ORIGINAL ARTICLE

Pre-emptive immunosuppression using tacrolimus monotherapy does not reduce the rate of early acute rejection in renal transplantation from live donors: a comparative cohort study

Michael L. Nicholson[1,2] (iD), Mekhola Hoff[1], Philippa Leighton[1], Ismail Mohamed[2] &
Sarah A. Hosgood[1] (iD)

1 Department of Surgery,
Addenbrooke's Hospital, University
of Cambridge, Cambridge, UK
2 Leicester General Hospital,
University of Leicester, Leicester, UK

Correspondence
Michael L. Nicholson, Department of
Surgery, University of Cambridge,
Box 202, Level 9, Addenbrooke's
Hospital, Cambridge CB2 0QQ, UK.
Tel.: 01223 339221;
e-mail: mln31@cam.ac.uk

SUMMARY

The planned nature of live donor kidney transplantation allows for immunosuppression to be initiated in the pretransplant period. The aim of this study was to determine the effect of pre-emptive immunosuppression on acute rejection rates after live donor kidney transplantation. In two consecutive cohorts of live donor kidneys transplants, 99 patients received pre-emptive immunosuppression with tacrolimus monotherapy for 2 weeks prior to transplantation (PET group – first era) and 100 patients received tacrolimus-based immunosuppression commencing on the day of transplantation (control group – second era). The main outcome measure was the incidence of biopsy-proven acute rejection (BPAR) in the first 3 months post-transplantation. Tacrolimus levels were significantly higher in the PET group at day 4 post-transplant (PET 9.08 ± 4.57 vs. control 5.92 ± 3.64 ng/ml; $P < 0.0001$), but there were no significant differences in tacrolimus levels at day 7 (PET 8.22 ± 3.58 vs. control 7.63 ± 3.56 ng/ml; $P = 0.2452$). BPAR was numerically higher in the PET group, but this difference did not reach statistical significance (PET 13/99 vs. control 6/100; $P = 0.097$). There were no differences in allograft function measured by serum creatinine at 1 year (PET 130 ± 36 vs. control 142 ± 69 μmol/l; $P = 0.6829$). Graft survival at 1 year was equivalent in both groups (PET 96.9 vs. control 97.0%; $P = 0.9915$). This study suggests that there is little role for the use of pre-emptive tacrolimus monotherapy in ABO blood group and HLA-compatible live donor kidney transplantation in patients on triple maintenance immunosuppression.

Introduction

The planned nature of live donor kidney transplantation allows for a period of pre-emptive immunosuppression, and the recent Kidney Disease: Improving Outcomes (KDIGO) guidelines advocate this approach [1]. The aim of pre-emptive immunosuppression is to reduce the rate of early acute rejection, and whilst it

seems intuitively logical to perform transplantation in patients who already have therapeutic levels of immunosuppression, there is no evidence base to support this strategy [2]. A number of different pre-emptive immunosuppressive strategies are possible. In this study, the use of tacrolimus monotherapy was chosen as calcineurin inhibitors are the mainstay of modern immunosuppressive regimens.

Acute rejection in renal transplantation is associated with reduced graft and patient survival as well as an increased rate of interstitial fibrosis and tubular atrophy [3]. Accordingly, the transplant community continues to strive for therapeutic measures that minimize acute rejection rates. The incidence of acute rejection in the first 6 months post-transplant varies according to the specific immunosuppressive regimen used, with quoted figures ranging from 3.8% for immunosuppression with a combination of tacrolimus and mycophenolate mofetil (MMF), 10.1% for low-dose cyclosporine and 35.3% for low-dose sirolimus [4–6]. There is good evidence to support the use of tacrolimus-based immunosuppression in renal transplantation [6], and by 2003, 67% of new kidney transplant recipients in the United States were receiving tacrolimus [7].

The aim of this study was to compare the outcome of live donor kidney transplantation in patients treated with (PET group) or without (control group) pre-emptive immunosuppression using tacrolimus monotherapy. The primary end point of the study was biopsy-proven acute rejection (BPAR) in the first 3 months post-transplant defined by the Banff criteria [8]. Secondary outcomes were tacrolimus levels, allograft function and allograft survival at 1 year.

Patients and Methods

This was a single-centre nonrandomized comparative cohort study of living-related and unrelated adult kidney transplants. The study involved retrospective analysis of data that were collected using a prospective computerized database that included demographic, biochemical, haematological, histopathological and graft survival information. Patients receiving pre-emptive immunosuppression with tacrolimus were reviewed twice per week by a live donor co-ordinator, and any adverse effects of the therapy were recorded prospectively.

Inclusion criteria

1. Patients undergoing kidney transplantation from a live donor.

2. Patients aged ≥18 years old.
3. Patients immunosuppressed with a regimen that included tacrolimus as the calcineurin inhibitor.
4. Patients receiving induction therapy with basiliximab.

Exclusion criteria

1. Patients who were already receiving immunosuppression for a failing renal transplant.
2. Patients immunosuppressed with cyclosporine as the initial calcineurin inhibitor.
3. HLA-incompatible renal transplantation because of pretransplant donor-specific antibodies with mean fluorescence index >1500 and/or a positive flow cytometric cross-match.

All patients underwent pretransplant HLA antibody screening at 3 monthly intervals, and pretransplant immunological cross-matches were performed by flow cytometry and where necessary using Luminex. Tacrolimus blood levels were performed onsite using an immunoassay technique (Dimension Xpand, Siemans). Trough levels were measured immediately before patients received their 10 a.m. dose of tacrolimus. For the purposes of this study, the therapeutic range for tacrolimus was defined as 6.0–12.0 ng/ml. Levels <6.0 ng/ml were considered subtherapeutic and levels >12.0 ng/ml were considered to be potentially toxic.

During the study period, a consecutive series of 218 patients received a live donor kidney transplant. In the first half of the series, the unit policy was to provide pre-emptive immunosuppression with tacrolimus. The unit policy then changed to performing live donor renal transplants with tacrolimus being introduced on the day of transplantation. Of the initial 218 patients, there were 19 exclusions as follows: seven patients in the PET and 5 in the control group were already receiving immunosuppression for a failing kidney transplant; two patients in the PET group and five patients in the control group did not receive tacrolimus in the initial post-transplant period. The study groups therefore consisted of a series of 99 patients receiving PET immunosuppression (first era) and 100 patients in the control group (second era).

Immunosuppressive protocol

In the PET group, tacrolimus was commenced 2 weeks prior to transplantation and was administered at a dose of 0.1 mg/kg/day in two divided doses. Tacrolimus trough blood levels were checked three times per week, and the therapeutic range was defined as 6.0-12.0 ng/ml.

In the control group, tacrolimus was commenced on the day of transplantation at the same dose and with the same target trough blood levels.

There was no immunological risk stratification in this series, and the immunosuppressive drug regimen was the same for patients in the PET and control groups. All patients received induction therapy with basiliximab 20 mg IV given on the day of transplantation and on the fourth post-transplant day. All patients also received 500 mg of methylprednisolone IV at induction of anaesthesia. MMF (CellCept, Roche, Welwyn Garden City, UK) was administered to both groups of patients, starting on the day of transplantation at a dose of 500 mg bd. Both groups of patients also received prednisolone at a dose of 20 mg daily in the first post-transplant month, 15 mg daily in the second post-transplant month, 10 mg daily in the third post-transplant month and 5 mg daily thereafter.

Cytomegalovirus (CMV) prophylaxis was identical in both groups throughout the study. Valganciclovir was used for 100 days if the recipient was CMV-negative and had received a kidney from a CMV-positive donor.

Graft function and survival

Primary nonfunction was defined as permanent lack of graft function from the time of transplantation because of any cause. Initial graft function was defined as ≥10% fall in serum creatinine on the first postoperative day. Allograft function was assessed using measurements of serum creatinine recorded pretransplant, on the first postoperative day and at 1 week and 1, 3, 6 and 12 months post-transplant. Graft failure was defined as loss of transplant function necessitating re-transplantation or a return to haemodialysis or peritoneal dialysis. Patients who died with a functioning graft were censored from the graft survival data.

Biopsy-proven acute rejection

Episodes of acute allograft dysfunction (serum creatinine rise of ≥10% or ≥30 μmol/l) and those patients who had an early plateauing of creatinine between 120 and 200 μmol/l were investigated by percutaneous needle core biopsy under ultrasound guidance. Surveillance biopsies were performed routinely at 1 week post-transplant. All transplant biopsies were examined by one of two experienced consultant histopathologists, and acute rejection was defined using the Banff criteria [8]. All BPAR episodes up to 3 months were recorded, as this was felt to best illustrate any difference in the use of pre-emptive tacrolimus.

Cost analysis

The acquisition cost of providing pre-emptive tacrolimus therapy was calculated using data obtained from the hospital renal pharmacy department. The direct costs of performing tacrolimus trough levels in the pre-emptive period were also calculated using data from the in-house tissue typing laboratory. The indirect costs of pre-emptive immunosuppression, including medical and laboratory staff time and overheads, were not calculated.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Normality testing was performed using the Kolmogorov–Smirnov test. For continuous variables, differences between groups were evaluated using either the Student's *t*-test or the Mann–Whitney *U*-test as appropriate. Categorical variables were analysed using the chi-squared test or Fisher's exact test. Graft survival was estimated using the Kaplan–Meier method, and differences between groups were compared using the log-rank statistic. All tests were two-tailed, and $P \leq 0.050$ was considered significant. Bonferroni's correction was applied to the multiple comparisons ($n = 5$) of serum creatinine. Each difference in serum creatinine was therefore tested at $\alpha = 0.01$.

Results

Demographics

The two study groups were well-matched for patient demographic details, although there were more HLA class I mismatches in the control group (Table 1). In addition, there were more 0-0-0 HLA mismatches in the PET group (15/99 vs. 5/100; $P = 0.0192$). There was no significant difference in the number of parental donors in the two groups (PET $n = 27$ vs. control $n = 34$; $P = 0.3569$). There were no differences in the overall level of antibody sensitization between the two groups (mean ± SD cRF was 7 ± 15 vs. 8 ± 13% for the PET and control groups, respectively). In the PET group, 89 recipients were White and 10 were Black or Asian. In the control group, 87 recipients were White and 13 were Black or Asian ($P = 0.6584$; Fisher's exact).

Kidney weight was significantly higher in the PET group, but the absolute difference was only 10 g. Anastomosis time was also significantly longer in the PET group, but the mean time in both groups was ≤30 min and the absolute difference was only 3 min (Table 2).

Table 1. Donor and recipient demographics.

Parameter	PET	Control	P value
Donor age (years)	47 ± 11	48 ± 11	0.5159
Donor gender (M/F)	43/56	37/63	0.3875
Recipient age (years)	42 ± 12	45 ± 13	0.2185
Recipient gender (M/F)	52/47	59/41	0.3933
Recipient BMI (kg/m^2)	25 ± 5	26 ± 4	0.2023
Dialysis status (Y/N)	56/43	48/52	0.2026
Previous transplant	5	6	0.7674
HLA A + B mismatches	1.5 ± 1	2.0 ± 1.0	0.0046
HLA DR mismatches	0.8 ± 0.6	1.0 ± 0.6	0.0576

Table 2. Transplant details.

Parameter	PET	Control	P value
Kidney weight (g)	173 ± 45	162 ± 32	0.0428
Warm ischaemic time (min)	4 ± 3	4 ± 2	0.0874
Cold ischaemic time (min)	207 ± 40	208 ± 52	0.6979
Anastomosis time (min)	30 ± 6	27 ± 6	0.0013
Graft function* (initial/slow)	94/5	98/2	0.2790

*Initial graft function defined as ≥10% fall in serum creatinine on the first postoperative day.

Tacrolimus levels

In the PET cohort of patients, the mean tacrolimus level at the time of transplantation was 9.5 ± 3.9 ng/ml. Seventy-six of these patients had therapeutic levels at the time of transplant (6–12 ng/ml), 19 patients had levels higher than the therapeutic range (>12 ng/ml) and only four patients had a subtherapeutic level of tacrolimus (<6 ng/ml). At 1 week post-transplantation, there were no differences in mean tacrolimus levels or the number of patients with levels in the therapeutic and nontherapeutic ranges (Tables 3 and 4).

Pre-emptive immunosuppression led to a number of tacrolimus-related side effects in the 2 weeks pretransplant treatment period. Twenty-two patients reported tremor, and 15 noted warm peripheries. There were no abnormalities in pretransplant serum potassium or

Table 3. Tacrolimus levels.

Time post-transplant	PET	Control	P
D1	9.53 ± 3.88	N/A	–
D4	9.08 ± 4.57	5.92 ± 3.64	<0.0001
D7	8.22 ± 3.58	7.63 ± 3.56	0.2452
6/52	8.96 ± 3.22	8.50 ± 2.20	0.2403

Values are mean ± SD.

blood glucose, and no operations were cancelled or delayed because of drug side effects.

Biopsy-proven acute rejection

The incidence of BPAR at 3 months was numerically higher in the PET group, but this did not reach statistical significance (13/99 vs. 6/100; $P = 0.097$; Table 5). There were also no significant differences in the incidence of borderline acute rejection, and the overall rejection rate in the two groups was very similar in the first 3 months (Table 5). There was only one episode of antibody-mediated acute rejection, and this occurred in the PET group (Table 5). The rates of rejection in kidneys from parental and nonparental donors were not significant in either group (PET: parental kidney BPAR 2/27 vs. nonparental kidney BPAR 11/72, $P = 0.5050$; and control: parental kidney BPAR 2/34 vs. nonparental kidney BPAR 4/66, $P = 1.000$).

Tacrolimus levels in the first post-transplant week were not predictive of the development of BPAR in the first 3 months post-transplantation. In the PET group, one of 19 patients with BPAR (including borderline rejection) had subtherapeutic drug levels (<6 ng/ml) in the first post-transplant week compared with one of 80 patients without BPAR (including borderline rejection) in the first 3 months ($P = 0.3486$, Fisher's exact test). Similarly, in the control group, subtherapeutic drug levels in the first week were noted in 4/17 vs. 13/83 patients with or without BPAR in the first 3 months, respectively ($P = 0.4808$, Fisher's exact test).

Post-transplant CMV disease and BK nephropathy

Despite the use of CMV prophylaxis with valganciclovir, CMV disease developed in two patients in the PET group and one in the control group ($P = 0.612$). These were treated by a reduction in immunosuppression and intravenous ganciclovir and did not result in any graft losses. BK viraemia (PCR >5000 copies) was detected in two patients in the PET group and 5 in the control group ($P = 0.4448$). There were no graft losses because of BK nephropathy.

Graft function and survival

There were no episodes of primary nonfunction, but one patient in the PET group suffered early graft loss because of recurrence of focal segmental glomerulosclerosis in the first week post-transplant. There were no episodes of allograft thrombosis in either group. Initial graft function

Table 4. Number of patients with subtherapeutic, therapeutic and supra-therapeutic tacrolimus levels.

Time post-transplant	Subtherapeutic <6.0 ng/ml	Therapeutic 6.0–12.0 ng/ml	Supra-therapeutic >12.0 ng/ml	P value Pre-emptive vs. control
D1 pre-emptive	4	76	19	–
D4 pre-emptive	9	78	12	–
D4 control	44	50	6	<0.00001
D7 pre-emptive	14	73	12	–
D7 control	22	66	12	0.3455
6/52 pre-emptive	5	86	8	–
6/52 control	2	94	4	0.2265

Table 5. Biopsy-proven acute rejection (BPAR) episodes in first 3 months post-transplantation.

	PET	Control	P value
BPAR (excl. borderline)	13	6	0.0970
Borderline	6	11	0.3106
BPAR (all types)	19	17	0.7161
Banff 1A	3	4	1.0000
Banff 1B	3	0	0.1212
Banff 2A	6	1	0.0649
Banff 2B	0	1	1.0000
Antibody-mediated rejection	1	0	0.4975

Fisher's exact test.

occurred in 94/99 patient in the PET group and 98/100 patients in the control group ($P = 0.2790$; Table 2). Serum creatinine fall over the first postoperative day was similar between the two groups (Table 2). There were no differences in the serum creatinine levels at 1, 3, 6 and 12 months (Table 6) and also no differences in 12-month allograft survival (PET 96.9 vs. control 97.0%; $P = 0.9915$; Fig. 1).

Cost analysis

Patients in the PET group required an average of 3.8 hospital visits to measure tacrolimus levels. The total additional cost of pre-emptive immunosuppression, in terms of drug and assay expenses, was £287 ± 90 per patient.

Discussion

This study shows that pre-emptive immunosuppression with tacrolimus monotherapy for a period of 2 weeks does not confer any advantages to the recipients of live donor kidney transplants. A period of pre-emptive immunosuppression achieved therapeutic tacrolimus drug levels at the time of transplantation in all but four of 99 patients, but this did not reduce the rate of BPAR in the first 3 months post-transplant. This may be related to the fact that by 1 week post-transplant therapeutic immunosuppressive drug levels were achieved in a large proportion of the recipients and there were no statistically significant differences between the two groups. Although subtherapeutic tacrolimus levels were present on day 4 post-transplant in significantly more patients in the control group compared with the pre-emptive group, this did not influence acute rejection rates. The use of 500 mg of methylprednisolone administered at induction of anaesthesia provides a potent level of immunosuppression for a number of days and this allows sufficient time to achieve therapeutic levels of tacrolimus, even when these are first administered on the day of transplantation. Similarly, the use of basiliximab in the first week is likely to have had a protective effect such that early tacrolimus levels are not predictive of acute rejection. In a small randomized controlled trial, Griffin *et al.* [9] demonstrated that calcineurin inhibitors do not need to be given for the first 24 h post-transplant. This delayed administration would further extend the time necessary to reach therapeutic drug levels, but this did not cause any increase in rejection episodes.

The triple maintenance immunosuppressive regimen used in this study included a higher and more
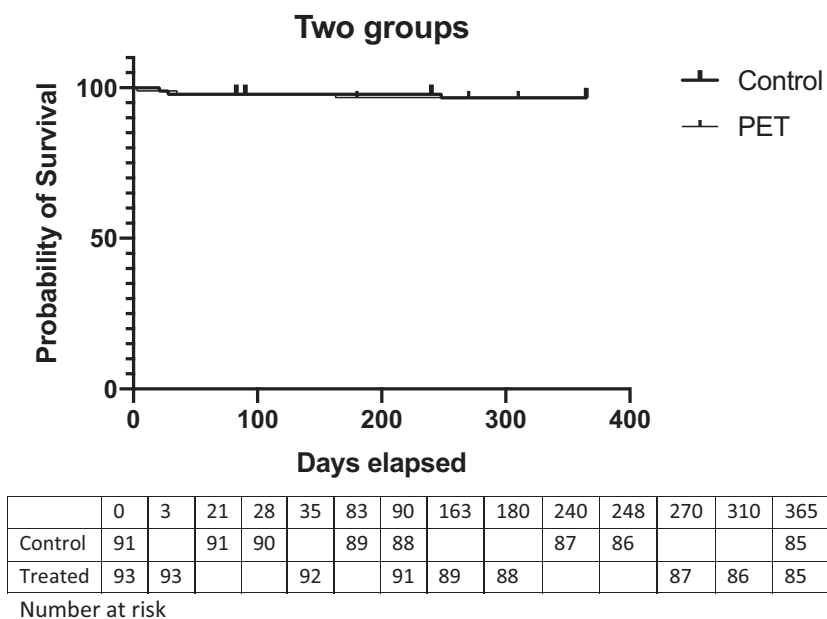
Table 6. Creatinine levels (μmol/l) post-transplantation.

Creatinine μmol/l (mean ± SD)	PET	Control	P value
1 week	143 ± 83	154 ± 107	0.5020
1 month	129 ± 37	136 ± 90	0.4045
3 months	126 ± 33	142 ± 63	0.1761
6 months	136 ± 39	153 ± 76	0.1562
1 year	130 ± 36	142 ± 69	0.6829

Figure 1 Renal allograft survival in patients with pre-emptive immunosuppression with tacrolimus (PET;*n* = 99) and patients starting immunosuppressive therapy on the day of transplantation (control;*n* = 100). Ticks represent censored events.

prolonged dosage of steroids than is used by some other units. Prednisolone was maintained at 20 mg daily for the first post-transplant month and only reduced to the baseline level of 5 mg daily by 3 months post-transplant. It is possible that pre-emptive tacrolimus monotherapy is useful in patients where steroids are withdrawn early, and this might form the basis of further study or a clinical trial in the future. The MMF dosage of 500 mg b.d. in this study was lower than the commonly used dosage of 1 g b.d. employed in many units. This was chosen against the background of using a more prolonged steroid taper in an attempt to balance the overall burden of immunosuppression.

We were surprised to find a paucity of literature in this field. We carried out MEDLINE, EMBASE and Cochrane Library searches up to 26 April 2020 using the search terms 'pre-emptive', "pre emptive" "immunosuppression", "immunosuppressive" "immunosuppressant" "tacrolimus" "living donor" "kidney transplantation" and "renal transplantation" but did not identify any relevant clinical studies. In a nonsystematic literature search, one xenotransplantation animal study suggested a beneficial effect of pre-emptive tacrolimus on the incidence of hyperacute rejection [10]. Several in vitro studies have suggested beneficial effects of pre-emptive tacrolimus on T-cell activity [11] and glucose metabolism [12], but these studies lack clinical correlation.

The administration of tacrolimus pre-emptively for a sustained period of 2 weeks may theoretically have a negative impact on initial graft function, owing to its nephrotoxic properties, if supra-therapeutic levels are achieved. Nonetheless, using the criterion of fall in serum creatinine by 10% in the first 24 h [13], we did not observe any difference in the rates of initial or slow graft function.

Pre-emptive immunosuppression has been used routinely in ABO blood group-incompatible patients and those with HLA antibody-incompatible kidney transplants [14–16]. In addition, some centres have reported starting immunosuppressive agents the day before or administering two doses orally prior to transplantation [17,18], but we were unable to find comparative studies of pre-emptive immunosuppression for a period of 2 weeks prior to routine live donor kidney transplants.

The series described here is a comparative cohort study from a consecutive series of transplants other than the exclusions described. Although patients were not randomized to pre-emptive immunosuppression or standard treatment, the numbers of patients studied should be sufficient to make valid conclusions. Data collection was complete for all patients, and selection bias was minimized because the two cohorts were consecutive series. The two study groups were well-matched for demographic and donor details, and although there were differences in HLA mismatching, if this had any effect, it should have been to bias against the control group not receiving pre-emptive immunosuppression. Moreover, the effect of HLA matching in live donor kidney

transplantation is still debated. A comprehensive study, undertaken on behalf of the Kidney Advisory Group of NHS Blood and Transplant, demonstrated that HLA matching was not a significant factor in graft survival after living donor kidney transplantation [19]. In contrast, data from the international collaborative transplant study suggest that HLA compatibility does affect the outcome of live unrelated kidney transplants [20].

Modern immunosuppressive therapy was used in the patients in this study, with tacrolimus as the primary immunosuppressant for the prevention of graft rejection. The addition of induction with basiliximab, along with MMF and steroids, represents one of the most common regimens used in transplantation in the UK and is in line with the recommendations of the National Institute of Clinical Excellence [21]. The use of triple immunosuppressive therapy achieves good patient and graft survival with a lower incidence of BPAR (44% vs. 27%) compared with dual immunosuppression using tacrolimus and prednisolone alone [22].

Tacrolimus has a narrow therapeutic index and a highly variable oral bioavailability among individuals [23]. Pre-emptive administration might improve optimization and efficacy, but there was no evidence for this in the current study. The use of pre-emptive immunosuppression does have its disadvantages, including an increased workload for unit staff and increased costs for drugs and drug assays. In this study, the mean increased cost of tacrolimus monotherapy for 2 weeks was just under £290.

The rate of BPAR in the control group not receiving pre-emptive immunosuppression was extremely low at 6%. The biopsy regimen employed in the unit was rigorous, and it is unlikely that clinically significant episodes of acute rejection were missed. A limitation of the study is that it only examined the effect of immunosuppressive monotherapy with tacrolimus. Whilst it is theoretically possible that full immunosuppression with tacrolimus, MMF and steroids might have had a greater effect, in reality it would be difficult to improve on the very low incidence of BPAR in the control group. In conclusion, this study suggests that there is little role for the use of pre-emptive immunosuppression with tacrolimus monotherapy in patients receiving triple maintenance immunosuppression after ABO- and HLA-compatible live donor kidney transplantation.

Authorship

MLN: designed the study, collected data, analysed data and wrote the manuscript. MH: analysed data, performed literature review and wrote the paper. PL: analysed data, performed literature review and wrote the paper. IM: collected data and analysed data. SAH: analysed data and wrote the manuscript.

Funding

Conflicts of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO: clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9**(Suppl 3): S1.
2. Morris PJ, Knechtle SJ. *Kidney Transplantation: Principles and Practice*, 6th edn. Philadelphia, PA: Saunders, 2008.
3. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med* 2003; **349**: 2326.
4. Silva HT Jr, Yang HC, Abouljoud M, *et al.* One-year results with Extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in de novo kidney transplant recipients. *Am J Transplant* 2007; **7**: 595.
5. Vincenti F, Friman S, Scheuermann E, *et al.* Results of an international randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transplant* 2007; **7**: 1506.
6. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Eng J Med* 2007; **357**: 2563.
7. Shapiro R, Young JB, Milford EL, Trotter JF, Bustami RT, Leichtman AB. Immunosuppression: evolution in practice and trends, 1993–2003. *Am J Transplant* 2005; **5**: 874.
8. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
9. Griffin PJA, Moore RH, Krishnan H, Fenn N, Salaman JR. Is there an optimal time for the first cyclosporine dose in renal transplantation? *Transplant Int* 1993; **6**: 223.
10. Masashi T, Valdivia LA, Rao AS, *et al.* Tacrolimus pre-treatment attenuates pre-existing xenospecific immunity and abrogates hyperacute rejection in a presensitized hamster to rat liver transplant model'. *Transplantation* 1996; **61**: 1730.
11. Wallin Elizabeth F, Hill DL, Linterman MA, Wood KJ. The calcineurin

inhibitor tacrolimus specifically suppresses human T follicular helper cells. *Front Immunol* 2018; **9**: 1184.

12. Van Duijnhoven EM, Boots JMM, Christiaans MH, Wolffenbuttel B, Van Hooff JP. Influence of tacrolimus on glucose metabolism before and after renal transplantation: a prospective study. *J Am Soc Nephrol* 2001; **12**: 583.

13. Stratopoulos C, Roberts IS, Brockmann J, et al. Risk factors for delayed graft function defined as need for dialysis or failure of creatinine to fall by 10% in the first 24 hours after transplant. *Exp Clin Transplant* 2008; **6**: 37.

14. Tydén G, Donauer J, Wadström J, et al. Implementation of a protocol for ABO-incompatible kidney transplantation – a three-center experience with 60 consecutive transplantations. *Transplantation* 2007; **83**: 1153.

15. Oettl T, Zuliani E, Gaspert A, Hopfer H, Dickenmann M, Fehr T. Late steroid withdrawal after ABO blood group-incompatible living donor kidney transplantation: high rate of mild cellular rejection. *Transplantation* 2010; **89**: 702.

16. Fuggle SV, Martin S. Toward performing transplantation in highly sensitised patients. *Transplantation* 2004; **78**: 186.

17. Rostaing L, Cantarovich D, Mourad G, et al. Corticosteroid-free immuno-suppression with tacrolimus, myco-phenolate mofetil and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807.

18. Hernández D, Miquel R, Porrini E, et al. Randomized controlled study comparing reduced calcineurin inhibitors exposure versus standard cyclosporine-based immunosuppression. *Transplantation* 2007; **84**: 706.

19. Fuggle S, Allen JE, Johnson RJ, et al. Factors affecting graft and patient survival after live donor kidney transplantation in the UK. *Transplantation* 2010; **89**: 694.

20. Opelz G. Impact of HLA compatibility on survival of kidney transplants from unrelated live donors. *Transplantation* 1997; **64**: 1473.

21. National Institute of Clinical Excellence. Immunosuppressive therapy for renal transplantation in adults. Technology appraisal guidance [TA481] 11 October 2017.https://www.nice.org.uk>guidance.ta481

22. Shapiro R, Jordan ML, Scantlebury VP, et al. A prospective randomized trial of tacrolimus/prednisone versus tacrolimus/prednisone/mycophenolate mofetil in renal transplant recipients. *Transplantation* 1999; **67**: 411.

23. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 2004; **43**: 623.