Excellent efficacy and beneficial safety during observational 5-year follow-up of rapid steroid withdrawal after renal transplantation (Harmony FU study)

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ABSTRACT

Background. We previously reported excellent efficacy and improved safety aspects of rapid steroid withdrawal (RSWD) in the randomized controlled 1-year "Harmony" trial with 587 predominantly deceased-donor kidney transplant recipients randomized either to basiliximab or rabbit antithymocyte globulin induction therapy and compared with standard immunosuppressive therapy consisting of basiliximab, low tacrolimus once daily, mycophenolate mofetil and corticosteroids.

Methods. The 5-year post-trial follow-up (FU) data were obtained in an observational manner at a 3- and a 5-year visit only for those Harmony patients who consented to participate and covered clinical events that occurred from the second year onwards.

Results. Biopsy-proven acute rejection and death-censored graft loss rates remained low and independent of RSWD. Rapid steroid withdrawal was an independent positive factor for patient survival (adjusted hazard ratio 0.554, 95% confidence interval 0.314–0.976; $P = .041$).
The reduced incidence of post-transplantation diabetes mellitus in RSWD patients during the original 1-year study period was not compensated by later incidences during FU. Incidences of other important outcome parameters such as opportunistic infections, malignancies, cardiovascular morbidity/risk factors, donor-specific antibody formation or kidney function did not differ during FU period.

Conclusions. With all the limitations of a post-trial FU study, the Harmony FU data confirm excellent efficacy and beneficial safety aspects of RSWD under modern immunosuppressive therapy over the course of 5 years after kidney transplantation in an immunologically low-risk, elderly population of Caucasian kidney transplant recipients.

Trial registration: Clinical trial registration number: Investigator Initiated Trial (NCT 00724022, FU study DRKS00005786)

Keywords: Harmony study, long-term follow-up, mortality, rapid steroid withdrawal, renal transplantation

INTRODUCTION

Corticosteroids are potent immunosuppressive agents that are still part of the current standard of immunosuppressive therapy after renal transplantation. Based on the extensive corticosteroid side effect profile such as accelerated cardiovascular risk factors (diabetes mellitus, hyperlipidemia, hypertension, weight gain), one-third of all kidney transplant recipients in the USA, but much fewer in Europe, have corticosteroids withdrawn mostly within the first week after transplantation [1].

KEY LEARNING POINTS

What was known:

- Short-term results of rapid steroid withdrawal (RSWD) 1 year after renal transplantation demonstrate excellent efficacy and improved safety aspects compared with the current gold standard of immunosuppressive therapy consisting of basiliximab induction followed by corticosteroids, mycophenolate and tacrolimus (Harmony study).
- Hereby, RSWD combined with induction therapy using either basiliximab or low-dose thymoglobulin led to equivalent 1 year results. RSWD allowed almost a bisection of the rate of posttransplantation diabetes mellitus but did not lead to an increase of biopsy-proven acute rejections (BPAR) compared with continuous corticosteroid use.
- In another study with relatively young and predominantly living donor–related transplant recipients, long-term results of RSWD were equivalently effective and safe compared with corticosteroid treatment.

This study adds:

- The observational 5-year follow-up (FU) investigation of our large Harmony trial shows translation of the equivalent efficacy and improved safety aspects of RSWD compared with the corticosteroid treatment arm from the 1-year results to the 5-year period.
- During 5-year FU, BPAR, death-censored graft loss as well as donor-specific antibody rates remained low and independent of RSWD. The reduced incidence of post-transplantation diabetes mellitus in RSWD patients during the 1-year study period was not compensated by later incidences during FU.
- With all the limitations of a post-trial FU study, the study data suggest RSWD as an independent positive factor for patient survival, which may relate to the elderly recipient population with a long time on dialysis exposed to predominantly elderly deceased-related donors, all of which are risk factors for patient survival after renal transplantation.

Potential impact:

- The long term results of the Harmony post-trial FU study demonstrate RSWD as a safe procedure and suggest even improved patient survival, supporting the RSWD approach to become at least a promising new alternative to the current corticosteroid-continuing gold standard treatment in an immunologically low-risk, elderly population of Caucasian kidney transplant recipients.
- This remarkable result likely relates to this typical elderly recipient population at risk, which has become the standard population in most European countries. These results fit with the long-term results of another RSWD study [16] demonstrating equivalent efficacy and safety in a much younger, living donor–related renal transplant population.
- Taken together, these data should secure and stimulate renal transplant centers to make use of the RSWD approach minimizing corticosteroid-induced side effects without losing long-term efficacy or safety for their patients.

Nevertheless, due to a scarcity of randomized controlled trials, the KDIGO recommendation for a rapid corticosteroid withdrawal in renal transplant recipients with low immunological risk is only based on evidence level 2B (2: suggested, B: moderate quality of evidence) [2]. Recent registry data are all influenced by a selection bias but suggest that steroid-withdrawal protocols lead to reduced post-transplantation diabetes mellitus (PTDM) rates as well as superior long-term graft and patient survival [3, 4]. Conversely, corticosteroid-free or -withdrawal regimens were frequently associated with increased T cell–mediated acute rejection rates [5, 6], while long-term data on the frequency of HLA-sensitization and humoral rejections are rare.

In the original investigator-initiated, randomized, multicenter Harmony trial, 587 kidney transplant recipients with low immunological risk profile were equally assigned to three different treatment arms to evaluate which of the two induction agents (basiliximab or rabbit ATG) was most efficacious and safe at permitting rapid steroid withdrawal (RSWD) in a prolonged release tacrolimus, mycophenolate mofetil (MMF) and prednisolone-based immunosuppressive therapy (control arm) within the first year after kidney transplantation [7]. Both induction therapies combined with RSWD were equivalent to permanent corticosteroid control patients for the prevention of biopsy-proven acute rejection (BPAR) within the first year after transplantation. Patient and death-censored graft survival rates as well as most safety parameters in the first year were excellent and similar in all groups independent of RSWD. In contrast, RSWD in both groups (24%/23%) markedly and

equivalently reduced the incidence of PTDM compared with control patients (39%).

The 5-year post-trial follow-up (FU) data of the Harmony trial reported here were obtained in an observational manner to evaluate how these excellent short-term surrogate parameters will translate into hard long-term outcome parameters like patient death/graft loss or will be complicated by increased rates of immunosensitization and humoral rejections.

MATERIALS AND METHODS

Study design and medication

This investigator-initiated, observational post-trial FU study was implemented to compare the 3- and 5-year safety and efficacy of basiliximab- or rATG-induced RSWD in immunologically low-risk kidney transplant recipients receiving low-dose tacrolimus and MMF/mycophenolic acid (MPA). The study was approved by the institutional review boards of all sites participating and written informed consent had to be obtained again from patients participating in the post-trial FU study (FU study DRKS00005786).

The original Harmony trial (NCT 00724022) was an investigator-initiated 1-year study in 21 German transplant centers performed as prospective, randomized, open-label, multicenter study in three parallel study arms of adult renal transplant recipients.

In the post-trial FU study, all patients alive after the Harmony trial and recruited by centers participating in the FU investigation were eligible. Transplant recipients were followed in a

non-interventional manner according to center standard. FU data were collected at 3- and 5-year visits for patients who consented to participate and covered clinical events that occurred from the second year onwards. In addition, deaths occurring after the original trial but before informed consent to FU investigation were included in the present analyses.

Medication, inclusion and exclusion criteria as well as study endpoints are described within the Supplementary material [8–12].

Statistical analysis

In this observational trial, all analyses were performed in a purely descriptive manner. As in the original trial, no imputation methods were applied for missing data caused by any reason. Time-to-event endpoints were analyzed for the whole intention-to-treat population with all events and censoring time points reported either in the original Harmony study or the FU period. Otherwise, analyses were based on the subset of intention-to-treat population participating in the FU trial. Categorical variables were summarized as counts and percentages, and continuous variables as means with standard deviations or median and interquartile range. Categorical data were analyzed by Fisher's exact test, and continuous variables by Kruskal–Wallis H Test or analysis of variance, as appropriate. Time-to-event endpoints, i.e. BPAR, graft loss or death, were calculated from date of transplantation to event or censoring time point. These endpoints were investigated by Kaplan–Meier method and tested by log-rank test. After checking the proportional hazard assumption, Cox regression analysis was used to investigate risk factors and estimation of hazard ratios (HR) for events. Adjusted models were obtained by forward selection of independent covariates. A significance level of 0.2 was used for inclusion of variables in the model.

If not otherwise stated, P-values for comparison of three arms are presented. By analogy with the original study, the impact of RSWD was investigated by comparing Arm A with Arms B and C. A P-value of <.05 was considered statistically significant. In this descriptive FU analysis, no adjustment of type I error for multiple comparison was planned as the confirmatory analysis was conducted in the original Harmony trial. Analyses were performed using SAS software 9.4. A FU for data collection was done whenever possible with patients who were prematurely eliminated from the original study.

RESULTS
Patient population characteristics

From originally 587 Harmony study participants, at the 1-year time point 144/133/126 patients in Groups A/B/C finished the full 12-month study period, respectively [7]. A total of 135/113/111 transplant recipients (including deceased participants) representative of the original Harmony study population in Groups A/B/C provided data for the observational FU analysis (Table 1). By year 5 after transplantation, 11 (6/3/2) recipients were lost to FU in the different groups, respectively (see flow chart in Supplementary data, Fig. S1).

Baseline characteristics of the three study arms excluding all patients lost to FU study were well balanced and representative of the original Harmony study, as indicated in Table 1.

Measurements of HLA-antibody activity was not part of the original Harmony trial and therefore not available at the 1-year time point. At the 1-year time point, 90%/16%/17% of patients

of the original Harmony study were receiving corticosteroids in Groups A/B/C.

At the 3- and 5-year FU investigations, the different steroid withdrawal frequency was still statistically significant, but only a minority of 38.5% of Group A patients remained on corticosteroids, while in Group B only 22.8% and in Group C 21.9% of patients received corticosteroid therapy (P = .012; see Table 2). More than 80% of the study patients remained on tacrolimus and MMF/MPA immunosuppressive treatment at either FU time point, equally distributed in all treatment groups. Only 4 patients received azathioprine (1.3%), 12 patients cyclosporine (3.9%), 15 patients mTOR inhibitors (4.9%) and 3 patients belatacept (1.0%) therapy during FU period (see Table 2).

Efficacy endpoints

From transplantation up to the 3/5-year visits of FU, the cumulative incidence of the primary endpoint BPAR (excluding or including borderline) was low and similar among the three study arms (Fig. 1A, Table 3). Severity of new acute rejections was also similar: in Group A, two Banff 1A and one acute antibody-mediated rejection (ABMR) grade III, in Group B three Banff 1A, and in Group C two ABMR grade II and one chronic active antibody-mediated rejection were noted. Only two steroid-resistant rejections (Banff grade IA/borderline) were documented during FU period, both of which occurred in Group B. From day of transplantation to year 5, a total of 99 patients with rejections including borderlines were equally distributed in the three treatment Groups A (36), B (31) and C (32). Hereby, 10 new borderline rejections in seven patients were counted during the FU period, while zero events were counted in Group A patients, three events in two Group B patients and seven events in five Group C patients.

In total, 52 deaths were observed from transplantation up to the full 5-year FU period, of which 25 occurred in Group A, 13 in Group B and 14 in Group C patients (Table 3, Fig. 1B, log-rank test for A vs B vs C P = .178). Median observation time was 4.7 years and similar in all study arms. In analogy with the original study, the impact of RSWD was investigated by comparing Arm A with Arms B and C. This numerical difference of RSWD groups vs Arm A almost reached statistical significance (log-rank test for A vs B + C P = .064) and relates to an overall survival rate of 89.2%/84.7% in Group A, 95.1%/89.4% in Group B, and 93.4%/90.4% in Group C after a 3- and 5-year FU period, respectively. Death-censored graft survival rates were similar in all three groups. From transplantation until 5 years of FU, 31 graft losses (9 in Group A, 9 in Group B, 13 in Group C) were observed, of which only 9 (1 in Group A, 3 in Group B, 5 in Group C) were new events during FU period (Table 3, Fig. 1C). Patient and graft survival rates for the three groups at the 5-year FU time point (33 events in Group A, 21 events in Group B, 26 events in Group C) were not different (Table 3, Fig. 1D).

Graft function was similar in all treatment groups at any time point (Table 2). Interestingly, the trend of an improved graft function in Arm C started early on during the first months after transplantation and remained at a comparable difference throughout all time points examined up to 5 years.

Safety endpoints

During the FU period, monitoring of anti-HLA antibodies was introduced gradually in clinical routine of the transplant programs. From year 1 on during the full FU period, any anti-HLA antibody screening was done in 45.8% (143/312) of patients, whereas 23.1% (33/143) tested positive. The detection of any or *de novo* anti-HLA

Table 1: Baseline characteristics and main outcome data at 12 months of Harmony FU patients.

Variable	Category	Arm A: basiliximab/steroids	Arm B: basiliximab/RSWD	Arm C: rATG/RSWD	FU-ITT[a]	Total-Harmony
Number	Evaluable	135	113	111	359	587
Age (years)	Mean ± SD	55.0 ± 11.0	55.0 ± 12.5	53.6 ± 11.8	54.6 ± 11.7	54.1 ± 12.2
Male sex	%	68	65	66	66	66
Cause of end-stage renal disease						
Hypertension/large vessel disease	%	38	38	34	37	37
Glomerulonephritis	%	29	29	26	28	27
Polycystic kidney disease	%	22	18	20	20	19
Diabetes	%	12	8	10	10	11
Interstitial nephritis	%	7	11	6	8	7
Type of donor						
Cadaveric Do-type	%	87	92	87	89	87
Donor with expanded criteria	%	42	41	39	41	44
Donor age (years)	Mean ± SD	53.5 ± 15.3	54.7 ± 14.9	50.9 ± 16.1	53.1 ± 15.5	54.0 ± 14.7
Antigen mismatches: A/B/DR	Mean	0.7/1.0/0.8	0.9/1.0/0.8	0.9/1.1/0.9	0.8/1.0/0.8	0.8/1.0/0.9
No panel reactive antibodies before Tx	%	84	90	89	88	88
Previous transplants	%	5	3	4	4	4
Diabetes mellitus before NTx	%	18	12	16	15	15
CMV serologic high-risk status	%	24	27	29	26	25
Endpoints at 1 year						
BPAR excl. borderline	1 year rate and 95% CI	11.1 (6.7–18.0)	11.2 (6.5–18.9)	11.2 (6.5–18.9)	11.1 (8.2–15.0)	10.8 (8.5–13.8)
Overall survival	1 year rate and 95% CI	91.3 (84.8–95.1%)	96.2% (90.1–98.6)	95.2 (88.9–98.0)	94.1 (90.9–96.1)	96.1 (94.1–97.5)
Death-censored graft survival	1 year rate and 95% CI	98.4 (93.9–99.6)	99.1 (93.6–99.9)	99.0 (93.2–99.9)	98.8 (96.9–99.6)	95.8 (93.6–97.2)
Graft loss or death	1 year rate and 95% CI	9.4 (5.4–16.0)	4.7 (2.0–11.0)	4.8 (2.0–11.1)	6.5 (4.3–9.7)	7.6 (5.6–10.2)
PTDM acc. ADA criteria	%	38.7	20.0	21.5	27.3	28.8
CMV infection	%	22.2	18.6	25.2	22.0	20.4
BKV infection	%	10.4	5.3	13.5	9.7	10.4
EBV infection	%	3.7	1.8	5.4	3.6	2.6

[a]Baseline characteristics described for participants of post-trial FU study. Hereby, non-survivors of original trial or before 3-year FU visit were included to avoid bias of characteristics by positive selection of survivors only.
rATG: rabbit ATG; ITT: intention-to-treat; Tx: transplant; CI: confidence interval; EBV: Epstein–Barr virus; Do-type: donor type; CMV: Cytomegalovirus; BKV: BK polyomavirus; PTDM: Post-transplantation diabetes mellitus; ADA: American Diabetes Association; BPAR: Biopsy-proven acute rejection; RSWD: Rapid steroid withdrawal.

antibody tended to be more frequent in the steroid withdrawal groups, although not statistically significant, compared with the control group (Table 2). The donor-specific occurrence of anti-HLA antibodies (DSA) was similar, with *de novo* occurrence particularly evident in study Arm B (basiliximab/RSWD) during the FU period (Table 2).

Metabolic profiles

In analogy with the original study, *de novo* occurrence of PTDM was defined according to the American Diabetes Association (ADA) criteria [11]. Nevertheless, no single oral glucose tolerance testing was performed during the FU period. A novel event of PTDM during FU was therefore based on former absence of diabetes/PTDM diagnosis followed by center diagnosis/judgement of new PTDM or diabetic blood values including fasting glucose and HbA1c measurements at years 3 or 5. Altogether, only 16 new cases of PTDM were seen during the FU period from years 1 to 5. Five cases were observed in Group A, seven in Group B and four in Group C, confirming that the significant reduction of PTDM with rapid steroid withdrawal (RSWD) within the first year after transplantation is not at all compensated by later PTDM events up to 5 years post-transplantation (Table 2).

Most of the other metabolic profile parameters such as blood pressure, lipids or weight/body mass index (BMI) did not differ substantially among the three study arms as described further in Supplementary results.

Cardiovascular disease, bone disease

The FU incidence of any cardio- or cerebrovascular disease event was around 10% and equally distributed in all study arms (Table 2).

FU incidence of bone fractures reported was low (2.6%) and similar in all study arms (Table 2).

Infections, anemia and cancer

During FU period from year 1 to 5, the overall incidence of any serious bacterial infection requiring hospitalization was decreased in Arm C compared with Arms A and B (Table 2) with similar differences at the first and second 2 years of FU. In addition, Arm C also showed a significant reduction in the incidences of repetitive bacterial infections. The incidence of any invasive opportunistic infection was similar in the three groups as was the overall incidence of cytomegalovirus (CMV) infection (Table 2). During the full 5-year FU period, the detected incidence of new BK virus (BKV) infection according to either polymerase chain reaction measurement or histological criteria for BKV nephropathy were similar in all groups (Table 2).

Table 2: Safety endpoints at 5 years after transplantation[a].

Variable	Arm A: basiliximab/ steroids n = 113	Arm B: basiliximab/ RSWD n = 101	Arm C: rATG/ RSWD n = 98	FU-ITT[a] n = 312	P-value[e]
Infections					
Severe bacterial infections, hospitalization required	29/112 (25.9)	35/101 (34.7)	14/98 (14.3)	78/311 (25.1)	.004
More than one severe bacterial infection	14/112 (12.5)	19/101 (18.8)	8/98 (8.2)	41/311 (13.2)	.086
Invasive opportunistic infection	4/112 (3.6)	7/101 (6.9)	8/98 (8.2)	19/311 (6.1)	.366
Any CMV infection	9/112 (8.0)	10/101 (9.9)	14/98 (14.3)	33/311 (10.6)	.348
Any BKV infection	3/112 (2.7)	2/100 (2.0)	5/98 (5.1)	10/310 (3.2)	.476
BKV viremia	3/112 (2.7)	2/100 (2.0)	5/98 (5.1)	10/310 (3.2)	.476
BKV nephropathy	1/112 (0.9)	0/100 (0.0)	3/98 (3.1)	4/310 (1.3)	.164
Malignancies					
All malignancies	15/109 (13.8)	11/97 (11.3)	10/96 (10.4)	36/302 (11.9)	.773
Skin malignancy	12/109 (11.0)	9/97 (9.3)	8/96 (8.3)	29/302 (9.6)	.831
Organ malignancy	4/109 (3.7)	2/97 (2.1)	2/96 (2.1)	8/302 (2.6)	.741
PTLD	0/109 (0.0)	0/97 (0.0)	0/96 (0.0)	0/302 (0.0)	
Allograft function					
Creatinine clearance (mL/min) (Cockroft–Gault formula)	n = 91, 60.3 ± 22.2	n = 88, 60.3 ± 24.6	n = 83, 64.0 ± 26.8	n = 262, 61.5 ± 24.5	.529
eGFR (mL/min/1.73 m^2) (CKD-EPI formula)	n = 94, 48.7 ± 17.4	n = 92, 48.1 ± 19.6	n = 87, 52.8 ± 22.3	n = 273, 49.8 ± 19.8	.226
Proteinuria/creatinine (mg/g) at 5 year visit (median and IQR)	n = 63, 112 [34–245]	n = 65, 117 [66–232]	n = 65, 87 [46–164]	n = 193, 108 [46–189]	.394
Proteinuria ≥1000 mg/g creatinine	7/82 (8.5)	4/76 (5.3)	6/77 (7.8)	17/235 (7.2)	.739
Albuminuria/creatinine (mg/g) at 5 year visit (median and IQR)	n = 58, 60 [15–120]	n = 55, 30 [13–102]	n = 60, 29 [13–135]	n = 173, 37 [14–117]	.446
Albuminuria ≥300 mg/g creatinine	8/70 (11.4)	9/65 (13.8)	12/70 (17.1)	29/205 (14.1)	.614
Anti-HLA antibodies[b]					
Screening performed	54/113 (47.8)	44/101 (43.6)	45/98 (45.9)	143/312 (45.8)	
All anti-HLA antibodies	7/54 (13.0)	11/44 (25.0)	15/45 (33.3)	33/143 (23.1)	.049
De novo Anti-HLA antibodies	3/54 (5.6)	6/44 (13.6)	9/45 (20.0)	18/143 (12.6)	.087
Donor-specific anti-HLA antibodies	4/54 (7.4)	5/44 (11.4)	5/45 (11.1)	14/143 (9.8)	.825
De novo DSA	0/54 (0.0)	3/44 (6.8)	1/45 (2.2)	4/143 (2.8)	.072
Diverse					
Any cardio- or cerebrovascular event	11/109 (9.7)	14/97 (13.9)	7/96 (7.1)	32/302 (10.3)	.281
Anemia	14/110 (12.7)	16/97 (16.5)	16/96 (16.7)	46/303 (15.2)	.671
Anemia requiring ESA	3/110 (2.7)	10/97 (10.3)	10/96 (10.4)	23/303 (7.6)	.041
Any fracture event	3/109 (2.7)	2/97 (2.0)	3/96 (3.1)	8/302 (2.6)	.910
Weight, relative change[c] (mean ± SD)	1.0 ± 0.13	1.0 ± 0.13	1.0 ± 0.11	1.0 ± 0.13	.190
BMI, relative change[c] (mean ± SD)	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	.203
PTDM[d]	5/58 (8.6%)	7/76 (9.2%)	4/67 (6.0%)	16/201 (8.0%)	.802
Current immunosuppressive medication					
Corticosteroids	42/109 (38.5)	23/101 (22.8)	21/96 (21.9)	86/306 (28.1)	.012
Tacrolimus	87/109 (79.8)	85/101 (84.2)	81/96 (84.4)	253/306 (82.7)	.626
MMF/MPA	87/109 (79.8)	82/101 (81.2)	83/96 (86.5)	252/306 (82.4)	.444
mTOR inhibitors	7/109 (6.4)	5/101 (5.0)	3/96 (3.1)	15/306 (4.9)	.602
Cyclosporine	4/109 (3.7)	3/101 (3.0)	5/96 (5.1)	12/306 (3.9)	.760

[a]Safety endpoints during FU reported for patients who gave informed consent for FU study and did not experience death by the first FU visit. Data presented as absolute and relative frequencies, mean ± SD or median and IQR.
[b]Anit-HLA antibodies were considered positive with detectability.
[c]Computed as value at 5-year visit divided by value at baseline (value 1 means constant weight).
[d]Among patients without diabetes mellitus at baseline and without PTDM during original Harmony study (203 patients in total; Arm A 59 patients, Arm B 76 patients, Arm C 68 patients).
[e]P-values calculated for comparison of Arm A vs B vs C. Fisher's exact test, analysis of variance or Kruskal–Wallis H test, as appropriate.
rATG: rabbit ATG; ITT: intention-to-treat; eGFR: estimated glomerular filtration rate; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; SD: standard deviation; IQR: interquartile range; RSWD: Rapid steroid withdrawal; CMV: Cytomegalovirus; BKV: BK polyomavirus; PTLD: Post-transplant lymphoproliferative disorder; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; DSA: Donor-specific antibody; ESA: Erythropoiesis-stimulating agents; BMI: Body mass index; PTDM: Post-transplant diabetes mellitus; MMF/MPA: Mycophenolates.

While within the first year of the Harmony trial, the frequency of anemia was significantly higher after RSWD compared with control patients, during the 3- and 5-year FU time, anemia rates were low (around 15%) and similar in all groups (Table 2). In contrast, anemia requiring erythropoiesis-stimulating agent (ESA) therapy was still significantly more frequent in both steroid withdrawal groups compared with the control group during 5-year FU (Table 2).

Cancer development occurred at similar rates in all groups during the FU study. During FU, 36 patients encountered 37 new malignancies (15 in Group A, 11 in Group B, 10 in Group C) since year 1, of which 29 were skin and 8 were solid organ-related (Table 2). No
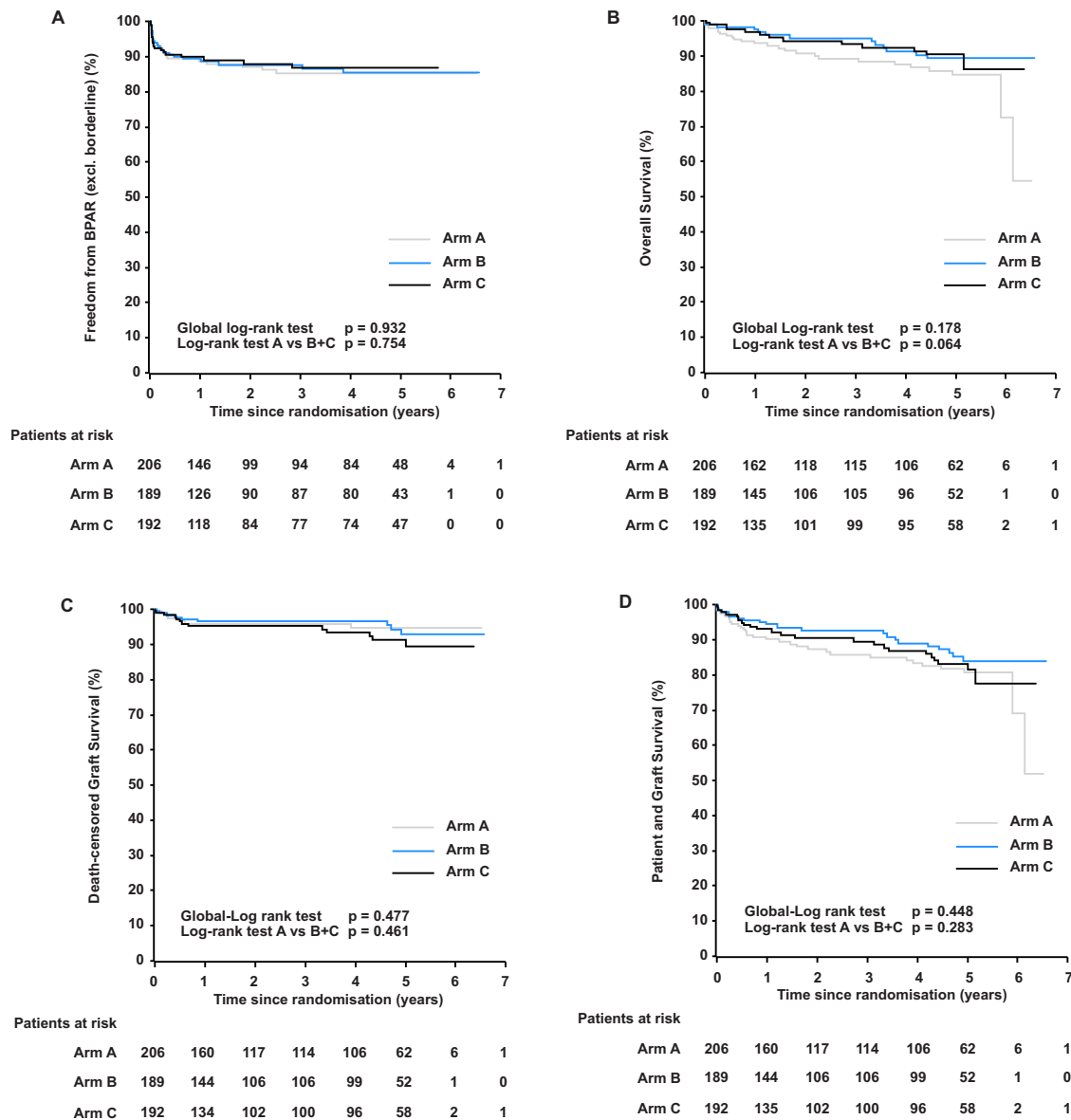
Figure 1: (**A**) BPAR. Arm A: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and prednisolone. Arm B: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Arm C: induction therapy with rabbit ATG and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Borderline acute rejections were excluded from the analysis of BPAR. Randomization took place before kidney transplantation after receiving informed consent from the patient. The analysis includes all patients in the intention-to-treat population of the original Harmony trial ($n = 587$). For the Kaplan–Meier estimates, data of patients who completed the study before having an event were censored at the time of their last visit. (**B**) Patient survival. Arm A: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and prednisolone. Arm B: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Arm C: induction therapy with rabbit ATG and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Randomization took place before kidney transplantation after receiving informed consent from the patient. The analysis includes all patients in the intention-to-treat population of the original Harmony trial ($n = 587$). For the Kaplan–Meier estimates, data of patients who completed the study before having an event were censored at the last day they are known to be alive. (**C**) Death-censored graft survival. Arm A: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and prednisolone. Arm B: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Arm C: induction therapy with rabbit ATG and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Randomization took place before kidney transplantation, after receiving informed consent from the patient. The analysis includes all patients in the intention-to-treat population of the original Harmony trial ($n = 587$). For the Kaplan–Meier estimates, data of patients who died or completed the study before having an event (graft loss) were censored at the corresponding study day. (**D**) Patient and graft survival. Arm A: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and prednisolone. Arm B: induction therapy with basiliximab and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Arm C: induction therapy with rabbit ATG and immunosuppression with tacrolimus extended-release once daily, MMF and RSWD within the first week after transplantation. Randomization took place before kidney transplantation, after receiving informed consent from the patient. The analysis includes all patients in the intention-to-treat population of the original Harmony trial ($n = 587$). For the Kaplan–Meier estimates, data of patients who completed the study before having an event (graft loss or death) were censored at the last day they are known to be alive.

Table 3: Efficacy endpoints at 3 and 5 years after transplantation.

| Variable groups | Category | 3 year rates[a] | | | 5 year rates[a] | | | P-value[b] |
		Arm A: basiliximab/ steroids	Arm B: basiliximab/ RSWD	Arm C: rATG/ RSWD	Arm A: basiliximab/ steroids	Arm B: basiliximab/ RSWD	Arm C: rATG/ RSWD	
BPAR	% (N + new events)[c]	14.7 (23 + 3)	12.4 (20 + 1)	13.2 (19 + 2)	14.7 (26)	14.5 (21 + 2)	13.2 (21)	.932
BPAR including borderlines	% (N + new events)[c]	19.4 (34 + 2)	17.8 (28 + 2)	19.6 (27 + 5)	19.4 (36)	20.8 (30 + 3)	20.7 (32 + 1)	.987
Patient survival	% (N + new events)	89.2 (11 + 7)	95.1 (4 + 3)	93.4 (6 + 3)	84.7 (18 + 7)	89.4 (7 + 6)	90.4 (9 + 5)	.178
Death-censored graft survival	% (N + new events)	95.8 (8 + 0)	96.6 (6 + 0)	95.3 (8 + 0)	94.9 (8 + 1)	93.0 (6 + 3)	91.4 (8 + 5)	.477
Patient and graft survival	% (N + new events)	85.8 (18 + 7)	92.7 (10 + 2)	89.5 (13 + 3)	80.7 (25 + 8)	84.0 (12 + 9)	83.2 (16 + 10)	.448

[a]Estimated by Kaplan–Meier method.
[b]P-values of log-rank test calculated for comparing Arm A vs B vs C.
[c]New events = number of patients with first BPAR event during corresponding FU period with or without counting borderline rejections.
rATG: rabbit ATG; RSWD: Rapid steroid withdrawal; BPAR: Biopsy-proven acute rejection.

Table 4: Cox regression analysis for time to death from any cause.

| Risk factor | | Unadjusted models | | | Adjusted model[a] | | |
		HR	95% CI	P-value	HR	95% CI	P-value
Age (per 10 years)		3.143	(2.163–4.567)	<.001	3.296	(2.094–5.187)	<.001
Sex	Female vs male	1.099	(0.617–1.958)	.750			
Rapid steroid withdrawal	Yes vs no	0.575	(0.330–1.003)	.051	0.554	(0.314–0.976)	.041
Induction therapy	rATG vs basilix.	0.773	(0.411–1.456)	.426			
Time on dialysis (per 6 month)		0.999	(0.958–1.043)	.981	1.047	(1.000–1.095)	.048
Donor with expanded criteria	Yes vs no	2.757	(1.529–4.970)	<.001			
Diabetes mellitus at transplant	Yes vs no	4.818	(2.731–8.498)	<.001	2.968	(1.617–5.447)	<.001
Cardiovascular disease at transplant[b]	Yes vs no	3.391	(1.922–5.981)	<.001	1.876	(1.028–3.426)	.041
Renal anemia at transplant[b]	Yes vs no	0.913	(0.484–1.720)	.778			
Arterial Hypertension at transplant[b]	Yes vs no	0.994	(0.358–2.764)	.991	0.461	(0.159–1.339)	.155
Hyperlipidemia at transplant[b]	Yes vs no	1.792	(1.027–3.129)	.040			
Elevated cholesterol at transplant[b]	Yes vs no	1.333	(0.746–2.381)	.332			
Elevated triglycerides at transplant[b]	Yes vs no	1.363	(0.749–2.482)	.311			

[a]Effect selection by forward selection. Significance level for entering a predictor into the model is 0.20.
[b]According to centre definition and local laboratory references.

post-transplantation lymphoproliferative disease (PTLD) occurred in any patient during the FU period (year 1–5).

Multivariable analysis of patient survival

To determine whether certain factors such as age, sex, RSWD, induction therapy, donor with expanded criteria, diabetes mellitus, cardiovascular disease, arterial hypertension, anemia, elevated cholesterol and hyperlipidemia at inclusion exert an independent impact on patient survival/death, a Cox regression analysis was performed evaluating the complete study period. No relevant collinearity was revealed among the independent risk factors. Hereby, affiliation of transplant recipients to RSWD Groups B + C compared with continued corticosteroid Group A recipients revealed as an independent protective factor for patient death (adjusted HR 0.554, 95% confidence interval 0.314–0.976; P = .041), but not the type of induction therapy. Multivariable analysis confirmed that recipient age, time on dialysis and diabetes mellitus,

as well as cardiovascular disease at inclusion were independent risk factors for patient death (Table 4).

DISCUSSION

This 5-year FU data of the randomized Harmony study comparing induction with rabbit ATG or interleukin-2 receptor antibody treatment describes one of the largest prospectively collected dataset to achieve RSWD in an immunological low-risk kidney transplant population with modern immunosuppression (low-dose once daily tacrolimus and MMF). At the low-dose of rabbit ATG used, this intervention did not show superiority over basiliximab induction for the prevention of BPAR after RSWD within 1 year after renal transplantation [7]. The original 1-year trial also demonstrated that RSWD generally could be safely performed with either induction agent without compromising efficacy with a tacrolimus/MMF-based regimen. Further, it leads

to an improved safety profile, especially by reducing the incidence of the important cardiovascular risk factor PTDM by 40%.

The Harmony FU study demonstrates that 1-year trial results are translated to the 5-year FU period confirming that steroid-free therapy remains safe and efficient over the course of 5 years after kidney transplantation. To interpret the 1- to 5-year Harmony FU study results, it is important to keep in mind that this study comprised an almost 100% Caucasian, elderly recipient population (mean by 55 years) with long dialysis vintage. Additionally, these recipients received in 87% of cases deceased and elderly donor kidneys (average donor age 53 years), almost 50% of whom met the expanded criteria. During the FU period, about 80% of transplant recipients remained on a tacrolimus/MMF/MPA combination therapy and about 80% in the RSWD arms remained corticosteroid-free. In the steroid control group, the percentage of steroid-free transplant recipients increased from 10% by year 1 to 63% of patients by year 3/5, indicating partially a late steroid withdrawal (LSWD) approach in the majority of FU patients.

During the complete FU period, only very few BPARs were diagnosed via indication biopsies in all treatment groups demonstrating great efficacy of a once daily tacrolimus and MMF/MPA-based immunosuppressive therapy independently of ongoing corticosteroids or RSWD. In addition, considering that two-thirds of all control patients were weaned off corticosteroids between years 1 and 3, it demonstrates that the LSWD approach was also not accompanied by a considerable risk for acute rejections as indicated by earlier results [13].

Multivariate analysis identified RSWD as an independent positive factor for patient survival within our study cohort. Nevertheless, due to the limitations of this study given its observational, post-trial FU design, this survival result should not be interpreted as RSWD superiority compared with corticosteroid use, but at least secure non-inferiority. The overall patient and death-censored graft survival rate (equivalent in all groups) within this study is excellent, especially when the elderly recipient and donor population with a very long waiting time on dialysis is being considered. SWD regimens have shown reduced cardiovascular as well as infectious events, and improved survival when large registry data sets were examined [4]. While large registry data are always complicated by selection bias, no randomized SWD study so far was large and long enough to show differences in survival. Although we could not find any evidence of selection bias in the present study, it can never be ruled out with certainty. Additionally, this study was not designed to detect a difference in graft or patient survival. In the largest RSWD study to date by Woodle and coworkers [14–16], RSWD appeared to be equivalently effective and safe to corticosteroid treatment. Five-year survival was around 94% and even after 10 years independent of SWD. In comparison with the aforementioned study [14–16], our patient population is characterized by a 9 years older mean age and a mean time on dialysis before transplantation of 64 ± 42 months, which is markedly higher than the usual times in the USA. In our cohort, multivariate analysis also identified recipient age, time on dialysis, diabetes mellitus and cardiovascular disease at inclusion, as well known independent risk factors for patient death, supporting the value of the new factor RSWD. Additional differences relate to the donor pool, since in their US study 57% of transplantations were living donor–related but only 13% in the Harmony trial (>40% marginal donor organs). These substantial differences between the two study populations may also be critical for the different results regarding the influence of RSWD on the relevant cardiovascular risk factor PTDM [16]. While RSWD had some limited beneficial influence on PTDM in a young, pre-

dominantly living donor–related transplant population [14–16], in the Harmony trial incidence of PTDM at the 1-year time point was generally higher and almost bisected by RSWD [7]. Irrespective of a substantial fraction of patients experiencing an LSWD as in our control patient group, this marked reduction of PTDM frequency via RSWD was not countervailed by later events suggesting that an early time point of steroid withdrawal is critical for beneficial effects on survival or PTDM in an elderly recipient population.

Safety with respect to CMV, BKV as well as invasive opportunistic infections did not differ significantly in all three groups during FU. Unexpectedly [17–19], in the rabbit ATG Group C, the incidence of any serious bacterial infection requiring hospitalization or repetitive bacterial infections during FU was reduced by about 50%. In comparison with many other studies using rabbit ATG, the very low cumulative mean dose of 4.6 mg/kg body weight may have played a role for this beneficial effect. Since the basiliximab induction Group B did not show this marked reduction in severe bacterial infections compared with control patients, no relation to the RSWD management can be established. Additionally, no PTLD and no difference regarding the development of cancers was noted in all treatment arms during the 5-year FU period.

In the original 1-year study, both RSWD groups were associated with a significantly higher incidence of anemia and ESA-requiring anemia [7]. Surprisingly, the ESA-requiring anemia effect at a much lower level was still translated into the FU period. Despite LSWD until year 3 in more than 60% of all control patients, this effect is most likely still attributable to corticosteroids, since kidney function during FU was not different in all groups.

Since corticosteroids are potent immunosuppressive agents, one major concern of corticosteroid withdrawal compared with corticosteroid continuation is the possibility of increased immunosensitization and its consequences, but no firm conclusions can be drawn from the transplant studies available so far [20]. According to our 5-year FU results, the frequency of neither rejection-related graft losses nor chronic cellular/humoral rejections nor donor-specific antibody formation increased in the RSWD groups. Nevertheless, some higher immunosensitization attributable to RSWD cannot be excluded, since the frequency of total or *de novo* anti-HLA antibody formation or borderline rejections tended to be increased (at a low level) compared with the control group but remained without any consequences for the trial outcome within the study period.

Our study has several limitations. The observational manner of the FU study with changes in medication according to center standard and patient course may introduce some bias and limits the equality of observation, but relates to real life treatment results. For interpretation of these 5-year FU study results, the frequent LSWD beyond year 1 in the control patient group needs to be considered. Measurement of anti-HLA antibody formation was not yet regularly established at every transplant center and was only available in up to half of patients. The lost to FU of patients from the original 1-year study potentially contributing to some selection bias relates to the fact that this FU study was not planned from the beginning due to financial restrictions. We also interpret our largely beneficial results of RSWD being restricted to an immunologically low-risk, elderly population of Caucasian recipients.

In conclusion, the 5-year FU period of our investigator-initiated Harmony trial confirms the excellent efficacy and beneficial safety aspects of RSWD under modern tacrolimus/MMF/MPA immunosuppressive therapy as already seen in the original 1-year study. A steroid-free therapy remains safe, efficient and in regard

to survival at least comparable to a steroid-containing therapy over a course of 5 years after kidney transplantation. With all the limitations given in an observational 5-year post-trial FU study, the results shown here support the RSWD approach to be more frequently used as an equivalent alternative to the current mostly corticosteroid-continuing therapy in an immunologically low-risk, elderly population of Caucasian kidney transplant recipients.

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

CONFLICT OF INTEREST STATEMENT

The following authors of this manuscript have conflicts of interest do disclose as described by the *American Journal of Transplantation*: C.H. received grant support, consulting fees and lecture fees from Astellas, Sanofi, Genzyme, Roche Pharma, Novartis and Wyeth/Pfizer. C.K. received consulting fees and lecture fees from Astellas, Chiesi, Novartis and Sanofi-Genzyme. J.W.-M. received grant support, consulting fees and lecture fees from Vifor, GSK, Novartis, AstraZeneca and Boehringer Ingelheim. O.W. has received research grants for clinical studies, speaker's fees, honoraria and travel expenses from Amgen, Alexion, Astellas, Basilea, Biotest, Bristol-Myers Squibb, Correvio, Chiesi, Gilead, Hexal, Janssen, Dr F. Köhler Chemie, MSD, Novartis, Roche, Pfizer, Sanofi, Takeda, TEVA and UCB. B.B. received grant support, consulting fees and lecture fees from Astellas, Bayer, Chiesi and Novartis. J.S. received lecture fees from Astellas, Boehringer-Ingelheim and Fresenius Medical Care.

All other authors of this manuscript have no conflicts of interest to be disclosed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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