multipotent adult progenitor cells (MAPc), during *ex-situ* preservation (Tab. II). Delivery of cells to the parenchyma was confirmed in some study, with visualization of cells in lung [96], liver [87,90], and kidney tissue [93,95], although migration from the vascular pole to, i.e., the peritubular space in the renal medulla or to the hepatocellular trabeculae was seldom observed [87,95]. Additionally, stem cell therapy during *ex-situ* dynamic organ preservation was already found to positively modulate the immune response of the recipient, to reduce inflammation, and to promote organ regeneration.

Thompson et al. perfused discarded human kidney pairs with NMP for 7 hours, with or without MAPc [95]. MAPc therapy significantly increased activity of indolamine-2,3-dioxygenase, which suppresses effector T cells and activate Foxp3-positive Treg. Additionally, MAPc secretome in the perfusate of treated kidneys was shown to significantly reduce the chemoattraction of peritoneal neutrophils in a mouse model, indicating, that MAPc exert immunomodulatory effects that may foster pro-tolerant changes when administered during NMP [95]. Cao et al. investigated the effect of MSCs delivery during NMP on the rate of acute cellular rejection in a rat model of DCD liver transplantation. Rat livers were preserved with NMP for 4 hours with or without the addition of MSCs and transplanted with or without immunosuppression [91]. Although with a follow-up of only 12 days, MSCs therapy during NMP significantly reduced the rate of acute cellular rejection compared to control, similarly to what achieved with post-transplant immunosuppression.[91]

A significant reduction in the inflammatory response during perfusion was observed during *ex-situ* stem cells therapy for lung [85], kidney [94,95], and liver [88] grafts. Indeed, a

Table II. Overview of studies investigating the feasibility and efficacy of cell therapy during *ex-situ* dynamic organ preservation.

Study	Species	Organ	Transplant model	Injury	Type and duration of machine perfusion	Stem cells type	Effect
Fang et al. (2014) [84]	Human	Lung (discarded)	No	CIT 10-64 hours	NMP, 4 hours	Human bone marrow derived MSCs	↑ alveolar fluid clearance
Borg et al. (2014) [85]	Human	Lung (discarded)	No	CIT 8 hours	NMP, 4 hours	MAPc	↓ cellularity and protein content in bronchoalveolar lavage fluid ↓ histological severity of inflammation
Gregorini et al. (2017) [93]	Rat	Kidney	No	WIT 20 minutes	HMP, 4 hours	Rat bone marrow derived MSCs	↓ markers of injury ↓ severity histological lesions
Martens et al. (2017)[109]	Pig	Lung	No	WIT 90 minutes	NMP, 6 hours	MAPc	↓ TNF-alpha, IL-1beta, IFN-gamma perfusate concentration No difference in lung oedema or histological severity of inflammation

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Table II. *continues.*

Study	Species	Organ	Transplant model	Injury	Type and duration of machine perfusion	Stem cells type	Effect
Stone et al. (2017) [110]	Mouse	Lung	No	CIT 60 minutes WIT 60 minutes	NMP, 1 hour	Human umbilical cord derived MSCs	↓ oedema ↑ increase dynamic compliance
Sasajima et al. (2018) [86]	Rat	Liver	No	CIT 4 hours WIT 30 minutes	NMP, 2 hours	Swine adipose derived MSCs	No effect on markers of hepatic injury No effect on inflammatory cytokines
Brasile et al. (2019) [94]	Human	Kidney (discarded)	No	CIT 29.4 ± 7.4 hours	NMP, 24 hours	MSCs	↑ ATP tissue concentration ↓ pro-inflammatory cytokines perfusate concentration ↑ perfusate concentration of growth factors and proliferation at histology
Thompson et al. (2020) [95]	Human	Kidney (discarded)	No	CIT 13-36 hours	NMP, 7 hours	MAPc	↓ urinary marker of injury Restoration medullar flow ↓ pro-inflammatory cytokines and ↑ IL-10 perfusate concentrations
Laing et al. (2020) [87]	Human	Liver (discarded)	No	CIT 8-13 hours	NMP, 6 hours	MAPc	↑ perfusate concentration of pro-inflammatory cytokines
Yang et al. (2020) [88,89]	Rat	Liver	No	WIT 30 minutes	NMP, 8 hours	Rat bone marrow derived MSCs	↓ perfusate concentration of markers of hepatic injury ↓ mitochondrial oxidative injury ↓ Suzuki score at histology
Cao et al. (2020) [88]	Rat	Liver	Yes	WIT 30 minutes	NMP, 4 hours	Rat bone marrow derived MSCs	↓ transaminase release at 14 days post-transplant ↓ cytokines release post-reperfusion ↓ Suzuki score at 14 days post-transplant ↑ survival at 14 days post-transplantation
Verstegen et al. (2020) [90]	Pig	Liver	No	WIT 30 minutes	HOPE, 1 hour	Human bone marrow derived MSCs	↑ perfusate concentration of IL-6 and IL-8 during 4 hours whole blood normothermic reperfusion
Cao et al. (2021) [91]	Rat	Liver	Yes	WIT 30 minutes	NMP, 4 hours	Rat bone marrow derived MSCs	↓ incidence of acute cellular rejection, effect similar to post-transplant immunosuppression ↓ markers of hepatic injury and Suzuki score post-transplantation ↑ survival at 14 days post-transplant
Sun et al. (2021) [92]	Rat	Liver	No	WIT 30 minutes	NMP, 6 hours	Rat bone marrow derived MSCs	↓ perfusate markers of hepatic injury and Suzuki score at histology ↓ lipidic oxidative stress and ferroptosis

ATP: adenosine triphosphate; CIT: cold ischemia time; HMP: hypothermic machine perfusion; HOPE: hypothermic oxygenated machine perfusion; IFN-gamma: interferon gamma; IL: interleukin; MAPc: multipotent adult progenitor cells; MSCs: mesenchymal stem cells; NMP: normothermic machine perfusion; TNF-alpha: tumour necrosis factor alpha; WIT: warm ischemia time

significant lower perfusate concentrations of typical pro-inflammatory cytokines were observed during kidney [95] and liver [88] NMP therapy with MAPc and MSCs, respectively. Borg et al. observed a significant reduction in inflammatory cells in broncho-alveolar lavage fluid and less severe inflammatory changes at histology at the end of *ex-situ* human lungs preservation supplemented with MAPc [85]. Furthermore, in a rodent study by Cao et al., MSCs therapy during NMP significantly downregulated the expression of interleukin 1 beta, tumour necrosis factor alpha, and interleukin 6 mRNA [88], whereas Thompson et al. observed a significant increase in the perfusate levels of the anti-inflammatory interleukin 10 with MAPc therapy during NMP of discarded human kidneys [95]. Overall, these preliminary findings indicate that stem cells delivery during *ex-situ* dynamic preservation effectively blunts the inflammatory response associated with IRI and provides a more favourable microenvironment during preservation by tipping the balance in favour of anti-inflammatory mediators.

The potential of stem cell therapy to promote tissue regeneration during *ex-situ* preservation has been explored in a single human preclinical study, in which discarded kidney pairs underwent 24 hours NMP [94]. MSCs therapy during *ex-situ* kidney NMP significantly increased the perfusate concentration of epidermal, fibroblasts, and transforming growth factors compared to untreated, matched controls. Additionally, histology after 24 hours of perfusion showed a significant increase in the number of proliferating renal cells. If replicated, these findings would indicate that MSCs therapy during *ex-situ* dynamic preservation promotes tissue regeneration relatively early during perfusion, and it is, therefore, plausible to assume that clinically meaningful organ repair and regeneration can be attained with prolonged dynamic preservation for multiple days [9].

An important question that needs answering is whether the immune-modulatory, anti-inflammatory, and pro-regenerative effects of stem cells therapy during *ex-situ* dynamic preservation are maintained also after organ transplantation and translate in significant improvement of clinical outcomes, especially after transplantation of high-risk grafts. To date, only two rat studies have transplanted livers after MSCs therapy during NMP [88,91], showing a significant reduction in transaminase and inflammatory cytokines release, severity of tissue damage at histology, and incidence of acute cellular rejection, with improved animal survival for up to 14 days after liver transplantation. These promising results suggest that MSCs therapy during *ex-situ* preservation may positively impact post-transplant results, but whether they will also improve long-term outcomes remains unknown.

Interestingly, the biological effects of stem cells were observed also when they were sequestered in the perfusion circuit or did not migrate out of the vascular space [87,93,94]. This observation suggests that stem cells effect during *ex-situ* preservation is mediated mostly by paracrine factors. These paracrine factors could be administered during *ex-situ* dynamic perfusion and replace the stem cells of origin, thereby avoiding the theoretic risk of immunogenic response and malignant transformation after cells engraftment. Among paracrine factors, Extracellular Vesicles (EVs) are particularly attractive. EVs are membrane-delimited particles secreted by stem cells that transfer bioactive molecules (lipids, proteins, and genetic information) to neighbouring or distant, target adult cells [97]. By interacting with adult cells, EVs change their biological processes and mediate the beneficial effects of the parental stem cells. Therefore, EVs are potential therapeutics candidate that can be used for cell-free organ therapy during *ex-situ* dynamic preservation. The feasibility of EVs delivery during machine perfusion has already been proven in rat models of liver NMP [98,99], as well as in discarded human lungs [100] and kidneys [101]. Rigo et al. showed that EVs derived from human liver stem cells are taken up by hepatocytes during 4-hours rat liver NMP, resulting in a significant reduction in transaminase release, as well as restoration of normal histology when compared to controls [98]. In a following study, the same group confirmed that these EVs reduce hepatocellular injury and enhanced liver regeneration at histology also in a rat model of DCD liver donation [99]. Gennai et al. investigated the effect of MSCs derived EVs therapy during *ex-situ* normothermic preservation of 30 human lungs discarded for transplantation [100]. Compared to controls undergoing NMP alone, MSCs-EVs reduced the inflammatory-induced pulmonary oedema by significantly increasing alveolar fluid clearance. Gregorini et al. took a different approach, performing MSCs-EVs therapy during 4-hours, *ex-situ*, hypothermic dynamic kidney preservation in a rat model of DCD donation [93]. Interestingly, some beneficial effects of EVs were observed during cold perfusion as well, such as a reduction of markers of renal injury, oxidative stress, and severity of histological damage. If reproduced, these findings would open a new appealing venue for cell free therapy during *ex-situ* organ preservation, as they suggest that cell-derived therapeutics can be administered in the cold as well. In a not-so-distant future, paracrine factors and EVs can be envisioned as a simpler, and, perhaps, safer therapeutic option to replace stem cell therapy to rescue grafts that are already too damaged for transplantation at the time of *ex-situ* preservation. Additionally, in the context of partial liver transplantation, paracrine factors and EVs can be envisioned as a supporting therapy to enhance the regeneration of small size grafts and prevent post-transplant failure. Nevertheless, because the purification of EVs is currently a lengthy process with only moderate efficiency, technological advancements are necessary before these ambitious goals can be reached.

Recently, organoids are also being investigated as potential therapeutics during *ex-situ* organ preservation, with

promising initial results. Organoids are three-dimensional organotypical structures grown *in vitro* that recapitulate the in vivo architecture of the organs from which they were derived. Although they are mostly used as a tool to replicate the complex biology of pathologic conditions with greater fidelity than traditional monolayer culture, their potential for organ repair has been recently highlighted during *ex-situ* liver preservation. In a feasibility study, human livers that were discarded during NMP evaluation because at high risk of biliary complications were treated with cholangiocyte organoids, which were delivered to the biliary tree during *ex-situ* liver preservation via the extra-hepatic bile duct [102]. Cholangiocyte organoids engrafted during perfusion and, whereas untreated bile ducts showed epithelial lining denudation, no evidence of cholangiopathy was observed in treated segments, where both native and infused cholangiocytes preserved bile duct integrity. Although it remains to be assessed whether this strategy will effectively prevent biliary complications after transplantation of grafts that were initially too damaged, these initial results are encouraging as they suggest attainable organ repair and regeneration during *ex-situ* preservation.

Tregs are a natural occurring sub-population of adaptive immune cells that mediate tolerance to self-antigens and limit the progression of immune response, thereby curbing tissue injury. As such, Tregs are an appealing therapy in the context of solid organ transplantation to reduce alloimmunity mediated chronic graft dysfunction, as well as to limit the need for toxic immunosuppressive drugs. Although clinical studies in the context of kidney transplantation demonstrated that Treg therapy is safe [103] and that reduction of immunosuppression is feasible [104], its efficacy in preventing graft rejection and promoting true tolerance is not yet established. Additionally, concerns exist regarding the possibility that their systemic infusion post-transplant may deliver Tregs at the graft site too late for efficient and clinically meaningful immunoregulation. Therefore, *ex-situ* preservation is the perfect platform to deliver Tregs to the graft before allorecognition has started. The possibility to increase the antigen specificity of Tregs by inserting a chimeric antigen receptor (CAR) construct in their genome holds the potential to boost and diversify the application of Treg therapy in solid organ transplantation [105], especially when combined to *ex-situ* organ preservation. Indeed, by targeting human leukocyte antigens (HLA) specific to the donor, CAR-Treg therapy can be tailored to the graft, thereby limiting the side effects of systemic immunesuppression with polyclonal Tregs (such as susceptibility to pathogens or *de novo* tumour formation). Alternatively, it can be envisioned to apply CAR-Treg therapy to downsize the effect of unfavourable or detrimental HLA mismatches between donor and recipients, where autologous Tregs can be engineered, expanded ex vivo, and banked while the recipient is on the waiting list, whereas *ex-situ* preservation would facilitate the loading of CAR-Tregs to the graft before reperfusion. CAR-Treg therapy may be advantageous even in the context of transplantation of immune-privileged organs, such as the liver, in the setting of paediatric transplantation, for instance. Indeed, CAR-Treg therapy delivered at the time of *ex-situ* liver preservation may foster tolerance and allow to reduce the exposure to immunosuppressive therapy of paediatric recipients. To date, only one study investigated the feasibility of Tregs delivery during *ex-situ* machine perfusion. Miyamoto et al. delivered Tregs to the lung during *ex-situ* perfusion in a mouse model, followed by lung transplantation. Tregs migrated from the vascular space to the pulmonary parenchyma, were detectable in the lung tissue for up to 3 days after transplantation, and showed signs of their immunomodulatory activity (such as CD4+ and CD8+ T cells infiltrates reduction) for up to one week post-transplant [106]. More importantly, Miyamoto et al. also provided proof of the feasibility and efficacy of the delivery of banked, *ex vivo* expanded, human Tregs to discarded human lungs during *ex-situ* dynamic preservation [106].

Finally, cell therapy during machine perfusion can also be envisioned to "recycle" grafts that did not respond to therapeutic interventions *ex-situ.* Indeed, these organs could undergo decellularization to create biocompatible scaffolds with integer extracellular matrix and vascular network, and subsequent recellularization, providing "new" organs for transplantation. Whereas the decellularization of human organs is feasible and can be aided by machine perfusion technologies [107], efficient and complete recellularization of human scaffolds requires additional research efforts because of the challenging task of repopulating all different cell types that constitute a solid organ. Machine perfusion technologies could be applied as well to facilitate cells seeding and scaffolds recellularization. Achieving efficient and complete recellularization of human solid organs suitable for transplantation could also signify the possibility of creating "autografts", where scaffolds obtained from discarded human organs will be repopulated with stem cells derived from individual patients with diseases progressing toward end-stage organ failure. These "autografts" could be engineered before the recipient reaches the stage in which a transplant is needed, and *ex-situ* dynamic preservation could be used to assess the suitability of these organs for transplantation. A future in which personalized human organs are available off-the-shelf and waiting time for a life-saving organ transplantation is negligible can currently only be imagined, but the advent of techniques for long term organ preservation [9,108] has moved the field a step closer.

CONCLUSIONS

Organ tailored interventions for grafts optimization before transplantation are becoming a reality, and extensive

evidence showing not only the feasibility, but also the efficacy of the delivery of therapeutics during *ex-situ* dynamic organ preservation has already been accumulated. Importantly, in contrast to what previously believed, there is already some evidence that there exists and additional window of opportunity to deliver gene therapy, NPs, and EVs therapy during *ex-situ* hypothermic dynamic preservation. The rationale for this approach is rendering the desired therapeutic readily available at the start of graft reperfusion and allorecognition by uploading the organ during the *ex-situ* preservation phase. However, in grafts at higher risk of failure, transplant physicians may want to evaluate response to treatment before deciding whether to proceed or not with a transplant, and normothermic *ex-situ* dynamic preservation may still be required. Therefore, the indication for therapy delivery during *ex-situ* hypothermic dynamic preservation may depend on the graft quality as well as on the desired effect of the therapeutic intervention (i.e., modulation of allorecognition versus organ repair/regeneration).

Most of the *ex-situ* therapeutic approaches reported herein were not assessed in the clinic yet. Therefore, their safety and clinical efficacy need to be carefully evaluated in future research. Additionally, specific criteria to assess treatment response during (prolonged) *ex-situ* preservation will also need to be identified before envisioning widescale clinical application.

Nevertheless, with the use of *ex-situ* dynamic preservation, pharmacological, gene, cell therapy, and nanotechnology, the potential to revolutionize the organ transplantation field is real. Unlike other disorders or diseases, organ transplantation may require only temporary genetic modulation or therapeutic interventions to personalize and optimize organs designed specifically to withstand injurious pathways that occur during transplantation. *Ex-situ* dynamic preservation offers a uniquely appropriate and clinically necessary period to realize that, while preventing the risk of off-target effects. In the future, customized graft therapy will create a reality where organs will be optimized, personalized, and likely be available on demand.

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