



## REVIEW ARTICLE

# Treatment of transplant kidneys during machine perfusion

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## SUMMARY

The increasing use of donation after circulatory death (DCD) and extended criteria donor (ECD) organs has raised awareness of the need to improve the quality of kidneys for transplantation. Treating kidneys during the preservation interval could improve early and long-term graft function and survival. Dynamic modes of preservation including hypothermic machine perfusion (HMP) and normothermic machine perfusion (NMP) may provide the functional platforms to treat these kidneys. Therapies in the field of regenerative medicine including cellular therapies and genetic modification and the application of biological agents targeting ischaemia reperfusion injury (IRI) and acute rejection are a growing area of research. This review reports on the application of cellular and gene manipulating therapies, nanoparticles, anti-inflammatory agents, anti-thrombolytic agents and monoclonal antibodies administered during HMP and NMP in experimental models. The review also reports on the clinical effectiveness of several biological agents administered during HMP. All of the experimental studies provide proof of principle that therapies can be successfully delivered during HMP and NMP. However, few have examined the effects after transplantation. Evidence for clinical application during HMP is sparse and only one study has demonstrated a beneficial effect on graft function. More investigation is needed to develop perfusion strategies and investigate the different experimental approaches.

*Transplant International* 2021; 34: 224–232

## Key words

cellular and regenerative therapies, hypothermic machine perfusion, kidney, normothermic machine perfusion

Received: 17 July 2020; Revision requested: 24 August 2020; Accepted: 15 September 2020;  
Published online: 19 October 2020

## Kidney transplantation

Kidney transplantation is the best treatment for end-stage renal failure but the reliance on more marginal donor organs, such as those from donation after circulatory death (DCD) and extended criteria donors (ECD), can result in early graft dysfunction and reduced long-term graft survival [1,2]. To increase the utility of these kidneys and improve outcomes research has focused on improving preservation techniques and

treating the kidneys to promote recovery and repair before transplantation.

Significant advancements have been made in the last decade in the development of regenerative therapies and therapies targeting ischaemia reperfusion injury (IRI). Many studies have demonstrated safety and feasibility but difficulties remain with systemic delivery. Toxicity or insufficient targeting to the damaged organ can limit their therapeutic potential [3]. The administration of cellular, genetic or biological therapies directly to the

kidney during preservation has been a topic of interest in recent years [4-9]. Dynamic methods of preservation that involve the circulation of a perfusion solution through the kidney either at hypothermic or normothermic temperatures are considered more advantageous particularly for the delivery of therapeutic agents. Treating a kidney in this way could ensure direct targeting of the organ and obviate any unwanted side effects in the recipient. This review focuses on the recent advances in the application of regenerative therapies and biological agents targeting IRI and acute rejection during kidney perfusion. The topics included are cellular and gene manipulating therapies, nanoparticles, anti-inflammatory agents, anti-thrombolytic agents and monoclonal antibodies. Table 1 lists the therapeutic strategies during HMP and NMP.

### Preservation strategies

During the last two decades emphasis has been placed on exploring different dynamic preservation strategies such as hypothermic (HMP) and normothermic machine perfusion (NMP) technologies. The main concept of both is to restore circulation albeit at different temperatures. During HMP a cold 4°C preservation solution is continually re-circulated through the kidney at a low pressure. The rate of metabolism is approximately 10% of that at normal physiological temperature [10]. Although metabolic activity is significantly reduced, the dynamic conditions support some cellular processes and can help to protect the endothelium [10].

NMP is still very much at an experimental stage but early clinical results are promising [11]. In contrast to hypothermia, the conditions are designed to replicate a more physiological environment at a near normal or subnormal body temperature. Oxygen carriers, normally

red cells, are supplemented with a crystalloid/colloid solution and various nutrients and oxygen added to support metabolism.

These perfusion platforms (HMP and NMP) are ideal for the direct administration of therapies to target IRI, acute rejection or instigate regeneration and repair processes to promote recovery.

NMP conditions could be more beneficial for the delivery of therapies compared to HMP conditions. In theory, cellular interactions and binding to target sites are more likely at normothermic temperatures than hypothermic conditions. Furthermore, any beneficial or detrimental effects of the agent/treatment may be more evident than during HMP.

### Mesenchymal stromal cells

One of the most researched cellular therapies is the application of mesenchymal stromal cells (MSCs). Clinically, their immunomodulatory properties have been trialled to reduce the immune response and induce allograft tolerance in kidney transplantation [12]. Experimental evidence has also demonstrated their ability to promote regeneration and repair, protecting against acute and chronic kidney injury [12].

MSCs are multipotent, nonhematopoietic fibroblast-like cells. They can be isolated from bone marrow, adipose tissue, umbilical cord and blood and can expand in cell culture conditions into a heterogeneous cell population [12]. MSCs are defined by plastic adhesion and the ability to differentiate into osteoblasts, adipocytes and chondrocytes. They express the surface markers CD105, CD90 and CD73 but lack expression of hematopoietic markers CD45, CD34 and endothelial marker CD31 [12]. MSCs facilitate repair and reduce inflammation by homing in on injured cells to release

**Table 1.** Experimental evidence of cellular and genetic, bioengineered, biological and antibody therapies administered during hypothermic machine perfusion (HMP) and normothermic machine perfusion (NMP) in rodent, porcine and human studies. Mesenchymal stromal cells (MSCs), multipotent adult progenitor cells (MAPCs)

	HMP	NMP
Cellular therapies	MSC/EVs [19] (rodent study)	MSCs [13,15,16] (porcine and human studies) MAPCs [17] (human)
Gene therapies	Small interfering RNA [24,25] (rodent study)	Short hairpin RNA [28] (rodent study)
Bioengineered therapies		Nanoparticles [30,31] (human studies) Nano-barrier membrane (NB-LVF4) [36] (human study)
Biological therapies	Corline heparin conjugate [33,34] (porcine studies) Thrombalexin [35] (porcine and human study)	
Antibodies		CD47 blocking antibody [37] (porcine study)

paracrine factors including growth factors and cytokines. At the time of writing, there have been no reports of the administration of MSCs during HMP.

### MSCs and normothermic machine perfusion

The MSC and normothermic perfusion consortium (MePEP) was formed in 2017 to evaluate this approach and encourage the development of well-designed clinical trials. The first report by the consortium investigated the feasibility of the administration of different concentrations of MSCs derived from human adipose tissue and bone marrow-derived prelabelled MSCs in porcine kidneys perfused with a red blood cell-based solution [13]. The human adipose MSCs were added to the kidney via the renal artery after 1h of perfusion. Only at a concentration of  $1 \times 10^7$  cells were most of the MSCs retained within the kidney, localized in clusters inside the lumen of the glomerular capillaries. This confirmed the group's previous findings that most of the MSCs infused into the renal artery remained in the kidney mainly localized in the renal cortex within the glomeruli. However, a small number could be found in the capillary network around the tubuli [14]. During the 7h of perfusion, it was apparent that the number of circulating cells decreased over time possibly due to the mechanical mode of perfusion. The effect of the MSCs on renal function or cellular injury was not examined in this study. However, in the most recent study by the group, they found that the MSCs increased reparative processes in porcine kidneys during NMP. There was an increase in immunomodulatory cytokines (IL-6 and IL-8) and hepatocyte growth factor, and a reduction in injury markers, lactate dehydrogenase and neutrophil gelatinase-associated lipocalin (NGAL) during perfusion [15].

Using a different approach, Brasile *et al.* treated non-transplanted ischaemically damaged human kidneys with MSCs delivered during NMP. The NMP conditions differed from those reported by Pool *et al.* in that kidneys were perfused for 24h using an acellular tissue engineering platform and exsanguinous metabolic support solution (EMS) at 32°C [16]. The administration of  $1 \times 10^8$  MSCs reduced inflammation and increased ATP synthesis and growth factors. There was also evidence of regeneration with restoration of the cytoskeleton and mitosis [16]. The authors concluded that several days of treatment and perfusion would be required to demonstrate more complete repair of the renal tubular damage.

An alternative to using MSCs is the application of multipotent adult progenitor cells (MAPCs). They are

derived from stromal cells of bone marrow mesenchymal origin. They are commercially available and lack major histocompatibility complex (MHC) class II or costimulatory molecules (CD80, CD86 and CD40) and therefore limit the expression of MHC class I. MAPCs release anti-inflammatory, immunomodulatory and pro-tolerogenic cytokines thereby limiting the infiltration and proliferation of pathogenic immune cells. In a recent report of the application of MAPCs during perfusion, Thompson *et al.* administered MAPCs to human nontransplanted kidneys during NMP [17]. Kidneys were perfused with a red cell-based solution at 36.5°C. Five pairs of kidneys were included in the study, one of each pair served as a control. The MAPCs ( $50 \times 10^6$  cells) were administered after 1h NMP followed by 6h of perfusion. MAPC-treated kidneys had improved tissue perfusion and lower levels of tubular injury and inflammation compared to controls [17]. The MAPCs were found mainly in the glomeruli within the cortex and in sections around the peritubular space within the medulla. A proportion also remained in the circulation and although only 21% of these were viable they may still have had a protective effect.

### Extracellular vesicles

Extracellular vesicles (EVs) are a population of doubled layered membrane fragments released by different types of stem cells and endothelial progenitor cells (EPC EVs) [18]. They differ in size, sedimentation rate and floating density and contain biologically active molecules such as proteins, lipids and nucleic acids (mRNAs, microRNAs and other noncoding small RNAs) [18]. They influence cells via interaction with surface receptors, direct stimulation and by the transferral of proteins and genetic material. Their properties are beneficial for angiogenesis, extracellular matrix remodelling, regulation of inflammation, cell cycle and proliferation, cell migration and morphogenesis [18]. In experimental models, EVs have been shown to protect against acute and chronic kidney injury [18]. EVs can home in on damaged cells within the kidney via the peritubular capillaries or glomeruli. EVs have been administered during HMP but not under NMP conditions.

### MSCs/EVs and hypothermic machine perfusion

Gregorini *et al.* compared the administration of bone marrow-derived MSCs and EVs derived from MSCs (MSC/EV) to rodent kidneys during HMP [19]. MSC/EV were most effective in reducing IRI with reduced

lactate dehydrogenase (LDH), oxidative damage and inflammatory markers in the effluent after perfusion. The authors conclude that EVs derived from MSCs better protected cell viability with the upregulation of genes involved in cell energy metabolism and ion membrane transport compared to MSCs [19].

### Gene therapies

Gene therapy involves the intentional transfer of genetic material into host cells in order to alter their original state and thereby treat the disease. It has recently been used in humans to enhance or alter the natural state of T cells to recognize antigens not naturally recognized by their endogenous T cell receptors [20]. While the application of gene therapy treatments in clinical transplantation is only just being realized, there have been a substantial number of animal studies that have been summarized comprehensively elsewhere [21]. These are directed towards immunomodulation such as induction of tolerance through exposure of recipient bone marrow to donor MHC gene, modulation of IRI and xenotransplantation [21,22].

Small interfering RNAs (siRNAs) have been more commonly used in transplantation to target IRI. siRNAs are able to regulate the expression of genes by a process called RNA interference (RNAi) [23]. siRNAs can be delivered by nonviral (peptides, polymers, lipids) or viral systems. There are four types of siRNA: siRNA, microRNA, short hairpin RNA (shRNA) and dicer substrate RNA [23]. siRNAs have been administered during HMP and NMP.

### Gene therapies during HMP and NMP

Moser *et al.* silenced matrix metalloproteinase (MMP-2) using a siRNA during HMP in a rodent kidney model [24]. MMPs are released in response to cellular injury and are involved in acute and chronic renal injury, IRI and the development of fibrosis. After 22h HMP, levels of cellular and tubular injury were significantly less than in untreated control kidneys. Of note, inhibition of MMP-2 and MMP-9 using the nonspecific inhibitor, doxycycline, had greater effect [24]. This highlighted the need to target both MMP-2 and MMP-9.

Zheng *et al.* used a cocktail of siRNAs targeting C3, Fas and RelB using a murine model. The cocktail was administered directly into the kidney via the renal artery followed by 4h of perfusion at 4°C [25]. Silencing of these genes during perfusion improved renal function, reduced inflammation and reduced apoptosis after

transplantation. Yang *et al.* used a similar approach to deliver caspase 3 siRNA, although the kidneys did not undergo perfusion after administration [26]. The siRNA was administered to porcine kidneys via the renal artery [26]. The caspase 3 targeted siRNA reduced levels of apoptosis. In a follow on study, the effect was not evident after transplantation and this was possibly due to instability of the siRNA [27].

It is well established that differences in the MHC antigens between donor and recipient adversely affect outcomes after transplantation. In a rat subnormothermic ex vivo perfusion model, Yuzefovych *et al.* delivered lentiviral vectors directly to the organ. This delivered encoding shRNA targeting beta2 microglobulin and the class II transactivator, in addition to a sequence for a secreted nanoluciferase [28]. After kidney transplantation and 6 weeks follow up, bioluminescence was detected in the plasma and urine of recipients of engineered kidneys, indicating stable gene expression. This has been a key hurdle in gene therapies for other diseases. The transcript levels of beta 2 microglobulin and the class II transactivator were decreased by 70% in kidneys expressing specific shRNAs. In addition, there was an alteration in cytokine secretion trending towards an immunoregulatory profile, and the vector was restricted to the graft as detected by biodistribution assays [28].

### Nanoparticle delivery during NMP

Nanoparticles are small particles with a size range of 1 and 100 nanometres. They can be used to deliver drugs to specific types of cell. Nanoparticles internalize into a cell and as they degrade by hydrolysis they slowly release the encapsulated drug to ensure direct targeting to the cell [29]. They provide an ideal delivery system as the effect may last more than 6 weeks after administration. Tietjen *et al.* recently used a surface conjugated anti-CD31 antibody to enhance the targeting of nanoparticles to endothelial cells by 5 to 10 fold during NMP of nontransplanted human kidneys with a red blood cell-based solution [30]. Although targeting was largely successful, the nanoparticles also nonspecifically accumulated within obstructed regions of the microvasculature during NMP. Further investigation revealed these as areas of red cell clumping related to the excess production of fibrinogen in the tubular epithelium during cold storage and subsequently released into the microcirculation during NMP [30]. To refine the NMP technique, tissue plasminogen activator (tPA) and plasminogen were administered during NMP to clear the microcirculation and allowed better nanoparticle

targeting during NMP [31]. The group plan to perform a clinical trial to assess the effects of tPA and plasminogen treatment during NMP to determine whether clearance of the microcirculation during NMP can enhance recovery after transplantation.

### Biological targeting of IRI and acute rejection during HMP and NMP

IRI occurring after transplantation involves a cascade of interlinked events involving the upregulation of inflammatory mediators, oxidative damage, infiltration of neutrophils, complement activation, apoptosis and necrosis [32]. Severe IRI can lead to significant graft dysfunction over a prolonged period [32]. This can reduce long-term graft function and survival. Many different therapies targeting specific pathways or broader aspects of IRI have demonstrated efficacy in experimental models, yet none have translated into clinical practice. A number of different therapies focused on targeting the endothelium have been investigated during HMP and NMP.

The endothelial glycocalyx is a mesh of membrane bound proteoglycans and glycoproteins that covers the entire surface of the endothelium. It serves as a barrier against cellular oedema, inflammation and leucocyte and platelet adhesion. Damage to the glycocalyx occurs during IRI causing it to shed [33]. This promotes the formation of thrombi, inflammation, reduces microcirculation and causes graft dysfunction. Sedigh *et al.* administered a Corline heparin conjugate (CHC) to porcine kidneys during HMP [33]. CHC consists of 70 heparin molecules that can adhere to biological tissues expressing heparin affinity such as the glycocalyx. The study concluded that by binding and coating the inner surface walls during HMP this could help to restore the endothelial glycocalyx and protect the vascular endothelium against IRI. The group went on to assess the effects of CHC during IRI using an *ex vivo* porcine kidney reperfusion model [34]. To ensure HMP did not damage the endothelium, kidneys were perfused at lower arterial pressure (15mmHg) than advocated in clinical use (30 mmHg). During HMP they found that CHC bound to the glomeruli vasculature within the first hour of HMP. CHC treated kidneys had improved function, lower intra-renal resistance and less tubular injury during reperfusion compared to paired control kidneys [34]. These studies provided proof of concept that CHC could effectively coat the endothelium and protect against IRI.

Hamaoui *et al.* assessed the effects of a cytotoxic endothelial localizing peptide (thrombalexin), a thrombin inhibiting anticoagulant delivered to porcine and human kidneys during HMP [35]. Thrombalexin was able to successfully adhere to endothelial cells to improve the microvasculature and organ tissue perfusion during NMP [35].

Brasile *et al.* used their EMS NMP system to deliver a receptor mediated bioengineered nano-barrier membrane (NB-LVF4) to coat the vasculature of canine kidneys [36]. NB-LVF4 is composed of laminin, vitronectin, fibronectin and type IV collagen. It attaches to vascular endothelial cells using several integrin complexes and is designed to provide a surface that maintains a barrier between the recipient's immune cells and the vasculature of the kidney. The aim of the study was to assess the feasibility of 'immunocloaking' to prevent allorecognition and protect against early graft rejection. They found that after 3h of NMP NB-LVF4 was uniformly dispersed on about 90% of the vascular luminal surface of small and large blood vessels without restricting the vessels, it did not elicit a proliferative response, did not adversely affect renal function after transplantation and delayed the onset of rejection by 24 days in the absence of any immunosuppression therapy [36].

Hameed *et al.* examined the effect of a CD47 blocking antibody ( $\alpha$ CD47Ab) delivered during NMP using a red blood cell-based solution in a porcine kidney model [37]. CD47 signalling is increased during IRI by inhibition of nitric oxide production, increased inflammation and oxidative damage [39]. It also inhibits renal tubular epithelial cell self-renewal and proliferation [39]. In kidneys treated with the CD47 blocking antibody, there was evidence of glomerular and tubular binding, increased blood flow, reduced inflammation and oxidative stress during NMP compared to control kidneys [38]. However, the effects were not assessed after transplantation.

### Clinical trials

Only a handful of reported clinical trials have assessed the effects of therapeutic agents administered during kidney perfusion in the last 10 to 15 years (Table 2). All of these studies have used agents to target the endothelium and inflammatory pathways. None have involved cellular or genetic therapies and at the time of writing there are no clinical trials to assess these during kidney perfusion registered on the approved trial databases.



**Table 2.** Clinical trials using hypothermic machine perfusion to deliver biological therapies

Author	Agent	Type number of kidneys	Main outcome
Polyak <i>et al.</i> 1999 [39]	Prostaglandin E <sub>1</sub> , trifluoperazine, verapamil, papaverine and mannitol	ECD kidneys HMP + treatment ( <i>n</i> = 150) vs control ( <i>n</i> = 150)	Prostaglandin E <sub>1</sub> , Improved early graft function
Woodside <i>et al.</i> 2015 [40]	tPA (Alteplase 50mg)	Paired study DCD kidneys HMP + treatment ( <i>n</i> = 12) vs control ( <i>n</i> = 12)	No effect on DGF but no adverse effects
Diuwe <i>et al.</i> 2017 [41]	TNF alpha inhibitor, etanercept	Paired study SCD/ECD kidneys HMP + treatment ( <i>n</i> = 47) vs control ( <i>n</i> = 47)	No effect on 12mth patient survival No effect on DGF or acute rejection rates

In one of the earliest reported clinical trials examining the administration of a therapy during HMP and static cold storage Polyak *et al.* investigated the effects of five vasoactive agents, prostaglandin E<sub>1</sub>, trifluoperazine, verapamil, papaverine and mannitol, in a series of 275 ECD kidneys [40]. During HMP, prostaglandin E<sub>1</sub> reduced cellular calcium release into the perfusate and reduced intra-renal resistance. After transplantation prostaglandin E<sub>1</sub> treated kidneys had improved early graft function compared to nontreated kidneys. None of the other agents had any effect during HMP or when administered during static cold storage. Prostaglandin E<sub>1</sub> increases adenylate cyclase activity to stabilize the endothelium and smooth muscle membranes [40]. There is also evidence of its ability to reduce the production of oxygen free radicals. There are no further reports of its use since this trial.

Woodside *et al.* reported the results of a small randomized clinical trial to investigate the effects of tPA during HMP to clear the microcirculation [40]. No significant differences in graft function up to 12 months were found compared to kidneys without tPA treatment. However, the study did demonstrate the feasibility of tPA treatment during HMP without complications [41] by eliminating the high risk of bleeding associated with the systemic administration of tPA. A possible explanation for the lack of effect is that the study did not include the addition of plasminogen to the perfusate during treatment with tPA. Plasminogen is produced by the liver and is present in plasma. It binds with tPA to form plasmin to breakdown the thrombi [31]. Without exogenous administration in the absence of plasma, tPA remains relatively inactive.

A randomized clinical trial using pairs of SCD and ECD kidneys (*n* = 94) investigated the administration of a p75 Fc receptor protein (TNF alpha inhibitor, etanercept) to target inflammation during HMP [42]. The

trial demonstrated no significant differences in patient survival at 12 months, rates of delayed graft function or incidence of acute rejection [42]. The authors concluded that the study might have been underpowered to detect a statistical difference in graft function at 12 months. Furthermore, although previous work in experimental models demonstrated that at 4°C etanercept could exert a protective effect [43], the efficiency in human kidneys during HMP may be reduced. The complexity of the interlinked inflammatory pathways upregulated during IRI is also a limiting factor.

## Discussion

Dynamic methods of machine perfusion have experienced a renaissance, in part due to the ever-increasing transplant waiting list, as well as the changing donor profile landscape. The use of more high-risk donors and increasing levels of ischaemic injury has necessitated ways of reducing organ discard rates and optimizing the outcomes of organs that are used through resuscitation and restorative processes.

HMP technologies are widely used and cost effective in kidney transplantation [44]. Therefore, they provide an ideal mode of delivery for therapeutic agents. However, one of the challenges is in delivering an effective therapy or agent at low temperatures. At 4°C the pharmacokinetics of many different agents are unknown. Encouragingly, in experimental models, regenerative therapies such as MSC/EVs [17] and siRNAs [24,25] have been delivered successfully to manipulate the kidney and reduce preservation injury. However, only a few have assessed the effects after transplantation [25,27,34]. The delivery of agents during HMP to coat and protect the endothelium has been particularly successful in reducing the level of IRI and therefore represents a promising new strategy for clinical trials [33–36].

Of the clinical trials reported, only the administration of prostaglandin E<sub>1</sub> during HMP had any beneficial effect on improving graft function after transplantation yet this has not been used since [41]. None of the clinical studies have examined the effects of cellular- or genetic-based therapies during HMP.

NMP provides a more a physiological environment and therefore has a theoretical advantage over HMP. Furthermore, kidneys may be preserved for longer and this could be beneficial in genuine repair as described by Brasile *et al.* [6,16]. The ability to regenerate and repair after prolonged ischaemic injury may have important implications for expanding the organ donor pool and promoting the use of uncontrolled DCD kidneys.

Experimental evidence for the delivery of MSCs [13–15], gene therapies [28], nanoparticles [30,31] and other IRI targeted [33–39] therapies during NMP is increasing and the results have been positive. However, none of the approaches have been tested in clinical practice. NMP poses a significant challenge in kidney transplantation. It is a new technology that requires significant resources and at present there is no consensus on the optimal strategy. The optimal time, pressure, flow, temperature, partial pressure of oxygen, sequence with cold static storage and perfusion solutions are all still under investigation [45]. Furthermore, the clinical application of NMP in kidney transplantation is sparse, with only a handful of single case reports [45,46] and one case series [11]. A randomized controlled trial is underway in DCD kidney transplantation to assess the effects of an end 1h period of NMP compared to static cold storage with the result expected next year [47].

Translation of the treatment of cellular and genetic therapies during kidney perfusion (HMP and NMP) into clinical practice remains a significant hurdle. Regulatory approval processes, pharmaceutical support and

cost are challenging [48]. Nonetheless, the application of these therapies to the kidney during perfusion may prove to be more ethically acceptable than treating the recipient, particularly in the case of genetic therapies. One of the most promising treatments is the application of MSCs. This is a growing area of research and expertise with their use in other medical fields will aid their application as an intervention during kidney perfusion. Furthermore, the availability of ‘off the shelf’ products may ease the regulatory legislation and provide a readily available treatment.

HMP and NMP strategies can be used to successfully deliver a range of therapeutic agents to the kidney. Nonetheless, more experimental evidence is needed to determine the efficacy of these therapies during HMP and NMP and refinement of NMP techniques is required before translation into clinical practice.

### Authorship

MH and SAH co-wrote the manuscript, MLN revised and reviewed the manuscript.

### Funding

Supported by National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU) in Organ Donation and Transplantation at the University of Cambridge in collaboration with Newcastle University and in partnership with NHS Blood and Transplant (NHSBT). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health and Social Care, or NHSBT.

### Conflicts of interest

The authors have declared no conflicts of interest.

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