

Supplementary Appendix

List of Participating Transplant Centers (Centers and Names)

Austria (4 centers, 17 recipients) – **Landeskrankenhaus Graz:** H. Müller, H. Holzer; **Universitätsklinik für Chirurgie Innsbruck:** R. Margreiter, C. Bösmüller, W. Mark, H. Fetz; **Allgemeines Krankenhaus der Stadt Linz:** C. Gross, B. Schmekal; **Allgemeines Krankenhaus Wien:** F. Mühlbacher, I. Kristo, M. Pones, G. Györi.

Belgium (7 centers, 183 recipients) – **Universitair Ziekenhuis Antwerpen:** D. Ysebaert, J.L. Bosmans, G. Van Beeumen, W. Van Donink; **Universitair Ziekenhuis Brussel:** J. Lamote, J. Sennesael, B. Amerijckx; **Hôpital Erasme Bruxelles:** A.D. Hoang, D. Mikhalski, D. Abramovicz, V. Brulein; **Universitair Ziekenhuis Gent:** C. Randon, P. Peeters, M. VanderVennet; **Cliniques Universitaires St. Luc Bruxelles:** M. Mourad, M. de Meyer, J. Malaise, L. De Pauw; **Centre Hospitalier Universitaire Liège:** J.P. Squifflet, L. Weekers, O. Detry, M.H. Delbouille; **Universitaire Ziekenhuizen Leuven:** J. Pirenne, Y. Vanrenterghem, F. van Gelder, B. Desschans.

Germany (38 centers, 327 recipients) – **Universitätsklinikum Aachen:** G. Jakse, D. Rohrmann, J. Floege, A. Homburg; **Knappschafts Krankenhaus Bochum:** R. Viebahn, O. Vonend, P. Schenker, A. Wunsch; **Universitätsklinik Bonn:** S.C. Müller, H. Klehr; **Universitätsklinikum Düsseldorf:** W. Sandmann, K. Ivens, A. Voiculescu, K. Balsler; **Universitätsklinikum Essen:** A. Paul, O. Witzke, J. Treckmann, A. Jonait-Borkenhagen; **Medizinische Universitätsklinik Köln-Lindenthal:** D. Stippel, Th. Benzing, K. Prenzel, B. Hoppe; **Städtische Krankenanstalten Köln-Merheim:** M. Ströhlein, W. Arns, R. Hackenberg, U. Lange; **Westfälische WU Klinikum Münster:** H. Wolters, B. Suwelack; **Zentralklinikum Augsburg:** E. Nagel, H. Weihprecht, R. Eser, T. Breidenbach; **Charité Berlin - Campus Benjamin Franklin:** K. Miller, M. Van der Giet, E. Krusic, M. Tölle; **Charité Berlin - Campus Mitte:** F. Fuller, K. Budde; **Charité Berlin - Campus Virchow:** J. Pratschke, P. Reinke, Th. Mehlitz; **Zentralkrankenhaus Bremen:** S. Melchior, F.A. Zantvoort, Ch. Bahrs, S. Meier; **Universitätsklinikum Carl Gustav Carus Dresden:** M. Wirth, P. Gross, S. Leike, J. Passemmer; **Klinikum der JW Goethe Universität Frankfurt:** M. Probst, E.-H. Scheuermann; **Klinikum der AL Universität Freiburg:** P. Pisarski, P. Gerke, M. Geyer, S. Hils; **Universitätsklinikum Halle:** A. Hamza, O. Rettkowski, K.

Fischer, A. Haberland; **Klinikum der Universität Heidelberg:** J. Schmidt, M. Zeier, B. Schmied, C. Sommerer; **Nephrologisches Zentrum Niedersachsen:** J. Küster, V. Kliem; **Medizinische Hochschule Hannover:** F. Lehner, A. Schwarz, M. Hiss, N. Mogilewskaja; **Universitätsklinik des Saarlandes Homburg/Saar:** M. Stöckle, M. Girndt, M. Janssen, U. Sester; **Medizinische Fakultät/Klinikum Jena:** Th. Steiner, O.H. Undine, J. Schubert, G. Wolf; **Universitätsklinikum Schleswig-Holstein Kiel:** D.C. Bröring, U. Kunzendorf, P. Glass, F. Braun; **Westpfalz-Klinikum Kaiserslautern:** W. Seybold-Epting, Th. Rath, A. Dahms; **Universitätskrankenhaus Leipzig:** J. Hauss, P. Martin, D. Weinert; **Universitätsklinikum Schleswig-Holstein Lübeck:** C. Bürk, M. Nitschke; **Klinikum der Stadt Mannheim:** M. Schwarzbach, P. Schnülle; **Klinikum Rechts der Isar München:** M.C. Raggi; **Klinikum Grosshadern München:** W.-D. Illner, M. Rentsch; **Klinikum Lahnberge Marburg/Lahn:** J. Geks, U. Kuhlmann, T. Maier, J. Hoyer; **Klinikum der Joh. Gutenberg Universität Mainz:** J. Thüroff, O. Schreiner, J. Jones, K. Allers; **Universitätskrankenhaus Erlangen-Nürnberg:** G. Schott, Ch. Hugo, K. Pressmar, K. Hirsch; **Medizinische Fakultät Rostock:** K. Stein, M. Burde; **Katharinenhospital Stuttgart:** M. Schock, G. Hasche, Ch. Olbricht, M. Kalus; **Chirurgische Universitätsklinik Tübingen:** W. Steurer, N. Heyne, Ch. Thiel, K. Knubben; **Universitätskrankenhaus Ulm:** J. Mayer, F. Keller, C. Brockschmidt, S. Stracke; **Klinikum der Bayerischen J-M-U Würzburg:** K. Lopau, R. Bonfig.

Luxemburg (1 center, 4 recipients) – **Centre Hospitalier de Luxembourg:** S. Lamy, P. Duhoux, E. Tasch, J. De Sousa.

The Netherlands (8 centers, 136 recipients) – **Academisch Medisch Centrum Amsterdam:** M.M. Idu, F.J. Bemelman, I. ten Berge, K. van Donselaar; **Universitair Medisch Centrum Groningen:** H.S. Hofker, V.B. Nieuwenhuijs, C. Krikke, M. van Dijk; **Leids Universitair Medisch Centrum:** J. Ringers, A.F.M. Schaapherder, J.W. de Fijter, J. Dubbeld; **Academisch Ziekenhuis Maastricht:** E. van Heurn, J. van Hooff, M. Christiaans; **UMC St. Radboud Nijmegen:** J.A. van der Vliet, A.J. Hoitsma; **Erasmus Medisch Centrum Rotterdam:** J.N.M. Yzermans, W. Weimar, J. Kal-van Gestel; **Universitair Medisch Centrum Utrecht:** R.W.H. van Reedt Dortland, R.J. Hené, V. Leydekkers, C. van Straalen; **Wilhelmina Kinderziekenhuis Utrecht:** M.R. Lilien.

Slovenia (1 center, 5 recipients) – **University Medical Center Ljubljana:** D. Kovac.

Ethics Committee Approval

Approval for the study was obtained from ethical review boards in each trial region, and from the Eurotransplant Ethical Advisory Committee and Kidney Advisory Committee. As the randomized intervention was limited to isolated organs before transplantation, according to national laws no informed consent from recipients was required. In addition, ethical rationale for not requiring informed consent for the organ preservation method was as follows: At the moment of randomization, as well as at the time point at which the randomized intervention had to be initiated, most kidneys would not yet be allocated to a potential recipient. Therefore, it would not be possible to obtain informed consent from the recipient before these important time points. As soon as organ allocation was known, no informed consent could be obtained from the potential recipient, as most national laws dictate a 24-hour consideration period, and it would be medically unacceptable to delay transplantation for this reason only. Moreover, should the potential recipient decide not to give informed consent, this would automatically imply that he or she would not receive the kidney, as the randomized intervention was already in progress at that moment. This would lead to an ethically unacceptable dilemma.

Two ethics committees (University Hospital Ghent and University Hospital of Leuven, Belgium) ruled that informed consent would be required to obtain follow-up data from recipients after transplantation in these hospitals, as this was a prospective study. This decision was respected by the steering committee. Other ethics committees did not require specific informed consent for this study's follow-up data retrieval, since the prospective randomized intervention was limited to isolated organs before allocation, and no more than standard clinical data were collected retrospectively without additional requirements due to the study that would affect the patient.

Randomization Process

To avoid regional imbalances between the two study arms due to slightly different allocation algorithms, a randomization scheme based on permuted blocks within regions was used with separate randomization lists for each of the three trial regions. Randomization lists were available only to the 24-hour Eurotransplant duty desk. Upon report of a kidney donor, the allocation officer first checked its eligibility and then assigned the left kidney to treatment with either machine perfusion or cold storage following one of the three randomization schemes, which automatically assigned the right kidney to preservation with the other method. Then both kidneys were offered according to the match list, without revealing the preservation method. Only if the kidney assigned to be machine perfused had a too small

aortic patch or too many renal arteries preventing a reliable connection to the machine perfusion device were surgical teams allowed to switch preservation methods during organ procurement, thus frustrating randomization.

Trial Safety Board

To prevent any bias in clinical decisions about transplanting or discarding an organ, machine perfusion dynamics data – such as intravascular resistance and flow readings – were never revealed to the transplant team. A safety board of experienced transplant surgeons was established and consulted on three occasions: In two out of three cases it felt the need to reveal machine perfusion dynamics data to the recipient center. In both of these cases the recipient centers saw no reason to discard the organ based on the additional information and transplanted the kidney.

Prespecified Covariates for the Logistic Regression and Cox Regression Analysis

- Machine perfusion vs. cold storage
- Panel-reactive antibody level (%)
- Recipient age (yr)
- Donor age (yr)
- Expanded criteria donor vs. standard criteria donor
- Cold ischemic time (h)
- HLA mismatches (no.)
- Duration of pretransplantation dialysis (yr)
- Retransplantation vs. first transplantation
- Non-heart beating donor vs. heart beating donor

Additional Non-Heart Beating Donor Inclusions

In September 2006, when donor inclusions into the study were nearly complete, the scientific steering committee expected that insufficient non-heart beating donors would be enrolled at trial completion to conduct a meaningful subgroup analysis for this donation type. At the suggestion of the steering committee and with permission of all centers, inclusions of only non-heart beating donors were extended, until a total of 82 were enrolled on 17 August 2007. Fig. S1 and Table S1 in this appendix present a Consort diagram and demographics for the two eras of non-heart beating donor inclusions. Solely for the heart beating/non-heart beating

subgroup analysis, these additional inclusions were added to the main set of cases to provide more statistical power. The main set of cases consisted of 336 kidney pairs (672 recipients), of which 42 (84 recipients) came from non-heart beating donors. The extended data set comprised a total of 376 kidney pairs (752 recipients). This total includes the 80 recipients of 40 non-heart beating kidney pairs who were later added to the main set for this analysis only. Therefore, the total number of non-heart beating kidney pairs in the extended data set was 82 (164 recipients). The same logistic regression model for delayed graft function with an interaction term for machine perfusion and non-heart beating donation was applied to the extended data set.

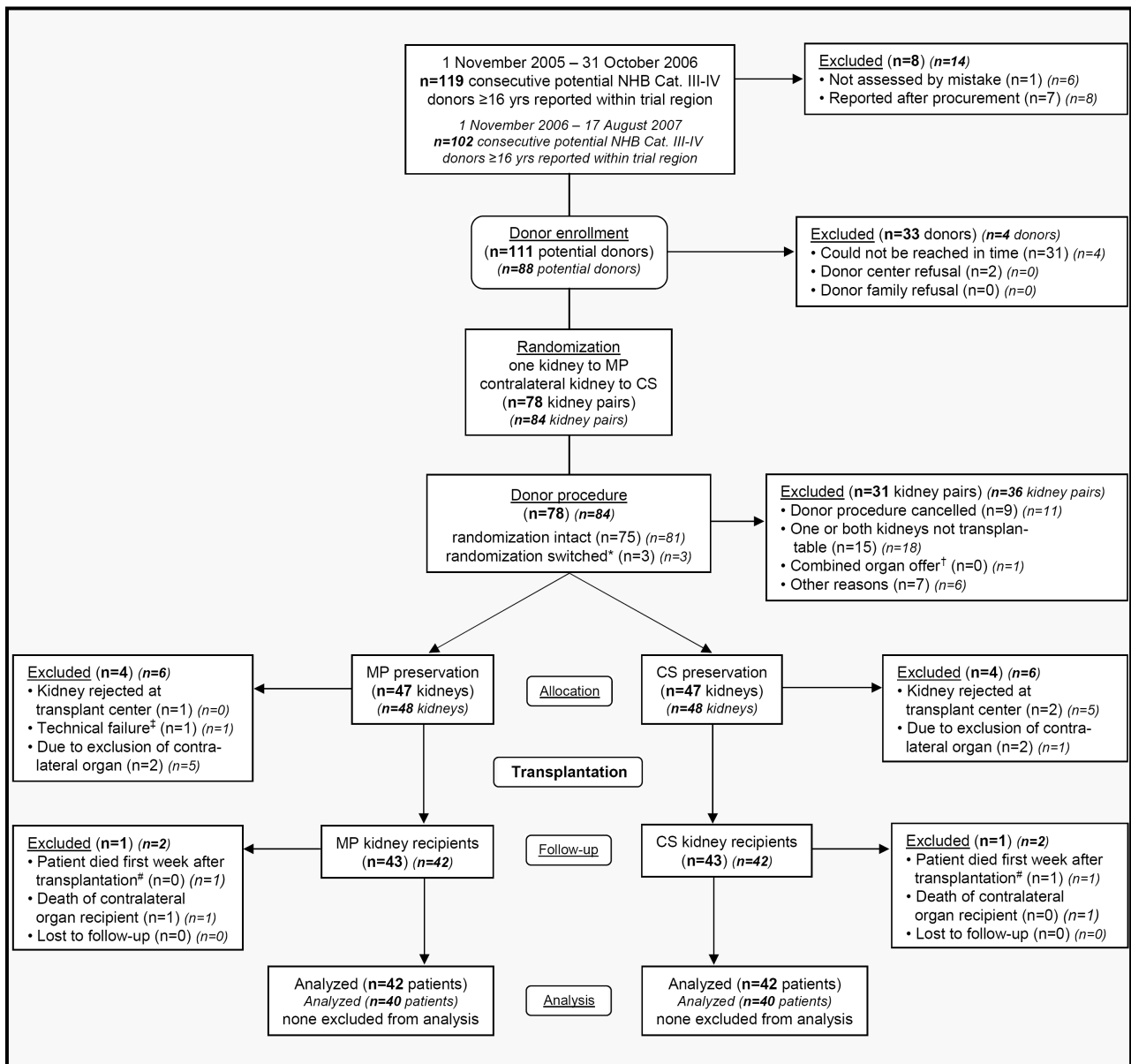


Figure S1. Enrollment, Group Assignment, Follow-up and Analysis of Non-Heart Beating Donor Inclusions.

Text and numbers in *italics* refer to the second era of non-heart beating donor inclusions, after main consecutive inclusions (heart beating and non-heart beating mixed) had ended. These extra non-heart beating donor cases were solely used for a subgroup analysis of the effect size of machine perfusion versus cold storage in non-heart beating versus heart beating kidney

grafts. NHB denotes non-heart beating, HB heart beating, MP machine perfusion, and CS cold storage.

* Switching randomization was only allowed when vascular anatomy made one kidney less suitable for the machine.

† Whenever one or both kidneys were offered together with another organ to one recipient, e.g. for combined pancreas–kidney or liver–kidney transplantation.

‡ None of these failures rendered the graft unsuitable for transplantation. When machine perfusion failed, the kidney was automatically cold stored inside the machine.

Causes of death were one transplant related and two non-transplant related events.

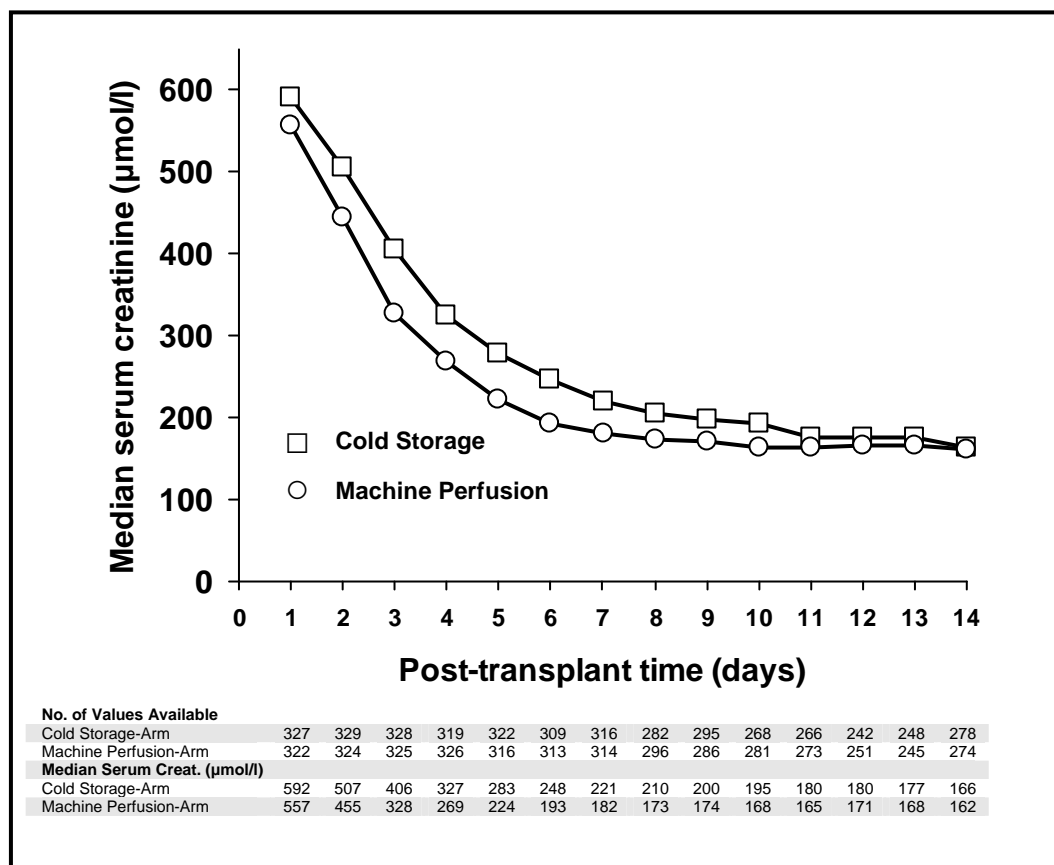


Figure S2. Time Course of Serum Creatinine after Transplantation.

Below the x-axis, the sample size available per post-transplant day, and median serum creatinine values per day are indicated. For each recipient, the area under the curve (AUC) was calculated. Missing values (12%) were imputed by means of linear interpolation. The median AUC of recipients in the machine perfusion-arm (1,456; range 385–5,782) was significantly lower than the median AUC of recipients in the cold storage-arm (1,787; range 288–6,500) (P=0.01; Wilcoxon signed rank test).

Each median value is based on all available cases for that group, and includes patients who were dialysis dependent.

Table S1. Demographics of Both Eras of Non-Heart Beating Donor Inclusions.*

| Variable | Era 1 (1 November 2005 – 31 October 2006) | | | Era 2 (1 November 2006 – 17 August 2007) | | | P value (Era 1 vs. Era 2) |
|--|---|------------------------------------|---|--|------------------------------------|---|------------------------------|
| | Machine Perfusion Arm (N = 42) | Cold Storage Arm (N = 42) | P value (Machine Perfusion vs. Cold Storage) | Machine Perfusion Arm (N = 40) | Cold Storage Arm (N = 40) | P value (Machine Perfusion vs. Cold Storage) | |
| Donor demographics | | | | | | | |
| Donor age (yr) | 41 (17–60) | | - | 48 (20–67) | | - | <0.001 |
| Maastricht category (III / IV) | 42 / 0 | | - | 40 / 0 | | - | - |
| SCD / ECD | 39 / 3 | | - | 29 / 11 | | - | 0.02 |
| Recipient demographics | | | | | | | |
| Recipient age (yr) | 49 (24–69) | 51 (27–77) | 0.66 | 48 (27–73) | 53 (24–76) | 0.94 | 0.45 |
| Duration of pre-transplant dialysis (yr) | 4.7 (1.1–18) | 4.4 (1.1–11) | 0.30 | 3.7 (1.0–10) | 3.5 (0.36–8.8) | 0.74 | 0.002 |
| Previous transplants (%)† | 19 | 19 | 0.61 | 0 | 0 | - | - |
| Panel-reactive antibodies (0–5% / 6–84% / >84%) | 90 / 10 / 0 | 81 / 17 / 2 | 0.17 | 83 / 17 / 0 | 93 / 7 / 0 | 0.16 | 0.46 |
| Prednisolon (%) | 98 | 98 | - | 95 | 100 | 0.25 | 0.67 |
| Cyclosporin (%) | 50 | 50 | - | 38 | 25 | 0.17 | 0.02 |
| Tacrolimus (%) | 50 | 52 | 0.50 | 53 | 75 | 0.03 | 0.12 |

| | | | | | | | |
|---|-----------|-----------|------|-----------|-----------|------|------|
| Azathioprine (%) | 2 | 2 | - | 0 | 0 | - | - |
| Mycophenolate mofetil (%) | 88 | 86 | 0.53 | 93 | 83 | 0.25 | 0.55 |
| Anti-thymocyte globulin (%) | 10 | 10 | - | 20 | 20 | - | 0.08 |
| Interleukin-2 receptor antagonists (%) | 31 | 40 | 0.25 | 45 | 25 | 0.05 | 0.53 |
| Transplant demographics | | | | | | | |
| Human leukocyte antigen mismatches (% of 0 mismatches)‡ | 2 | 0 | 0.10 | 3 | 8 | 0.83 | 0.31 |
| Cold ischemic time (h) | 16 (4–25) | 16(10–23) | 0.54 | 15(10–29) | 18 (9–47) | 0.41 | 0.70 |

* If not indicated otherwise, values are expressed as median (range). P values were obtained by Fisher’s exact test for discrete variables and by the Mann–Whitney test for continuous variables. SCD denotes standard criteria donation, and ECD expanded criteria donation.

† Indicates the percentage of recipients who had undergone one or more renal transplants prior to the one included in this analysis.

‡ Indicates the percentage of transplants with zero human leukocyte antigen A/B/DR mismatches.

Table S2. Multivariate Risk Analysis for Delayed Graft Function.*

| Variable | Hazard Ratio (95% Confidence Interval) | P value |
|--|---|----------------|
| Delayed Graft Function | | |
| Machine perfusion vs. cold storage | 0.59 (0.38–0.92) | 0.02 |
| Aberrant vascular anatomy† | 1.00 (0.56–1.77) | 0.98 |
| Panel-reactive antibody level (%) | 1.01 (0.99–1.02) | 0.28 |
| Recipient age (yr) | 1.01 (0.99–1.03) | 0.37 |
| Donor age (yr) | 1.03 (1.00–1.06) | 0.05 |
| ECD donor vs. SCD donor | 1.08 (0.47–2.45) | 0.86 |
| Cold ischemic time (h) | 1.08 (1.02–1.14) | 0.005 |
| HLA mismatches (no.) | 1.12 (0.93–1.35) | 0.23 |
| Duration of pre-transplant dialysis (yr) | 1.15 (1.02–1.29) | 0.02 |
| Retransplant vs. first transplant | 3.05 (1.77–5.25) | <0.001 |
| NHB donor vs. HB donor | 17.3 (8.15–36.9) | <0.001 |

* Logistic regression model for the risk of delayed graft function, with aberrant vascular anatomy as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Aberrant vascular anatomy was defined as >1 renal artery of the kidney graft.

Table S3. Multivariate Risk Analysis for Graft Failure.*

| Variable | Hazard Ratio (95% Confidence Interval) | P value |
|---|---|----------------|
| Graft Failure within One Year Post-transplant† | | |
| Machine perfusion vs. cold storage | 0.52 (0.29–0.92) | 0.03 |
| NHB donor vs. HB donor | 0.87 (0.27–2.81) | 0.82 |
| Recipient age (yr) | 0.97 (0.95–1.00) | 0.02 |
| Duration of pre-transplant dialysis (yr) | 1.00 (0.87–1.15) | 0.97 |
| Panel-reactive antibody rate (%) | 1.01 (0.99–1.03) | 0.30 |
| Cold ischemic time (h) | 1.04 (0.97–1.10) | 0.29 |
| Donor age (yr) | 1.05 (1.01–1.10) | 0.02 |
| ECD donor vs. SCD donor | 1.17 (0.42–3.27) | 0.76 |
| HLA mismatches (no.) | 1.23 (0.98–1.55) | 0.07 |
| Aberrant vascular anatomy‡ | 1.35 (0.66–2.74) | 0.41 |
| Retransplant vs. first transplant | 1.73 (0.89–3.36) | 0.11 |

* Cox proportional hazards model for the risk of graft failure within one year post-transplant, with aberrant vascular anatomy as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Censored upon death with a functioning graft.

‡ Aberrant vascular anatomy was defined as >1 renal artery of the kidney graft.

Table S4. Multivariate Risk Analysis for Graft Failure.*

| Variable | Hazard Ratio (95% Confidence Interval) | P value |
|---|---|----------------|
| Graft Failure within One Year Post-transplant† | | |
| NHB donor vs. HB donor | 0.48 (0.16–1.48) | 0.20 |
| Machine perfusion vs. cold storage | 0.60 (0.34–1.06) | 0.08 |
| Duration of pre-transplant dialysis (yr) | 0.96 (0.84–1.10) | 0.56 |
| Recipient age (yr) | 0.97 (0.95–0.99) | 0.005 |
| Panel-reactive antibody level (%) | 1.01 (0.99–1.02) | 0.54 |
| Cold ischemic time (h) | 1.02 (0.95–1.08) | 0.61 |
| Donor age (yr) | 1.04 (1.00–1.08) | 0.03 |
| ECD donor vs. SCD donor | 1.10 (0.44–2.72) | 0.84 |
| Retransplant vs. first transplant | 1.13 (0.58–2.18) | 0.72 |
| HLA mismatches (no.) | 1.19 (0.96–1.47) | 0.12 |
| Delayed graft function‡ | 1.69 (1.35–2.11) | <0.001 |

* Cox non-proportional hazards model for the risk of graft failure within one year post-transplant, with delayed graft function as extra covariate, added post hoc. SCD denotes standard criteria donation, ECD expanded criteria donation, NHB non-heart beating, and HB heart beating.

† Censored upon death with a functioning graft.

‡ Delayed graft function was added to the model as a time dependent covariate.