Oxygenated versus standard cold perfusion preservation in kidney transplantation (COMPARE): a randomised, double-blind, paired, phase 3, superiority trial.

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Research in context

Evidence before this study

Although oxygen is vital to cellular survival, donor kidneys are preserved in cold hypoxic conditions as preservation solutions are currently not actively oxygenated. Recent animal studies suggest that providing oxygen during preservation, by means of machine perfusion techniques, might improve post-transplant outcomes by reducing the ischaemia-reperfusion injury cycle. Our systematic review (registered in PROSPERO 2013 with final searches carried out in 2016) of the evidence for supplemental oxygen during hypothermic preservation for deceased donor kidneys suggests that the effects of oxygen on restoring kidney function during preservation may be of value for kidneys donated after circulatory death and/or those that have undergone a period of hypotension, warm ischaemia or poor perfusion in the donor. The review highlighted the need for high-quality clinical studies in this area. In 2009, our randomised controlled trial comparing static cold storage of deceased donor kidneys with standard hypothermic machine perfusion preservation (HMP) showed improved short-term kidney function with HMP. In 2012, a follow-up of this study showed that HMP also improved long-term graft survival in kidneys donated after brain death but not for kidneys donated after circulatory death. These findings have been supported by several subsequent meta-analyses. However, there remains some controversy regarding the benefit of HMP in the subgroup of deceased donor kidneys that are donated after circulatory death. As donation after circulatory death is the fastest growing source of deceased donor organs, we set out to investigate whether supplemental oxygen during HMP could improve preservation of kidneys donated after circulatory death. Especially because many centres have already introduced HMP in their clinical practice.

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Added value of this study

This double-blind, randomised, paired design trial is the first to investigate the value of supplemental oxygen during hypothermic organ preservation. The trial randomised kidney pairs from circulatory-dead donors of at least 50 years old, comparing standard, non-actively oxygenated HMP to oxygenated hypothermic machine perfusion ($HMPO_2$). The results show that $HMPO_2$ is feasible, safe and easy to administer. $HMPO_2$ leads to reduced severe post-operative complications. When the beneficial effect of $HMPO_2$ on graft survival is considered, $HMPO_2$ is associated with improved one-year graft function, as measured by the estimated glomerular filtration rate, an established predictor of long-term graft survival. We also found a significant decrease in biopsy-proven acute rejection rates in the first post-transplant year. Exploratory analysis suggests that the reduction of rejection might be the underlying mechanism of the beneficial effect of oxygen.

Implications of all the available evidence

As $HMPO_2$ is a simple and minimal-cost extension to current preservation strategies, it has the potential for quick implementation in clinical practice with a direct beneficial effect improving outcomes for many patients.

The findings of this study underpin increasing evidence suggesting a close link between hypoxia, innate and adaptive immunity that lead to chronic scarring and loss of kidney function which needs further in-depth investigation. Furthermore, as the beneficial mechanisms are likely similar in other organs, future studies investigating the effect of supplemental oxygen during hypothermic organ preservation may want to include organ rejection and any confounding factors in their design.

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Summary

Background: Deceased donor kidneys are preserved in cold hypoxic conditions. Providing oxygen during preservation might improve post-transplant outcomes, particularly for kidneys subjected to greater degrees of preservation injury.

Methods: This double-blind, randomised, paired design trial was undertaken in 19 European transplant centres. Kidney pairs of donors ≥50 years who donated after circulatory death (DCD) were eligible if both kidneys were transplanted into two different recipients. One kidney from each donor was randomly assigned to oxygenated hypothermic machine perfusion ($HMPO_2$), the other to hypothermic perfusion without oxygenation (HMP). Perfusion was maintained from organ retrieval to implantation. The main outcome was 12-month CKD-EPI estimated glomerular filtration rate (eGFR) in pairs of which both kidneys were functioning at end of follow-up. Safety outcomes were reported for all transplanted kidneys. Intention-to-treat analyses were performed. (ISRCTN32967929, closed)

Findings: Between March 15, 2015 and April 11, 2017, 197 kidney pairs were randomised with 106 pairs transplanted into eligible recipients. Twenty-three pairs were excluded from primary analysis because of kidney failure or patient death. Mean eGFR at 12-months was 50·5 ml/min/1·73m² (SD: 19·3) in $HMPO_2$ versus 46·7 ml/min/1·73m² (SD: 17·1) in HMP [mean difference: 3·7 ml/min/1·73m^2 (95% CI: -0·99 to 8·43, p=0·12)]. Fewer severe complications (Clavien–Dindo grade ≥IIIb) were reported in $HMPO_2$ versus HMP [46/417 (11%, 95% CI: 8%–14%) versus 76/474 (16%, 95% CI: 13%–20%), p=0·032)]. Graft failure was lower after $HMPO_2$ compared to HMP [3/106 (3%) versus 11/106 (10%); hazard ratio 0·27 (95% CI: 0·07 to 0·95), p=0·028].

Interpretation: $HMPO_2$ of DCD kidneys ≥50 years is safe and reduces Grade ≥IIIb post-transplant complications. The 12-month eGFR difference between $HMPO_2$ and HMP did not

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reach significance when both kidneys of the same donor were still functioning one year post-transplant, but potential beneficial effects were observed in important secondary outcomes.

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Introduction

Globally, over 90.000 kidney transplantations took place in 2018.[1] Compared to dialysis, kidney transplantation improves survival and quality of life, making it the preferred treatment for end-stage renal disease, but many grafts fail prematurely. The introduction of effective immunosuppressants in the 1980s resulted in a significant improvement of kidney graft survival with current 1-year graft survival rates of 90% and higher.[1] However, the observed improvement in short-term graft survival has slowed considerably since 2000 while overall graft failure rates remains high at approximately 5% per year after the first year.[1] Ischaemia-reperfusion injury, the universal consequence of the organ donation process, is an important non-immunologic modifiable contributor to this kidney graft failure. Hypothermic preservation, as the cornerstone of organ preservation, mitigates the detrimental effect of ischaemia by reducing cellular metabolism and oxygen demand of the donor organ. Two methods of hypothermic preservation, i.e. static cold storage and hypothermic machine perfusion (HMP), are used clinically. In static cold storage, the kidney is submerged in cold preservation solution after which it is placed on melting ice in an icebox. During HMP, a device pumps cold preservation solution through the renal vasculature, and this has been shown to improve post-transplant outcomes.[2-4]

Despite reduced metabolic needs, there is residual ongoing metabolism during hypothermic preservation. Hypoxia prevails in both cold storage and HMP because the preservation solution is not actively oxygenated. Recent animal experiments, modelling donation after circulatory death, suggest that active oxygenation during hypothermia is essential to reduce oxidative stress and improve cellular energy status.[5-10] Oxygenated HMP ($HMPO_2$) leads to better early kidney function and reduced fibrosis in these models.[5-10] To date, there are no well-designed clinical studies assessing the effect of $HMPO_2$ in kidney transplantation.[11]

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This study aims to assess the effect of oxygen delivery to donor organs and it does so in the setting of kidney donation after circulatory death (DCD) in donors of at least 50 years of age. This donor population was chosen as it represents the fastest growing source of donor kidneys (see Tables S1, S2). DCD-kidneys are more susceptible to the ischaemia-reperfusion injury cascade compared to kidneys donated after brain death, resulting in higher post-transplant complication rates.[12] The challenge is to increase organ utilisation and transplant these kidneys without compromising organ function and survival. To achieve this, considerable efforts must focus on further optimisation of kidney preservation and HMP. Indeed, although HMP is frequently used to preserve DCD-kidneys,[13,14] and meta-analyses have shown HMP to reduce the risk of delayed graft function (DGF) in all types of deceased donor kidneys when compared to static cold storage, evidence showing that HMP improves long-term graft function or survival of DCD-kidneys is currently lacking.

Methods

Study design

This investigator-driven, international, randomised, paired, double-blind, controlled, phase 3, superiority trial involved 12 organ procurement teams and their associated hospitals in Belgium, The Netherlands, and the South of the United Kingdom. Kidneys were transplanted in 19 kidney transplant centres in the same countries (Appendix). The EU-funded Consortium for Organ Preservation in Europe (COPE, http://www.cope-eu.org) ran this trial. Approval was obtained by the institutional review boards or independent ethics committees in each trial region. The trial is reported in accordance with the CONSORT statement. One major amendment was made to the trial design after the start of recruitment (see 'Outcomes').

Participants

Inclusion was limited to kidney pairs procured from controlled DCD donors of at least 50 years of age when both kidneys were deemed transplantable by the donor surgeon. This donor group

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was deliberately chosen as it represents the fastest growing source of deceased donor kidneys (Tables S1-S2) that also suffer most from ischaemia-reperfusion and preservation injury.[12,15] When national law required, informed consent was obtained from the donor's relatives. The Declaration of Istanbul was adhered to. Recipients were eligible provided they were at least 18 years of age and listed for a kidney-only transplant in one of the participating centres. Belgian and Dutch donor kidneys were offered by Eurotransplant, donor kidneys from the United Kingdom were offered by NHS-Blood and Transplant services. As randomisation took place early in the donation process, recipients were informed that the kidney they had been offered was included in this trial and they gave written consent to use follow-up data stored in coded fashion in a secure online database established by the COPE Consortium. The consent also included collection and storage of biological samples. Organ allocation followed established organ allocation service rules(Eurotransplant, NHS-Blood and Transplant).

Procedures, randomisation and masking

Once the donor surgeon confirmed transplantability of both donor kidneys, the kidney pair was randomised. All clinical decisions made thereafter, including graft suitability, were made independently of the trial team. Donors, organ transport, and recipients were managed according to local protocols. Using a computer-generated randomisation scheme with permuted blocks, stratified by organ allocation region, one kidney was randomly assigned to $HMPO_2$ and the contralateral kidney to standard HMP. The unit of randomisation was donor kidney pairs, analyses are reported for transplant recipients.

Immediately following removal from the donor, the kidney was connected to the Kidney Assist Transporter device (Organ Assist BV, Groningen, NL) to be perfused for the duration of the preservation period with actively oxygenated ($HMPO_2$ group) or non-actively oxygenated (HMP group) University of Wisconsin machine preservation solution (Bridge to Life Ltd, Columbia, USA) at 1 to 4°C with a fixed mean perfusion pressure of 25 mmHg. No changes in

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perfusion settings were made and all involved were blinded to perfusion characteristics. Oxygen (100%) was given at 100 ml/min, resulting in perfusate partial oxygen tensions around 600 mmHg (Appendix) in $HMPO_2$.

For standardised trial purposes, trained technicians were involved and responded when a potential donor was announced. The technicians transported the perfusion device to the donor hospital, randomised kidney pairs, supported surgeons with connecting the kidney to the device, controlled oxygenation, and collected baseline, donation, and transplantation data. Clinicians were blinded to treatment allocation by use of dummy empty oxygen bottles in the control arm. Follow-up data were collected by the transplant centres.

Donor blood and urine, recipient blood, and a kidney tissue biopsy were collected at pre-specified time points from every donor/kidney/recipient transplanted in the study when consent was in place. In addition to these, samples of perfusate fluid were collected from every kidney. Samples were stored in a central biobank established by the COPE Consortium for ongoing mechanistic studies. No patient identifiable data were associated with the sample.

Outcomes

The primary endpoint was renal function at 12 months after transplantation which is independently associated with an increased risk of graft failure.[16,17] It was planned to obtain creatinine clearance calculated from a 24-hour urine collection at 12 months post-transplant to determine renal function. During recruitment, while data were still blinded, a high proportion of creatinine clearance values was observed to be missing as many centres had abandoned this method of assessing renal function. Therefore, the data monitoring committee, investigators, and trial statistician jointly decided to change the way that renal function was determined. The primary endpoint changed to an estimated glomerular filtration rate (eGFR) using the CKD-EPI equation (Chronic Kidney Disease Epidemiology Collaboration)[18] at 12 months post-transplant, which was originally a secondary endpoint (Appendix).

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Secondary endpoints were: (1) survival of the graft and patient up to 12 months post-transplant. Graft failure was defined as return to chronic dialysis or pre-emptive re-transplantation; (2) short-term outcomes as determined by primary non-function (permanent lack of function of the graft from time of transplantation resulting in (re)institution of chronic dialysis), DGF (dialysis during the first week post-transplant preceding return of kidney function), and functional DGF (absence of a decrease in serum creatinine level by a minimum of 10% per day during three consecutive days in the first postoperative week excluding those with acute rejection of calcineurin inhibitor toxicity); (3) renal function as determined by the CKD-EPI equation at 3 and 6 months and by the 4-variable MDRD equation (Modification of Diet in Renal Disease)[18] at 3, 6 and 12 months; (4) renal function as determined by creatinine clearance from a 24-hour urine collection at 12 months; (5) biopsy-proven acute rejection up to 12 months, and (6) safety events. Reporting of adverse events was in accordance with the MEDDEV guidelines.[19] Following trial completion, these were reviewed by two clinicians blinded to treatment and graded according to the Clavien-Dindo system.[20] The proportion of Clavien-Dindo grade ≥ IIIb was compared between groups. Safety and adverse events are reported for all randomised kidneys.

Statistical analysis

Previous data from the University Hospitals Leuven, Belgium (Supplementary Methods), demonstrated a correlation coefficient of 0·4 for the function of two kidneys from the same donor, a standard deviation of 17 ml/min/1·73m² and an expected mean eGFR in the control group of 46 ml/min/1·73m² at 12 months post-transplant. The study was powered to detect an 8 ml/min/1·73m² difference in the eGFR (CKD-EPI equation[18]), considered the minimum clinically important difference, with a 90% power at a 5% significance level, requiring 81 kidney pairs available for analysis of the primary endpoint (Appendix). The use of eGFR as a primary endpoint needed careful consideration as eGFR overestimates true renal function when

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the graft fails, and patients return to dialysis. Also, eGFR at 12 months is not available when a patient dies with a functioning graft prior to that time. For the primary analysis, only kidney pairs for which both grafts were still functioning at 12 months post-transplant were considered. As this could introduce an undesired bias towards either group, a pre-specified sensitivity analysis of the primary endpoint was carried out in which kidneys that failed before 12 months follow-up were accounted for. In this sensitivity analysis, kidneys that experienced graft failure, with the patient receiving chronic dialysis treatment, were given a nominal eGFR value of 10 ml/min/1·73m², matching the start of chronic dialysis in patients with end-stage renal failure during the IDEAL trial.[21] Guidelines promote dialysis initiation when patients have symptoms or signs of advanced chronic kidney disease that are likely to occur with GFR values in the 5-10 ml/min/1·73m² range. When the patient died with a functioning graft, the last known eGFR was carried forward.

Primary analyses were performed according to the intention-to-treat principle, with a pre-specified per-protocol analysis performed as a sensitivity analysis. All reported p-values are two sided and unadjusted for multiple testing. $p<0·05$ was considered to indicate statistical significance; 95% confidence intervals are reported. The primary endpoint was compared between treatment allocation using a paired t-test. Secondary endpoints were compared using paired t-tests or Wilcoxon rank-sum tests for continuous variables or McNemar's test for discrete variables. Time to graft failure and patient death were compared using Kaplan-Meier and log-rank methods. Multivariate analyses were performed using generalised estimating equation models with either a binomial or Gaussian distribution. No interim analyses of study endpoints were carried out. Throughout this report percentages may not precisely reflect the absolute figures due to rounding to whole numbers. Analyses were performed with SAS version 9·4 and STATA version 15. At regular intervals, an independent data monitoring

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committee reviewed confidential reports covering recruitment, safety parameters and endpoint data. The trial was pre-registered (ISRCTN-32967929).

Role of the funding source

The European Commission's 7th Framework Programme, funder of the study, had no role in study design and collection, analysis, interpretation of data, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between 15 March 2015 and 11 April 2017, 197 kidney pairs were randomised, with 91 pairs subsequently excluded (Figure 1). Of 394 randomised kidneys, 16 (4%) were subsequently considered not-transplantable by the retrieving donor surgeon upon additional inspection after randomisation [8 (4%, 95% CI: 2%-8%) in both groups] and 12 (3%) considered not-transplantable by the transplant surgeon after they had been perfused [$HMPO_2$: 8 (4%, 95% CI: 2%-8%); HMP: 4 (2%, 95% CI: 1%-5%)]. Due to graft failure or patient death, 83 kidney pairs were available for primary outcome analysis, with 106 available for sensitivity analysis and secondary outcome analyses. The allocation groups were well-balanced according to donor and recipient characteristics (Tables 1, 2). Eleven kidneys (11/212, 5%) were cold-stored because machine perfusion was not possible (Table S3) and in nine machine-perfused kidneys (9/201, 5%) randomised allocation was accidently switched (Table S4); these organs were included in the intention-to-treat analysis. As this was a paired trial, some kidneys that were randomised were not eligible for analysis of the main outcome as their partner kidney had been excluded ($HMPO_2$: N=35; HMP: N=27).

At 12-months post-transplant, the eGFR was 3·7 ml/min/1·73m^2 higher in the $HMPO_2$ group compared to the HMP group (mean difference: 3·7, 95% CI: -0·99 to 8·43, p=0·12) for those

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pairs of which both donor kidneys were still functioning. The sensitivity analysis of the main outcome, which accounts for donor kidneys that failed before 12 months follow-up and patient death, showed a 5·0 ml/min/1·73m^2 mean difference in favour of $HMPO_2$ (95% CI: 0·35 to 9·68, p=0·035) (Table 3). Posthoc sensitivity analyses utilising different imputation methods, confirmed these findings (Tables S5-S9). The multivariate regression analyses, including clinically relevant baseline variables, demonstrated that donor age was the only independent predictor of eGFR at 12 months (Table S10).

Graft failure was significantly lower in the $HMPO_2$ group compared to the HMP group [3/106 (3%) versus 11/106 (10%); hazard ratio 0·27 (95% CI: 0·07 to 0·95), p=0·028 (Table 4, Figure S1)]. Grafts failed because of preservation injury (N=2), immunological reasons (N=3), arterial (N=3) or venous thrombosis (N=1), or other reasons (N=5) (Table S11). No graft failures beyond three months post-transplant occurred in $HMPO_2$, while 36% of graft failures in HMP occurred beyond three months post-transplant. There was no significant difference in patient survival; seven patients died over the course of 12 months in the $HMPO_2$ group while eight patients died in the HMP group [7% versus 8%, HR: 0·88 (95% CI: 0·32 to 2·41), p=0·80; (Table 4, Figure S2-S3)]. Patients died from infection (N=5), cardiovascular disease (N=4), cerebrovascular event (N=1), cancer (N=1), multiple organ failure (N=1), and unknown causes (N=3) (Table S12). In each group, five patients died with a functioning graft. Rates of primary non-function, DGF and functional DGF were comparable between the two groups (Tables 3, S13).

Renal function (calculated by the MDRD and CKD-EPI equations) improved over time and at all time-points the renal function was numerically better in the $HMPO_2$ group (Table 3, Figures S4-S5). CKD-stages are given in Tables S14-S15. Creatinine clearance from a 24-hour urine collection was significantly higher in $HMPO_2$ compared to HMP at 12 months (Table 3).

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The relative risk reduction of biopsy-proven acute rejection was 44% (relative risk ratio 0·59; 95% CI: 0·31 to 0·98) in the $HMPO_2$ group (14%) compared to HMP group (26%) (absolute risk difference: -11%, 95% CI: -22 to -0,01, p=0·040, Table 3) and rates of biopsy-proven acute rejection occurring beyond three months post-transplant were higher in the HMP group (Table S6, Figure S6). Recipients in both groups were found to be well-matched for induction therapy and maintenance immunosuppression (Tables 2, S17). Post-hoc analysis of those patients receiving induction therapy showed a similar reduction in rejection rates (Table S18). Exploratory analysis showed no difference in Banff-grading or response to steroid pulse treatment (Table S19). As preclinical evidence suggested a link between $HMPO_2$ and acute rejection,[22] an exploratory multivariate analysis looking at determinants of biopsy-proven acute rejection was carried out. This showed that $HMPO_2$ was the only independent factor protecting against biopsy-proven acute rejection suggesting that the effect of $HMPO_2$ on eGFR may be mediated via a reduction in biopsy-proven acute rejection. The adjusted odds of biopsy-proven acute rejection occurring in the $HMPO_2$ group was approximately 55% lower than in the HMP group (Table S20).The results of the per-protocol analysis, which included 88 kidney pairs, supported the intention-to-treat analysis, though, apart from a lower graft failure rate in $HMPO_2$, the findings were not statistically different (Tables S21-S23).

The proportion of recipients with reported adverse events was similar in the two groups [26% in the $HMPO_2$ group (95% CI: 19%–34%) versus 28% in the HMP group (95% CI: 20%–36%)]. Table 5 shows documented adverse events for all randomised kidneys. Of 891 adverse events reported for recipients (417 in $HMPO_2$, 474 in HMP), fewer severe (Clavien–Dindo grade ≥IIIb) complications[20] were reported in the $HMPO_2$ group versus the HMP group [46/417 (11%, 95% CI: 8% to 14%) versus 76/474 (16%, 95% CI: 13% to 20%), p=0·032)]. One kidney that underwent $HMPO_2$ was not transplanted following a technical issue with a leakage of perfusion fluid. Modifications to the device were made to avoid re-occurrence (Appendix).

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Discussion

This international double-blind, paired, multicentre randomised controlled trial is the first to test the effect of oxygenation during hypothermic kidney preservation. The trial was embedded in the standard practice of organ donation and allocation and included 106 paired donor kidneys of older DCD-donors with one kidney preserved by $HMPO_2$ and the other by standard HMP. The results showed that $HMPO_2$ is feasible, safe, and easy to deliver. Severe (Clavien-Dindo grade ≥ IIIb) post-transplant complications were reduced by $HMPO_2$ compared to HMP. When both kidneys of the same donor were still functioning one-year post-transplant, $HMPO_2$ did not result in a significantly improved eGFR. When considering the beneficial effect of $HMPO_2$ versus HMP on graft survival, $HMPO_2$ demonstrates a significant improvement in renal function of 5 ml/min/1·73m² at 12 months post-transplant. A significant relative risk reduction of acute rejection by 44% was observed after $HMPO_2$ compared to preservation with standard HMP.

The clinical benefits observed in this trial are consistent with results from previous animal work in which active oxygenation during preservation improves kidney function and reduces fibrosis which affects long-term graft survival.[8,10,23] These effects appear to be mediated via a blunting of the immune response to ischaemia-reperfusion. Innate and adaptive immunity are activated upon reperfusion when a superoxide burst from the mitochondrial respiratory chain induces tissue injury and damage-associated molecular patterns.[24] A sterile inflammatory response with a maladaptive injury repair initiates later alloreactive T and B-cells responses, priming the organ for rejection and fibrosis.[25-27] $HMPO_2$ reduces damage-associated molecular patterns and prevents mitochondrial superoxide production, while it also reduces endothelial, macrophage and T-cell activation after reperfusion. Oxygen is essential to obtain these effects and for this, supra-physiological oxygen tensions are required under hypothermic conditions in the absence of oxygen-carriers.[8,9] Concern has been expressed about possible increased oxidative stress,

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due to supra-physiological oxygen levels during perfusion as higher levels of lipid peroxidation have been reported after oxygenated perfusion.[23] However, we found no evidence that oxygenation at these levels increased post-transplant morbidity. The incidence rate of complications post-transplant was low in this group of patients, with a higher proportion of severe complications reported in the HMP group. Only one adverse event was attributed to the device, with effective measures taken to prevent such further events. Mortality rates at 12 months were also low for this relatively elderly patient population, with no differences observed between the treatment groups.

The observed 44% relative risk reduction in acute rejection episodes in the $HMPO_2$ group is in line with recent work showing a reduction in acute rejection rates in rodent kidney and liver transplantation.[22] Together with the finding that $HMPO_2$ was the only independent predictor of acute rejection when correcting for other known risk factors such as human leukocyte antigens (HLA) mismatches and the use of induction therapy, these results suggest that the effect of $HMPO_2$ on eGFR may be mediated via a reduction in acute rejection. More work is needed to unravel the effects of $HMPO_2$ on kidney immunogenicity and the immunologic mechanisms of rejection, which was outside the scope of this trial. We cannot exclude that uncontrolled confounders in this real-world trial may have influenced the observed reduction in acute rejection as immunosuppressive regimens followed standard practice and were not always fully identical while detailed information on calcineurin trough levels as well as the presence and development of donor specific antibody formation was not recorded. Reduced immunogenicity with a dampened inflammatory response leading to lower rejection rates may be the reason for improved graft survival in these older and higher risk donor kidneys. Persistent inflammation in scarred areas after T-cell mediated rejection has been associated with chronic scarring and fibrosis due to maladaptive injury responses which are important risk factors for long-term graft

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failure.[26] Over one third of graft failures (36%) in the HMP group occurred beyond three months post-transplant, making it more likely that immunological factors have played a role. Graft failures in the $HMPO_2$ group all occurred in the first three months post-transplant. Although the one-year graft failure of 10% in the control group might appear high, a thorough analysis showed that this rate matches graft failure rates in comparable kidney transplant cohorts (Appendix). Due to the limited number of graft failures, it was not possible to ascertain whether organ rejection was an independent determinant of graft failure in this cohort. A post-hoc analysis of biopsies showed no difference in Banff-grading. As biopsies were scored as part of clinical routine, we cannot exclude that interobserver variability may have masked any differences.

Unlike in animal studies, this trial did not show a difference in early renal function as was assessed by the presence or absence of DGF. Due to the reported association between DGF and acute rejection, the lack of a difference in DGF between both groups is intriguing and we can only speculate on the reasons for this observation. The association between DGF and acute rejection has been described mostly for kidneys donated after brain death. A recent Canadian study showed that DGF is an important risk factor for acute rejection in a contemporary cohort of kidney transplant recipients.[28] This association was less pronounced in recipients who were older, diabetic, unsensitised, and received expanded-criteria donor kidneys .[28] This might explain the lack of an association as kidneys in our trial were often expanded-criteria kidneys going to older, unsensitised recipients. In addition, the physiology of DGF in DCD kidney transplantation is different from that in donation after brain death, with selective activation of resilience-associated pathways in DCD grafts.[15] The pathways leading to DGF and acute rejection in donation after brain death may therefore differ from those in DCD which might explain the observed difference in acute rejection despite similar DGF rates.

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For $HMPO_2$ to be supported by healthcare funders, a health-economic analysis is needed. Adding oxygen to current standard HMP generates low additional cost while the cost-effectiveness of HMP has already been demonstrated.[29] Furthermore, our results suggest that considerable benefits will accrue, not only from reduced severe complications but also from reduced diagnostic procedures and hospital readmissions associated with acute rejection, and most important from improved graft survival reducing cost of chronic dialysis.

This preservation trial in kidney transplantation is not without its limitations. As no preclinical evidence had investigated a potential effect of $HMPO_2$ on acute rejection at time of trial design, information on calcineurin inhibitor trough levels, donor specific antibody titres, proteinuria, and independent scoring of biopsies was not collected. Future trials investigating oxygenation in organ preservation should consider collecting the necessary data allowing in-depth analysis of any effect on acute rejection.

We suggest that using eGFR as a primary endpoint in kidney transplantation trials requires some consideration. In this preservation trial, the primary endpoint should be interpreted with its sensitivity analysis. Indeed, when reporting eGFR only in those pairs of which both kidneys are still functioning at 12 months post-transplant excludes informative dropouts, possibly relevant and associated with the trial intervention, which are considered in the sensitivity analysis. One could argue that the sensitivity analysis of the primary outcome should have been the primary outcome from the start, although, as an effect on graft survival was not anticipated, underlying assumptions would have weakened the sample size calculation.

While we had anticipated to determine renal function from a 24-hour urinary creatinine clearance, it proved necessary to change this to eGFR, originally a secondary endpoint, during the trial. During recruitment, while data were still blinded, a high proportion of creatinine clearance values was observed to be missing as many centres had abandoned this method of

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assessing renal function in favour of the CKD-EPI equation. Therefore, the data monitoring committee, investigators, and trial statistician jointly decided to change the way that renal function was determined. eGFR at 12 months is independently associated with longer-term graft survival in all donor types.[17] Also, eGFR presents a clinically important outcome measure already integrated in daily practice and was reproducibly attainable in all participating centres. The CKD-EPI equation was therefore chosen to replace the 24-hour urinary creatinine clearance to estimate GFR as this better reflects true GFR of the transplanted kidney compared to other calculations of GFR or serum creatinine values.[16] Our findings are supported by the analysis of the original primary endpoint that showed improved 24-hour urinary creatinine clearance at 12 months post-transplant in the $HMPO_2$ group available in 77 out of the 106 kidney pairs.

As this trial focused on older DCD kidneys, it remains to be demonstrated to which extent the benefits of $HMPO_2$ will apply to kidneys donated after brain death, and whether oxygenation during the entire preservation period is necessary. In future studies, comparing $HMPO_2$ with other emerging perfusion strategies, such as normothermic perfusion, will be important.

This international multicentre trial in kidney preservation has demonstrated that $HMPO_2$, compared to standard HMP, confers a clinically relevant benefit. $HMPO_2$ improved renal function when keeping improved graft survival into account and reduced severe post-operative complications as well as kidney rejection after transplantation of DCD-kidneys. As cost for additional oxygen is low and benefits for patients appear considerable, this new and rather simple extension to the current preservation strategy has the potential for quick implementation in clinical practice to improve patient outcomes and reduce healthcare cost.

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Contributors

IJ, SRK, HL, JP, RJP designed this study with help from other authors. RJP is Coordinator of the COPE Consortium. IJ, AB, HSH, FvdL, JP, RJP oversaw data collection. IJ, HSH, RJP. were responsible for the clinical conduct of the study in the respective trial regions. IJ, LD, SRK were responsible for the statistical design and analysis. SRK provided governance oversight to ensure the study adhered to all regulatory and ethical requirements. IJ wrote the manuscript with input from all authors. All authors reviewed the manuscript. JP and RJP contributed equally to this work.

Declaration of interests

AB and HL report support from the European Commission 7th Framework Programme. SRK reports personal fees from OrganOx Ltd, outside the submitted work. HL is board member of Dutch Transplantation Society and member of the implementation group for Machine Perfusion in the Netherlands.

All other authors declare no competing interests. Organ Assist provided the perfusion device and disposables and were not involved in study design, conduct, data analysis, or manuscript preparation.

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Data sharing

The COPE consortium supports wider dissemination of information from the research it conducts to increase cooperation between investigators. De-identified individual participant data and data dictionary will be made available to researchers on request as detailed in the Appendix.

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Reference: Jochmans et al, *Lancet.* 2020; 396: 1653-62.

References

1. Coemans M, Susal C, Dohler B, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int* 2018; **94**(5): 964–73.

2. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**(1): 7–19.

3. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg* 2010; **252**(5): 756–64.

4. Tingle SJ, Figueiredo RS, Moir JA, Goodfellow M, Talbot D, Wilson CH. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. *Cochrane Database Syst Rev* 2019; **3**: Cd011671.

5. Treckmann J, Nagelschmidt M, Minor T, Saner F, Saad S, Paul A. Function and quality of kidneys after cold storage, machine perfusion, or retrograde oxygen persufflation: results from a porcine autotransplantation model. *Cryobiology* 2009; **59**(1): 19–23.

6. Buchs JB, Lazeyras F, Ruttimann R, Nastasi A, Morel P. Oxygenated hypothermic pulsatile perfusion versus cold static storage for kidneys from non heart-beating donors tested by in-line ATP resynthesis to establish a strategy of preservation. *Perfusion* 2011; **26**(2): 159–65.

7. Thuillier R, Allain G, Celhay O, et al. Benefits of active oxygenation during hypothermic machine perfusion of kidneys in a preclinical model of deceased after cardiac death donors. *J Surg Res* 2013; **184**(2): 1174–81.

8. Darius T, Gianello P, Vergauwen M, et al. The effect on early renal function of various dynamic preservation strategies in a preclinical pig ischemia-reperfusion autotransplant model. *Am J Transplant* 2019; **19**(3): 752–62.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process. https://doi.org/10.1016/S0140-6736(20)32411-9
Reference: Jochmans et al, *Lancet.* 2020; 396: 1653-62.

9. Patel K, Smith TB, Neil DAH, et al. The Effects of Oxygenation on Ex Vivo Kidneys Undergoing Hypothermic Machine Perfusion. *Transplantation* 2019; **103**(2): 314–22.

10. Venema LH, Brat A, Moers C, et al. Effects of Oxygen During Long-term Hypothermic Machine Perfusion in a Porcine Model of Kidney Donation After Circulatory Death. *Transplantation* 2019; **103**(10): 2057–64.

11. O'Callaghan JM, Pall KT, Pengel LHM, Consortium for Organ preservation in E. Supplemental oxygen during hypothermic kidney preservation: A systematic review. *Transplantation reviews (Orlando, Fla)* 2017; **31**(3): 172–9.

12. Heylen L, Jochmans I, Samuel U, et al. The duration of asystolic ischemia determines the risk of graft failure after circulatory-dead donor kidney transplantation: A Eurotransplant cohort study. *Am J Transplant* 2018; **18**(4): 881–9.

13. l'Agence de la biomédecine. Le rapport médical et scientifique du prélèvement et de la greffe en France, greffe rénale. 2017. https://www.agence-biomedecine.fr/annexes/bilan2017/donnees/organes/06-rein/synthese.htm (accessed 08/02/2019).

14. Cannon RM, Brock GN, Garrison RN, Marvin MR, Franklin GA, Davis EG. Machine perfusion: not just for marginal kidney donors. *Am Surg* 2015; **81**(6): 550–6.

15. de Kok MJ, McGuinness D, Shiels PG, et al. The Neglectable Impact of Delayed Graft Function on Long-term Graft Survival in Kidneys Donated After Circulatory Death Associates With Superior Organ Resilience. *Ann Surg* 2019; **270**(5): 877–83.

16. Shaffi K, Uhlig K, Perrone RD, et al. Performance of creatinine-based GFR estimating equations in solid-organ transplant recipients. *Am J Kidney Dis* 2014; **63**(6): 1007–18.

17. Smith-Palmer J, Kalsekar A, Valentine W. Influence of renal function on long-term graft survival and patient survival in renal transplant recipients. *Curr Med Res Opin* 2014; **30**(2): 235–42.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process. https://doi.org/10.1016/S0140-6736(20)32411-9
Reference: Jochmans et al, *Lancet.* 2020; 396: 1653-62.

18. Levey AS, Stevens LA, Schmid CH, et al. A New Equation to Estimate Glomerular Filtration Rate. *Annals of internal medicine* 2009; **150**(9): 604–12.

19. European Commission. Guidelines on Medical Devices. Clinical Investigations: Serious Adverse Event Reporting. Report No. MEDDEV 2.7/3: European Commission, 2010.

20. Clavien PA, Barkun J, de Oliveira ML, et al. The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009; **250**(2): 187–96.

21. Cooper BA, Branley P, Bulfone L, et al. The Initiating Dialysis Early and Late (IDEAL) study: study rationale and design. *Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis* 2004; **24**(2): 176–81.

22. Kron P, Schlegel A, Muller X, Gaspert A, Clavien PA, Dutkowski P. Hypothermic Oxygenated Perfusion: A Simple and Effective Method to Modulate the Immune Response in Kidney Transplantation. *Transplantation* 2019; **103**(5): e128–e36.

23. Hoyer DP, Gallinat A, Swoboda S, et al. Influence of oxygen concentration during hypothermic machine perfusion on porcine kidneys from donation after circulatory death. *Transplantation* 2014; **98**(9): 944–50.

24. Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. *Nat Immunol* 2017; **18**(5): 488–98.

25. Fuquay R, Renner B, Kulik L, et al. Renal ischemia-reperfusion injury amplifies the humoral immune response. *J Am Soc Nephrol* 2013; **24**(7): 1063–72.

26. Lefaucheur C, Gosset C, Rabant M, et al. T cell-mediated rejection is a major determinant of inflammation in scarred areas in kidney allografts. *Am J Transplant* 2018; **18**(2): 377–90.

27. Cippa PE, Liu J, Sun B, Kumar S, Naesens M, McMahon AP. A late B lymphocyte action in dysfunctional tissue repair following kidney injury and transplantation. *Nat Commun* 2019; **10**(1): 1157.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process. https://doi.org/10.1016/S0140-6736(20)32411-9
Reference: Jochmans et al, *Lancet.* 2020; 396: 1653-62.

28. Wu WK, Famure O, Li Y, Kim SJ. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. *Kidney Int* 2015; **88**(4): 851–8.

29. Groen H, Moers C, Smits JM, et al. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. *Am J Transplant* 2012; **12**(7): 1824–30.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process. https://doi.org/10.1016/S0140-6736(20)32411-9
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Tables

Table 1: Baseline donor characteristics of the intention-to-treat population

Variable	HMPO$_2$ (N=106)	HMP (N=106)
Donor characteristics		
Age (y), median (IQR)	58·0 (54·0 - 63·0)	
Gender, N (%)		
Female	40 (38%)	
Male	66 (62%)	
Body mass index (kg/m²), median (IQR)	25 (23 - 28)	
Condition leading to death, N (%)		
Trauma	16 (15%)	
CVA	42 (40%)	
Hypoxia	39 (37%)	
Other	9 (9%)	
CMV status		
Positive	44 (42%)	
Negative	61 (58%)	
Unknown	1 (1%)	
Arterial hypertension, N (%) (missing: 6)	29 (27%)	

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Last creatinine (mg/dL) , median (IQR)	0·7 (0·6 - 0·9)
Donor warm ischaemic time (min) , median (IQR)	28·5 (22 - 36)

CMV, cytomegalovirus; CVA, cerebrovascular accident; HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion

Median (range) is given for continuous variables

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Table 2: Baseline recipient and transplant characteristics of the intention-to-treat

Recipient characteristics	**HMPO$_2$ (N=106)**	**HMP (n=106)**	**p value***
Age (y), median (IQR)	60 (53 - 68)	61 (51 - 65)	0·30
Gender, N (%)			
Female	37 (35%)	39 (37%)	0·12
Male	69 (65%)	67 (63%)	
Previous transplant, N (%)	4 (4%)	4 (4%)	0·99
Panel-reactive antibody level, N (%)			
0-10%	90 (85%)	89 (84%)	0·80
10-84%	8 (8%)	9 (9%)	
≥85%	2 (2%) **	0 (0%)	
Missing	6 (5·7%)	8 (8%)	
CMV status			
Positive	61 (58%)	64 (61%)	0·79
Negative	42 (40%)	36 (34%)	
Unknown	2 (2%)	1 (1%)	
Missing	1 (1%)	5 (5%)	
Immunosuppressive drugs, N (%)			
Prednisolone	100 (94%)	98 (93%)	0·34
Cyclosporine	2 (2%)	1 (1%)	-
Tacrolimus	103 (97%)	104 (98%)	0·65
Azathioprine	1 (1%)	1 (1%)	-
Mycophenolate mofetil	104 (98%)	103 (97%)	0·94

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Antithymocyte globulin	9 (9%)	14 (13%)	0·13
Interleukin-2 receptor antagonists	52 (49%)	56 (53%)	0·34
Transplant characteristics			
HLA mismatches, N (%) (missing = 1)			
0	5 (5%)	5 (5%)	
1	8 (8%)	5 (5%)	
2	20 (19%)	20 (19%)	
3	32 (30%)	34 (32%)	
4	29 (27%)	33 (31%)	
5	7 (7%)	5 (5%)	
6	5 (5%)	3 (3%)	
CMV mismatch			
Yes	53 (50%)	50 (47%)	0·89
No	49 (46%)	49 (46%)	
Missing	4 (4%)	7 (7%)	
Cold ischaemia time (h), median (IQR) (missing = 4)	11·0 (8·7 - 13·7)	10·3 (8·9 - 14·0)	0·41
Perfusion time (h), median (IQR) (missing = 21)	6·85 (4·5 - 9·1)	7·40 (4·8 - 9·9)	0·21

CMV, cytomegalovirus; HLA, human leukocyte antigen; HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion

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* p values were calculated with the use of a paired t-test or Wilcoxon rank-sum test for continuous variables and McNemar's test for discrete variables. The unit of randomisation was donor kidney pairs and not recipients.

** These recipients did not develop biopsy-proven acute rejection

Median (range) is given for continuous variables

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Table 3: Univariable differences between the groups

Variable	$HMPO_2$ Mean (SD)	HMP Mean (SD)	Mean Difference (95% CI)	p value*
Primary endpoint				
GFR at 12 months post-transplant (ml/min/1·73m²); (CKD-EPI equation[29])				
Primary comparison (N=83)	50·5 (19·3)	46·7 (17·1)	3·7 (-1·0 to 8·4)	0·12
Sensitivity analysis (N=106)	47·6 (20·1)	42·6 (20·3)	5·0 (0·4 to 9·7)	0·035
Secondary endpoints	**$HMPO_2$ Mean (SD)**	**HMP Mean (SD)**	**Risk Difference (95% CI)**	**p value***
Primary non function, N (%) (N=106)	3 (3%)	5 (5%)	-2 (-7% to 3%)	0·48
Delayed graft function, N (%) (N=106)	38 (36%)	38 (36%)	0 (-14% to 14%)	0·99
Functional delayed graft function (N; %) (N=106)	76 (72)	76 (72)	0 (-13% to 11%)	0·99
Biopsy-proven acute rejection (N; %) (N=106)	15 (14)	27 (26)	-11 (-22% to -0·01%)	0·040

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Renal function	**$HMPO_2$ Mean (SD)**	**HMP Mean (SD)**	**Mean Difference (95% CI)**	**p value***
GFR 3 months post-transplant (ml/min/1·73m²)				
CKD-EPI equation (N=88)	46·5 (18·2)	45·0 (16·9)	1·5 (-3·2 to 6·3)	0·53
MDRD equation (N=89)	44·8 (15·7)	44.3 (23·8)	0·5 (-5·2 to 6·1)	0·87
GFR 6 months post-transplant (ml/min/1·73m²)				
CKD-EPI equation (N=83)	50·1 (18·5)	47·1 (19·6)	3·0 (-1·8 to 7·7)	0·22
MDRD equation (N=85)	48·1 (17·7)	44·7 (17·9)	3·4 (-1·2 to 8·0)	0·15
GFR 12 months post-transplant				
MDRD equation (ml/min/1·73m²) Primary comparison (N=83)	48·8 (19·5)	44·4 (15·4)	4·4 (-0·2 to 9·1)	0·062
MDRD equation (ml/min/1·73m²) Sensitivity analysis (N=106)	46·1 (19·9)	40·7 (18·8)	5·4 (0·8 to 10·0)	0·021
Creatinine clearance in 24-hour urine	58·2 (21·4)	51·1 (21·9)	7·1 (1·1 to 13·0)	0·021

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collection (ml/min) (N=77)				

CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate; HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion; MDRD, Modification of Diet in Renal Disease; SD, standard deviation. * p values were calculated with the use of a paired t-test or Wilcoxon rank-sum test for continuous variables and the McNemar's test for discrete variables.

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Table 4: Survival probability for graft and patient at each follow-up timepoint

Variable	$HMPO_2$ Survival probability (95% CI)	HMP Survival probability (95% CI)
Graft survival		
7 days	0·98 (0·93 to 0·99)	0·95 (0·89 to 0·98)
3 months	0·97 (0·91 to 0·99)	0·92 (0·85 to 0·96)
6 months	0·97 (0·91 to 0·99)	0·91 (0·83 to 0·95)
12 months	0·97 (0·91 to 0·99)	0·89 (0·82 to 0·94)
Patient survival		
7 days	1·00 (-)	0·99 (0·94 to 0·99)
3 months	0·94 (0·88 to 0·97)	0·95 (0·89 to 0·98)
6 months	0·94 (0·88 to 0·97)	0.95 (0·89 to 0·98)
12 months	0·93 (0·87 to 0·97)	0·93 (0·83 to 0·96)

CI, confidence interval; HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion

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Table 5: Adverse events and Serious Adverse Events according to the MEDDEV guidelines.

	$HMPO_2$ (N=141)	**HMP (N=133)**
Any adverse event during donor procedure (N)	20	7
Damaged polar artery	8	2
Massive atherosclerosis preventing safe connection to the device	5	1
Multiple renal arteries and no appropriate patch holder available	1	1
Device issue preventing correct set-up	6	3
Any adverse event during organ preservation (N)	6	8
Oxygen (not) administered erroneously	5	7
Perfusate leakage	1*	1
Serious adverse events in recipients (N)	213	209
Cardiovascular		
Cardiac failure	8	2
Myocardial infarction	3	3
Diarrhoea / vomiting	28	12
Electrolyte disturbances	5	8
Infection		
Abdomen	3	2
Chest	16	7
CMV infection / reactivation	5	8

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Sepsis	13	10
Urinary tract	34	20
Wound	4	2
Kidney dysfunction	57	59
Malaise	7	10
Permanent graft failure	7	13
Related to surgery		
Arterial stenosis	2	4
Arterial thrombosis	0	2
Bleeding	4	9
Lymphocele	0	3
Ureteral stenosis	7	11
Ureteral necrosis	3	4
Venous thrombosis	1	1
Seroma	2	1
Surgical revision within 12 months	4	18
Respiratory failure	4	9
Suspicion of rejection	32	32
Transfusion	11	11
Deaths and cause of death		
Cardiac event	3	1
Infection leading to sepsis	2	4
Cerebrovascular event	0	1
Cancer	2	0

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Multiple organ failure	0	1
Death from unknown cause	1	3
Adverse events in recipients (N)	204	265
Diarrhea / vomiting	8	10
Electrolyte disturbances	9	12
Infection		
Abdomen	2	0
Chest	3	5
CMV infection / reactivation	4	9
Urinary tract	15	11
Wound	5	4
Kidney dysfunction	11	22
Lymphocele	0	4
Malaise	4	8
Seroma	1	1
Suspicion of rejection	7	12
Transfusion	1	2
Ureteral stenosis	0	1
Other	139	179

* leading to kidney discard (See Appendix for further details)

CMV, cytomegalovirus; HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion

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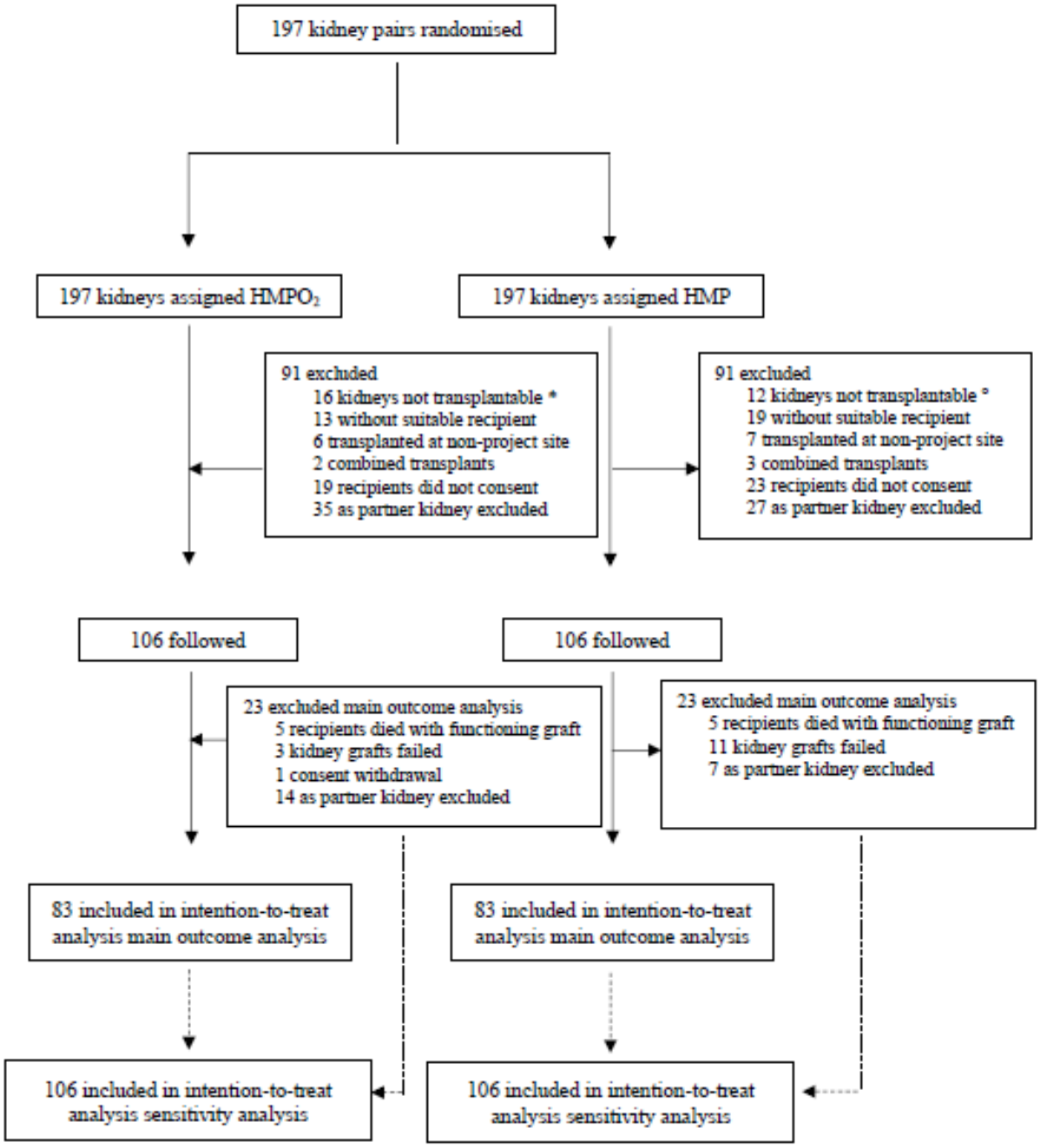

Figure 1: Trial profile. The drop-out rate between randomisation and final analysis is the direct consequence of the paired design of the trial and matches the predicted drop-out rate. *, 7 at donor centre, 9 at recipient centre; °, 6 at donor centre, 6 at recipient centre

HMP, hypothermic machine perfusion; $HMPO_2$, oxygenated hypothermic machine perfusion

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Reference: Jochmans et al, *Lancet.* 2020; 396: 1653-62.