ORIGINAL ARTICLE

Decreased incidence of acute rejection without increased incidence of cytomegalovirus (CMV) infection in kidney transplant recipients receiving rabbit anti-thymocyte globulin without CMV prophylaxis – a cohort single-center study

Mayara Ivani de Paula[1,2], Mary Grace Bowring[2], Ashton A. Shaffer[2,3], Jacqueline Garonzik-Wang[2], Adrieli Barros Bessa[1], Claudia Rosso Felipe[1], Marina Pontello Cristelli[1], Allan B. Massie[2,3], Jose Medina-Pestana[1], Dorry L. Segev[2,3] & Helio Tedesco-Silva[1]

1 Department of Nephrology, Hospital do Rim, Universidade Federal de São Paulo, São Paulo, Brazil
2 Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA
3 Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD, USA

Correspondence
Helio Tedesco-Silva MD, PhD, Nephrology Department, Hospital do Rim, Universidade Federal de Sao Paulo, Borges Lagoa Street, 960 – 04038-002 Sao Paulo, Brazil.
Tel.: +55 11 50878113;
fax: +55 11 50878145;
e-mail: heliotedesco@medfarm.com.br

SUMMARY

Induction therapy with rabbit anti-thymocyte globulin (rATG) in low-risk kidney transplant recipients (KTR) remains controversial, given the associated increased risk of cytomegalovirus (CMV) infection. This natural experiment compared 12-month clinical outcomes in low-risk KTR without CMV prophylaxis (January/3/13–September/16/15) receiving no induction or a single 3 mg/kg dose of rATG. We used logistic regression to characterize delayed graft function (DGF), negative binomial to characterize length of hospital stay (LOS), and Cox regression to characterize acute rejection (AR), CMV infection, graft loss, death, and hospital readmissions. Recipients receiving 3 mg/kg rATG had an 81% lower risk of AR (aHR $_{0.14}0.19_{0.25}$, $P < 0.001$) but no increased rate of hospital readmissions because of infections ($_{0.68}0.91_{1.21}$, $P = 0.5$). There was no association between 3 mg/kg rATG and CMV infection/disease (aHR $_{0.86}1.10_{1.40}$, $P = 0.5$), even when the analysis was stratified according to recipient CMV serostatus positive (aHR $_{0.94}1.25_{1.65}$, $P = 0.1$) and negative (aHR $_{0.28}0.57_{1.16}$, $P = 0.1$). There was no association between 3 mg/kg rATG and mortality (aHR $_{0.51}1.25_{3.08}$, $P = 0.6$), and graft loss (aHR $_{0.34}0.73_{1.55}$, $P = 0.4$). Among low-risk KTR receiving no CMV pharmacological prophylaxis, 3 mg/kg rATG induction was associated with a significant reduction in the incidence of AR without an increased risk of CMV infection, regardless of recipient pretransplant CMV serostatus.

Transplant International 2021; 34: 339–352

Key words
acute rejection, CMV infection, low immunological risk, thymoglobulin

Received: 21 August 2020; Revision requested: 28 September 2020; Accepted: 7 December 2020; Published online: 31 December 2020

doi:10.1111/tri.13800

Introduction

The goal of immunosuppression in kidney transplantation is to prevent acute rejection and maintain long-term allograft function without causing adverse effects. Currently, the majority of kidney transplant recipients (KTR) receive induction therapy in order to avoid early acute rejection (AR), which has historically been associated with graft loss [1]. Recent guidelines recommended basiliximab as the first-line agent for patients at lower risk and rabbit anti-thymocyte globulin (rATG) for those at higher risk of AR [2,3].

The combination of tacrolimus, mycophenolate and prednisone is considered the standard of care therapy for kidney transplant recipients. Yet, in our local population, tolerability and safety of mycophenolate is limited by the disproportional incidence of gastrointestinal, bone marrow and cytomegalovirus (CMV) infection adverse events, leading to premature drug discontinuation [4]. Hence, this drug combination is limited to higher risk patients, namely highly sensitized patients and those at higher risk to develop delayed graft function [5]. Accordingly, the combination of tacrolimus (TAC), azathioprine (AZA), and prednisone (PRED) has been used in low immunological risk KTR. In this group of patients, the use of induction therapy with basiliximab [6,7] or substitution of azathioprine by mycophenolate [8] may not provide substantial efficacy, safety and cost advantage. In two independent cohorts of patients receiving TAC, AZA, and PRED without induction therapy, we recently showed that the incidence of AR was 32% and of CMV infection was 30% [9], and that the incidence of CMV infection after treatment of AR was 47% [10].

While induction therapy with rATG is standard of care for KTR at high risk of AR, the use in patients at low immunological risk remains controversial [11,12]. Interestingly, utilization of rATG in low immunological risk patients has recently increased to over 40%, perhaps to further reduce the incidence of rejection [13,14]. On the other hand, the appropriate dose, which balances side effects and efficacy, remains debated, given the potential increase in the incidence of infections, particularly CMV infection [15,16]. Depending on recipient's immune status, 40–90% of the patients develop CMV viremia, and, in immunological naïve patients, up to 65% of the individuals develop symptomatic disease [17]. In a recent randomized trial, we observed a low incidence of AR (9.4%) and CMV infection (4.7%) in patients receiving 3 mg/kg of rATG induction, low-dose TAC, and everolimus in KTR receiving no pharmacological prophylaxis for CMV infection [18]. This immunosuppressive regimen, previously with basiliximab and currently with rATG induction, has also been used in low immunological risk KTR [19].

Therefore, we hypothesized that the use of a single 3 mg/kg of rATG would be associated with significant reduction in the incidence of AR in patients receiving TAC, AZA, and PRED and no CMV prophylaxis. This strategy may reduce the need for treatment of CMV infection that occurs after the treatment of AR, providing an effective and safe strategy compared with no induction.

Materials and methods

Study design

This was a natural experiment in which all recipients of kidney transplants, except living donor HLA identical, started receiving 3 mg/kg of rATG for induction therapy from June 17, 2014, at our institution, regardless of the maintenance immunosuppressive drug combination as per institutional protocols (Fig. S1). Therefore, two retrospective cohorts of consecutive low immunological risk adult KTR who received maintenance immunosuppression therapy with TAC, AZA, and PRED were constructed. From June 17, 2014, to September 16, 2015, there were 1126 kidney transplants of which 466 were included in the experimental group (r-ATG 3 mg/kg). For the control group (no-induction), we then identified 466 consecutive kidney transplants who did not receive induction therapy before June 17, 2014 (Fig. 1). Data were collected up to September 16, 2016.

We compared the 12-month incidence of acute rejection and CMV infection, patient and graft survival, and hospital readmissions among patients receiving single 3 mg/kg of ATG versus no induction with tacrolimus (TAC), prednisone (PRED), azathioprine (AZA), and no pharmacological CMV prophylaxis. The study was approved by the local Research Ethics Committee at Universidade Federal de São Paulo (UNIFESP) under registration C.A.A.E ID: 49776815.4.0000.5505.

Study population

All patients had a negative complement-dependent cytotoxicity cross-match, a panel-reactive antibody (PRA) lower than 50%, no preformed A, B, and DR donor-specific antibodies with mean fluorescence intensity higher than 1500, and received an ABO-compatible renal allograft from living or standard deceased donors. We excluded KTR with pretransplant serologies positive for human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus

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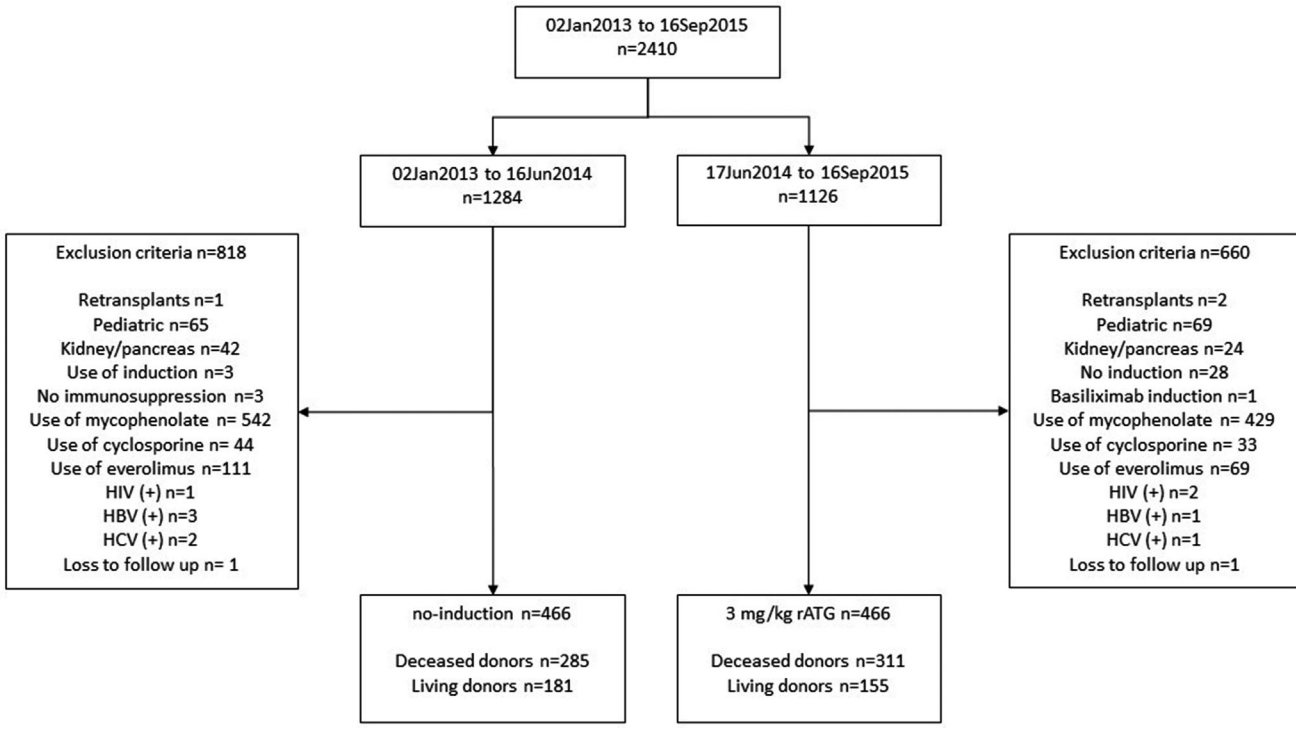

Figure 1 Disposition of the study population.

surface antigen (HBsAg) and those who underwent simultaneous pancreas–kidney and pediatric transplantation. Recipients who did not receive immunosuppression therapy because of early graft loss, received induction therapy with basiliximab, or received initial immunosuppressive therapy including cyclosporine, everolimus, and mycophenolate were excluded from the analysis.

Immunosuppression protocol

Induction therapy consisted of a single 3 mg/kg dose of rATG administered intravenously over 8 h beginning within the first 24 h after graft revascularization.

All recipients in both groups received TAC 0.1 mg/kg twice daily, with doses adjusted to maintain blood concentrations between 5 and 15 ng/ml, combined with 2 mg/kg of AZA. All recipients were initiated on intravenous methylprednisolone (500 mg) intraoperatively. Recipients received 0.5 mg/kg oral PRED, and the dose was reduced to a daily 5 mg doses by the end of the first month.

Prophylaxis

All patients received a 5 day course of 400 mg of albendazole and continuous use of sulfamethoxazole–trimethoprim. None of the patients received pharmacological prophylaxis for CMV infection. Preemptive strategy was used only for donor (+) and recipient (−) CMV serostatus combination (D+/R−), and after treatment of AR episodes. The other patients were monitored at the physician discretion. This targeted strategy was based on the low incidence of CMV disease in this patients receiving AZA [9] but higher incidence after treatment of AR [10]. The preemptive therapy consisted of every other week monitoring the viral replication from the third week after transplantation until the end of the third month, using the CMV pp65 antigenemia assay.

Primary and secondary outcomes

The primary outcome of this analysis was to compare the incidence of biopsy-confirmed acute rejection (BPAR) between the no-induction and the rATG 3 mg/kg groups. Secondary outcomes included the incidence of CMV infection and disease, incidence of delayed graft function, severity of BPAR according to Banff classification and response to treatment, all treated acute rejection episodes, and treatment of acute rejection, length of hospital stay, and incidence of hospital readmissions.

DGF and acute rejection in the first year

Delayed graft function was defined as the need of dialysis during the first week after transplantation,

excluding a single dialysis for hypervolemia and/or hyperkalemia. AR was defined as any report of treated AR episode in the first year post-transplant including clinical acute rejections and biopsy-proven acute rejection (BPAR). Clinical acute rejections were defined as graft dysfunction without histological evidence of rejection, including borderline changes, and treated with methylprednisolone for at least three days. The episodes of BPAR were graded according to the Banff 2009 criteria (IA or higher). Three pathologists, unaware of the details of the clinical scenario, including the immunosuppressive regimen, graded the biopsies. As a sensitive analysis, we also examined those with BPAR only. Severe rejections were defined as those episodes treated with rATG based on histological severity or resistant to steroid treatment. We used a Cox proportional hazards model to estimate the association in an adjusted framework. The final model was adjusted for pretransplant CMV serologic status, donor type, donor terminal creatinine, cold ischemia time (CIT), donor history of hypertension, and HLA mismatches.

CMV infection or disease

Cytomegalovirus infection was defined as the presence of more than 10 infected cells in a total of 200 000 peripheral blood neutrophils in asymptomatic patients based on CMV pp65 antigenemia assay. CMV disease was diagnosed based on the presence of CMV-related signs or symptoms including fever, asthenia, myalgia, leukopenia, thrombocytopenia, or liver enzymes abnormalities, and the presence of any number of CMV pp65-infected cells. CMV infection or disease was treated with intravenous ganciclovir for at least 14 days with weekly monitoring of viral replication. Treatment was continued for 1 week after the first negative CMV pp65 antigenemia test.

Length of stay for the transplant hospitalizations and post-KT hospital readmissions

We estimated the independent association between 3 mg/kg ATG and length of stay (LOS) using negative binomial regression (ratio of time). Post-KT readmissions were defined as hospital admissions after discharge from the transplant hospitalization. Recipients who died ($n = 7$) before initial discharge after KT were excluded. KT recipients who had graft loss ($n = 9$) before discharge, but did not die, were also excluded from the analysis.

Death-censored graft loss and mortality

The cumulative incidence of death-censored graft loss and mortality was measured within the first year post-KT and compared between 3 mg/kg rATG and no induction. Graft loss was defined as the need for permanent return to dialysis. Loss to follow-up was defined by the lack of information for more than 6 months.

Statistical analysis

Continuous variables were presented as mean and standard deviation or medians [interquartile range (IQR)], depending on normality. Differences among the groups were identified using independent-samples Mann–Whitney tests or *t*-test student. Categorical variables were presented as frequency and percentage, and differences among groups were identified using the chi-square or Fisher's exact test. We used logistic regression to estimate the association between rATG and DGF. Cox proportional hazards models were used to estimate the association between ATG and CMV infection, treated acute rejection, BPAR, post-KT readmission graft loss, and mortality. The final model was adjusted for pretransplant CMV serologic status, donor type, donor terminal creatinine, CIT, donor history of hypertension, and HLA mismatches. We also tested for effect modification for the association between 3 mg/kg ATG and CMV infection by recipient pretransplant CMV serologic status using the similar model describe above. All hazard ratios and 95% confidence intervals were reported as recommended by Louis and Zeger [20]. Survival curves were obtained by the Kaplan–Meier method. Differences among the groups were identified by the log-rank test. All statistical analyses were performed using STATA version 14.0 (StataCorp, College Station, TX, USA) with two-sided hypothesis testing and $P < 0.05$ as the criteria for statistical significance.

Results

Study population

There were 2410 transplants between January 2013 and September 2015, 1284 before and 1126 after June 17. Patients with pediatric transplants, combined kidney and pancreas transplants, and retransplants, recipients treated with mycophenolate, cyclosporine, and everolimus, and those with positive viral serology were excluded. Also, patients receiving induction therapy in the first period and those who did not receive rATG

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induction in the second period were excluded. The distribution of these patients in each period is listed in Fig. 1. The final study cohort included 466 KTR who received 3 mg/kg rATG and 466 KTR receiving no induction.

Characteristics of recipients by induction category

Recipients who received 3 mg/kg ATG and no induction were similar regarding age, sex, race, PRA, time on dialysis, and total number of HLA mismatches. They received a similar proportion of live donor kidney transplants (33.3% vs. 38.8%, $P = 0.08$), and for those who received standard deceased donors, a similar Kidney Donor Profile Index (KDPI; 51.0 vs. 52.0%, $P = 0.9$) was obtained. Compared with recipients receiving no induction, those who received 3 mg/kg ATG had slightly longer cold ischemia time (22.0 vs. 21.0 h; $P < 0.001$), higher mean terminal creatinine of deceased donors (1.5 vs. 1.3 mg/dl; $P < 0.001$), and lower number of donors with history of hypertension (20.6% vs. 27.7%). The most prevalent pretransplant CMV serologic status was donor (+)/ recipient (+) in both groups (85.6% vs. 86.1%). KT recipients receiving ATG 3 mg/kg had higher number of recipients with the high-risk pretransplant CMV serostatus combination, donor (+)/recipient (−; 10.7% vs. 6.0 %, $P = 0.003$; Table 1).

Delayed graft function and acute rejection

Recipients receiving 3 mg/kg rATG had similar DGF rates compared with those receiving no induction (48.2% vs. 44.9%, Table 2) even after adjusting for donor and recipient factors (Table 3).

There were no differences in median tacrolimus whole blood trough concentrations at 1, 3, 6, and 12 months (Fig. S1). The incidence of first treated acute rejection (13.7% vs. 50.6%, $P < 0.001$) and first BPAR (5.8% vs. 22.7, $P < 0.001$) was lower among patients receiving 3 mg/kg rATG (Table 2). The median times to first treated acute rejection were 7 days (IQR 5–16) in the no-induction group and 22.5 (IQR 9.5–63) days in the 3 mg/kg rATG group. Overall, recipients who received 3 mg/kg ATG were 80% less likely to have AR (Fig. 2a) and BPAR (Fig. 2b). These findings were consistent after adjusting for donor and recipient characteristics (Table 3) and also with time-varying CMV infection (Table S2). There were no Banff grade III and antibody-mediated acute rejections among those who received 3 mg/kg ATG (Table 2). Severe rejection was 74% less frequent among KT recipients receiving 3 mg/kg rATG than among those receiving no induction (Table 3).

CMV Infection or disease

The overall incidence of CMV infection/disease was 33.7% in the 3 mg/kg rATG group and 27.3% in the no-induction group, and there was no tissue-invasive CMV disease. The median times to first CMV infection were 53 (37–74) days in the no-induction group and 44 (IQR 31–64) in the 3 mg/kg rATG group. There were no differences in the incidence of CMV infection/disease in patients receiving preemptive therapy, either in D+/R− CMV patients or after treatment of acute rejection. On the other hand, among patients monitored at physician discretion, the incidence of CMV infection/disease was higher in those receiving rATG (Table 4). There were no differences in treatment duration [18 (IQR 14–25) vs. 20 (IQR 14–22), $P = 0.705$] and recurrence rate (29.1% vs. 35%, $P = 0.291$).

Overall, recipients receiving 3 mg/kg rATG had 1.33-fold (HR: $_{1.05}1.33_{1.68}$, $P = 0.016$) increased risk of CMV infections, but after adjusting for donor and recipient characteristics, including pretransplant CMV serostatus, this risk was no longer observed (aHR $_{0.86}1.10_{1.40}$, $P = 0.5$) compared with patients receiving no induction (Table 5). We found no association between pretransplant CMV serologic status and rATG on the occurrence of CMV, except for the high-risk (D+/R−) individuals (aHR $_{2.48}3.41_{4.71}$, $P < 0.001$; Table 5).

The cumulative incidences of CMV infection/disease among recipients with pretransplant serostatus positive (31.6% and 23.9%) and negative (50% and 68.6%, $P = 0.086$) in the 3 mg/kg rATG group and in the no-induction group are shown in Fig. 3, respectively. In a stratified unadjusted analysis, the risk of CMV infections associated with 3 mg/kg rATG differed between recipients who were pretransplant CMV serostatus positive and negative (p-interaction = 0.006). Among those who were positive, 3 mg/kg rATG was a 1.44-fold increased risk of CMV infections (HR: $_{1.11}1.44_{1.86}$, $P = 0.006$, Fig. 3a). Among recipients who were negative, 3 mg/kg rATG was not associated with any increased risk of CMV infections (HR: $_{0.39}0.68_{1.19}$, $P = 0.2$, Fig. 3b). After adjustment, risk of CMV infection still varied between CMV-negative and CMV-positive recipients (p-interaction = 0.001). However, the hazard ratio of CMV infection was no longer statistically significant in the positive (aHR $_{0.94}1.25_{1.65}$, $P = 0.1$) and in the negative (aHR $_{0.28}0.57_{1.16}$, $P = 0.1$) groups. When we adjust for time-varying AR in the time-to-CMV model, the new

Table 1. Demographic characteristics of the study population.

Parameters	No induction (n = 466)	rATG 3 mg/kg (n = 466)	P-value
Recipient age (years), median (IQR)	44.0 (33.0, 55.0)	43.0 (31.0, 54.0)	0.2
Recipient gender, male, %	61.4	66.1	0.1
Recipient race, %			
Caucasian	43.3	40.6	0.8
Black	15.5	16.1	
Mixed	38.8	40.3	
Others	2.4	3.0	
Cause of ESRD, %			
Glomerulonephritis	24.5	20.0	0.2
Hypertension	9.0	9.9	
Diabetes mellitus	11.2	14.4	
Polycystic disease	6.9	8.6	
Undetermined	39.1	40.6	
Others	9.4	6.7	
Type of dialysis, %			
Hemodialysis	82.8	87.1	0.2
Peritoneal	4.3	4.3	
No dialysis	9.2	5.6	
Hemodialysis + peritoneal	3.6	3.0	
Time on dialysis (months), median (IQR)	27.4 (11.0, 49.0)	28.1 (14.2, 50.8)	0.4
PRA class I, median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.6
PRA class II, median (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.3
HLA-MM, median (IQR)	3.0 (2.0, 3.0)	2.0 (2.0, 3.0)	0.066
CMV IgG serologic status, %			
D+/R+	86.1 (n = 401)	85.6 (n = 399)	0.003
D+/R−	6.0 (n = 28)	10.7 (n = 50)	
D−/R+	6.4 (n = 30)	3.2 (n = 15)	
D−/R−	1.5 (n = 7)	0.4 (n = 2)	
Donor age (years), median (IQR)	44.0 (33.0, 51.0)	42.0 (33.0, 49.0)	0.2
Donor gender, male, %	51.1	56.2	0.1
Donor race, %			
Caucasian	54.5	53.4	0.4
Black	10.7	11.4	
Mixed	34.3	33.7	
Others	0.4	1.5	
Donor type, %			
Living	38.8	33.3	0.08
Deceased	61.2	66.7	
Donor death, %			
Cerebrovascular	45.3	42.8	0.6
Trauma	42.8	46.9	
Others	11.9	10.3	
Terminal creatinine (mg/dl), median (IQR)	1.3 (0.9, 1.9)	1.5 (1.0, 2.7)	<0.001
History of hypertension			
Yes	27.7	20.6	0.041
No	72.3	79.4	
Cold ischemia time (h), median (IQR)	21.0 (18.0, 26.0)	22.0 (19.0, 28.0)	<0.001
KDPI, median (IQR)	52.0 (31.0, 68.0)	51.0 (33.0, 64.5)	0.9

ESRD, end-stage renal disease; KDPI, kidney donor profile index.

adjusted associations between ATG and CMV showed similar results, with a HR 1.71 (95% CI 1.28–2.29, $P < 0.001$) for the overall population, a HR 2.14 (95% CI 1.56–2.93, $P < 0.001$) for CMV-positive recipients, and a HR 0.66 (95% CI 0.31–1.39, $P = 0.3$) for CMV-negative recipients (Table S1).

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Table 2. Clinical outcomes.

	No induction $N = 466$	rATG 3 mg/kg $N = 466$	*P*-value
Delayed graft function*, *N* (%)	128 (44.9)	150 (48.2)	0.417
1st treated acute rejection, *N* (%)	236 (50.6)	64 (13.7)	<0.001
Biopsy-proven acute rejection, *N* (%)	106 (22.7)	27 (5.8)	<0.001
IA, *N* (%)	42 (39.6)	9 (33.4)	
IB, *N* (%)	41 (38.7)	6 (22.2)	
IIA, *N* (%)	18 (17.0)	10 (37.0)	
IIB, *N* (%)	2 (1.9)	2 (7.4)	
III, *N* (%)	1 (0.9)	0	
ABMR, *N* (%)	2 (1.9)	0	
Clinical acute rejection, *N* (%)	130 (27.9)	37 (7.9)	<0.001
Borderline changes	43 (9.2)	16 (3.4)	
Clinical assessment	87 (18.7)	21 (4.5)	
Severe acute rejection, *N* (%)	37 (7.9)	13 (2.8)	<0.001
Biopsy scores	9	7	
Steroid resistant	28	6	
Treatment, *N* (%)	238	64	
MP	197 (83.5)	51 (79.7)	
MP + rATG	28 (11.9)	6 (9.4)	
MP + PF + IVIG	1 (0.4)	0	
rATG	8 (3.4)	7 (10.9)	
rATG + PP	1 (0.4)	0	
PP	1 (0.4)	0	

ABMR, antibody-mediated rejection; IVIG, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis; rATG, rabbit anti-thymocyte globulin.

Table 3. Risk of delayed graft function, treated acute rejection, biopsy-proven acute rejection, and severe acute rejection within the first year by induction therapy.

	rATG 3 mg/kg vs. no induction			
	Unadjusted	*P*-value	Adjusted*	*P*-value
Logistic regression model				
DGF	$_{0.83}1.14_{1.58}$	0.4	$_{0.67}0.95_{1.34}$	0.8
Cox proportional hazards models				
Treated acute rejection	$_{0.15}0.20_{0.26}$	<0.001	$_{0.14}0.19_{0.25}$	<0.001
Biopsy-proven acute rejection	$_{0.12}0.19_{0.29}$	<0.001	$_{0.12}0.19_{0.30}$	<0.001
Severe acute rejection	$_{0.14}0.26_{0.50}$	<0.001	$_{0.14}0.26_{0.50}$	<0.001

*Adjusted for pretransplant CMV serologic status, donor type, donor terminal creatinine, donor history of hypertension, cold ischemia time, and HLA mismatches.

Length of stay for the transplant hospitalizations and Post-KT hospital readmissions

The median LOS for the transplant hospitalizations was 7 days in the 3 mg/kg rATG group and 8 days in the no-induction group (Table 6). Overall, recipients receiving 3 mg/kg rATG had 0.12-fold shorter LOS for the transplant than those who received no induction (aRR $_{-0.19}-0.12_{-0.04}$, $P = 0.002$, Table 7). Among KTR receiving 3 mg/kg rATG, 42.7% had a post-KT hospital readmission compared with 41.2% of those who did not receive induction (HR $_{0.82}1.00_{1.23}$, $P = 0.9$). After adjustment, we found no associations between 3 mg/kg ATG and post-KT hospital readmissions (aHR $_{0.75}0.93_{1.14}$, $P = 0.5$; Table 7). Compared with the most prevalent pretransplant (D+/R+) CMV serostatus, the high-risk (D+/R−) serostatus combination had 1.62-fold increased risk of post-KT hospital readmissions during first year post-KT (aHR $_{1.17}1.62_{2.24}$, $P = 0.003$). The 3 mg/kg rATG was not associated with an increased

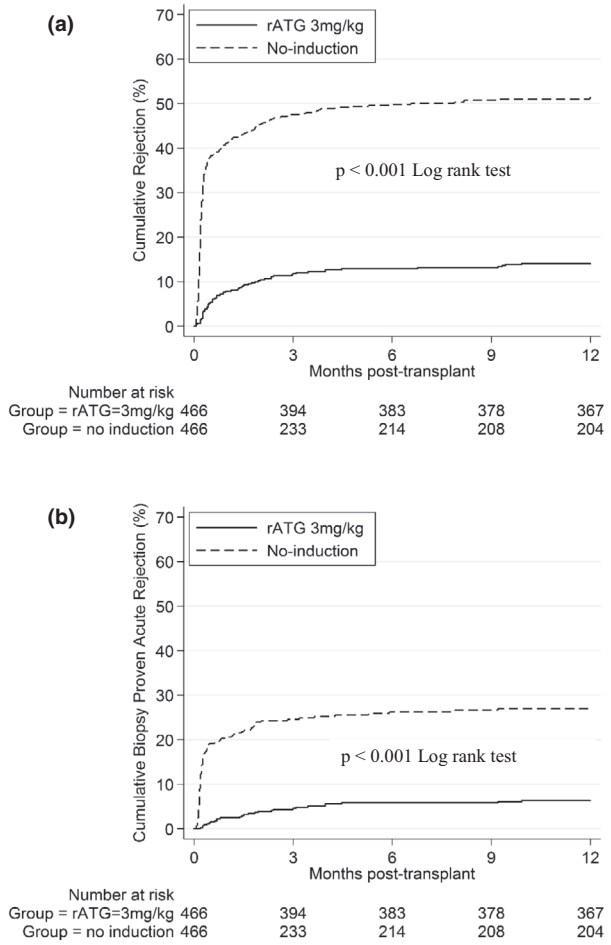

Figure 2 Cumulative incidence of (a) treated acute rejection and (b) biopsy-proven acute rejection in the first year after kidney transplant in low-risk recipients who received rATG 3 m/kg compared with those who received no induction.

risk of post-KT hospital readmissions because of infection complications compared with no induction (22.7% vs. 21.2%, HR: $_{0.79}1.04_{1.37}$, $P = 0.8$), and remained non-significant after adjustments (aHR: $_{0.68}0.91_{1.21}$, 0.5). Recipients receiving 3 mg/kg rATG were 57% less likely to have experienced post-KT readmissions because of AR (4.3% vs. 9.6%, HR: $_{0.25}0.43_{0.73}$, $P = 0.002$). This was consistent after adjustments (aHR: $_{0.24}0.41_{0.71}$, $P = 0.002$; Table 7).

Table 5. Risk factors associated with CMV infection after kidney transplantation.

	CMV infections	
	Unadjusted	Adjusted
rATG 3 mg/kg	$_{1.05}1.33_{1.68}$	$_{0.86}1.10_{1.40}$
CMV serologic status		
D+/R+ (reference)		
D+/R−		$_{2.48}3.41_{4.71}$
D−/R+		$_{0.49}$ 0.88 $_{1.59}$
D−/R−		$_{0.25}1.02_{4.13}$
Deceased donors		$_{0.81}1.45_{2.62}$
Donor terminal creatinine (mg/dl)		$_{1.04}1.12_{1.20}$
Cold ischemia time (h)		$_{0.97}0.99_{1.02}$
History of donor hypertension		$_{0.62}0.87_{1.23}$
HLA mismatches		$_{0.91}0.99_{1.09}$

All adjusted hazard ratios are from a single adjusted Cox proportional hazards model for CMV infections.

Mortality and death-censored graft failure

There were no differences in patient (97.2% vs. 98.0%) and death-censored graft (97.1% vs. 96.2%) survivals comparing recipients receiving 3 mg/kg rATG and no induction. Patients receiving 3 mg/kg rATG showed no increased risk for mortality (HR: $_{0.51}1.25_{3.08}$, $P = 0.6$, Fig. 4a) and death-censored graft failure (HR: $_{0.37}0.76_{1.56}$, $P = 0.5$, Fig. 4b) at 1 year post-KT. These findings were consistent after adjusting for donor and recipient characteristics for both mortality (aHR $_{0.61}1.44_{3.37}$, $P = 0.4$) and death-censored graft failure (aHR $_{0.34}0.73_{1.55}$, $P = 0.4$).

Table 4. Incidence of first CMV event according to preemptive therapy group.

	No induction (n = 466)		rATG 3 mg/kg (n = 466)	
Preemptive group	Patients, n	1st CMV event, n (%)	Patients, n	1st CMV event, n (%)
D+/R- CMV	28	11 (39)	50	21 (42)
Infection		6 (54.5)		15 (71.4)
Disease		5 (45.5)		6 (28.6)
After AR	236	84 (36)	64	26 (41)
Infection		53 (63.1)		16 (61.5)
Disease		31 (36.9)		10 (38.5)
Physician discretion	202	32 (16)	352	110 (31)
Infection		19 (59.4)		56 (50.9)
Disease		13 (40.6)		54 (49.1)
Total	466	127 (27.3)	466	157 (33.7)

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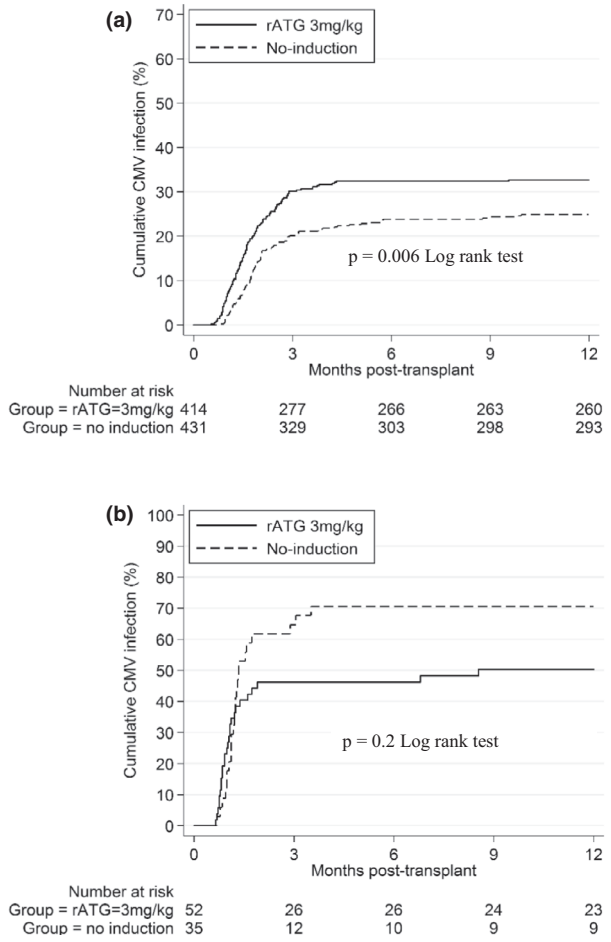

Figure 3 Cumulative incidence of CMV infections according to pre-transplant recipient CMV serostatus (a) positive and (b) negative in low-risk recipients who received rATG 3 m/kg compared with those who received no induction.

Table 6. Length of stay and hospital readmissions according to induction therapy.

	No induction (n = 466)	rATG 3 mg/kg (n = 466)	P-value
Length of stay for KT (IQR)	8 (6–13)	7 (5–12)	0.075
Hospital readmissions	192 (41.2)	199 (42.7)	0.216
Readmissions because of infections	99 (21.2)	106 (22.7)	0.580
Readmissions because of acute rejection	45 (9.6)	20 (4.3)	<0.001

Discussion

In this study, we present the results of our retrospective analysis, comparing outcomes for low-risk kidney transplants performed in two distinct induction therapy eras. We observed that KTR receiving a single 3 mg/kg dose of rATG experienced 80% lower risk of AR during the first year post-KT compared with those receiving no-induction therapies despite the perceived lower risk of AR in this selected population. Furthermore, we found that the use of rATG dose was not independently associated with an increased risk of CMV infections, despite recipients not receiving pharmacological prophylaxis. In addition, risk of mortality, death-censored graft survival, and post-KT hospital readmissions were similar among KT recipients in both regimens.

The combination of tacrolimus, azathioprine, and prednisone is not considered the standard of care therapy. We are still using this drug combination based on the large US cohort analysis showing similar long-term outcomes compared with mycophenolate, despite higher incidence of acute rejection [8]. Furthermore, in our kidney transplant population, the tolerability of mycophenolate is lower [4] and the incidence of CMV infection/disease is higher, thus limiting its use to higher risk patients [5].

Traditionally, nonsensitized, living and standard deceased donor KTR are presumed to be at lower risk for acute rejection than sensitized and expanded criteria donor recipients. Yet, the observed high incidence of biopsy-confirmed acute rejection among patients receiving no induction (22.7%) is comparable to earlier published studies, between 17% [21] and up to 35% [22–24]. On the other hand, such a low incidence of biopsy-proven acute rejection (5.8%) among patients receiving 3 mg/kg rATG has not been observed in low-risk patients receiving basiliximab induction, TAC, and mycophenolate [6,7]. The single 3 mg/kg dose of rATG is lower than that recently reported (5–6 mg/kg) [25,26], and still was associated with a 80% lower risk of AR compared with no induction, consistent with previous studies that generally reported lower rejection rates with rATG [13,27]. Although some center mentions r-ATG may not be justified in low-risk transplant recipients, we and previous studies [28,29] document a low incidence of AR associated with rATG, providing support to the idea that rATG costs may be offset by reduced clinical costs associated with diagnosis, management and hospital readmissions of fewer patients with less severe rejection episodes.

In our study, where 36% of recipients received allografts from living donors and 64% from standard deceased donors, 3 mg/kg rATG administered within 24 h after revascularization was not associated with

Table 7. The association between rATG 3 mg/kg induction and length of stay and post-KT readmissions.

	rATG 3 mg/kg vs. no induction			
	Unadjusted	*P*-value	Adjusted*	*P*-value
LOS for KT†	$_{-0.10}-0.03_{-0.05}$	0.5	$_{-0.19}-0.12_{-0.04}$	0.002
Post-KT hospital readmissions‡	$_{0.82}1.00_{1.23}$	0.9	$_{0.75}0.93_{1.14}$	0.5
Post-KT readmissions because of infections‡	$_{0.79}1.04_{1.37}$	0.8	$_{0.68}0.91_{1.21}$	0.5
Post-KT readmissions because of AR‡	$_{0.25}0.43_{0.73}$	0.002	$_{0.24}0.41_{0.71}$	0.002

AR, acute rejection; KT, kidney transplant; LOS, length of stay; rATG, anti-thymocyte globulin.

Subscript indicates upper and lower bounds of a 95% confidence interval.

*Adjusted for CMV serologic status, donor type, donor terminal creatinine, donor history of hypertension, cold ischemia time, and HLA mismatches.

†Relative rate derived from negative binomial regression.

‡Hazard ratios derived from Cox proportional hazards models.

reduced incidence of DGF as observed previously [30]. The timing of rATG administration and the longer CIT might be involved in this discrepancy. We also observed a shorter LOS among those receiving 3 mg/kg rATG than no induction, consistent with a recent published study who reported that 3 mg/kg rATG administrated intraoperatively was associated with a reduction in LOS. It is possible to be because of a less frequent dosing and reduced treatment for acute rejection in the transplant hospitalization [14].

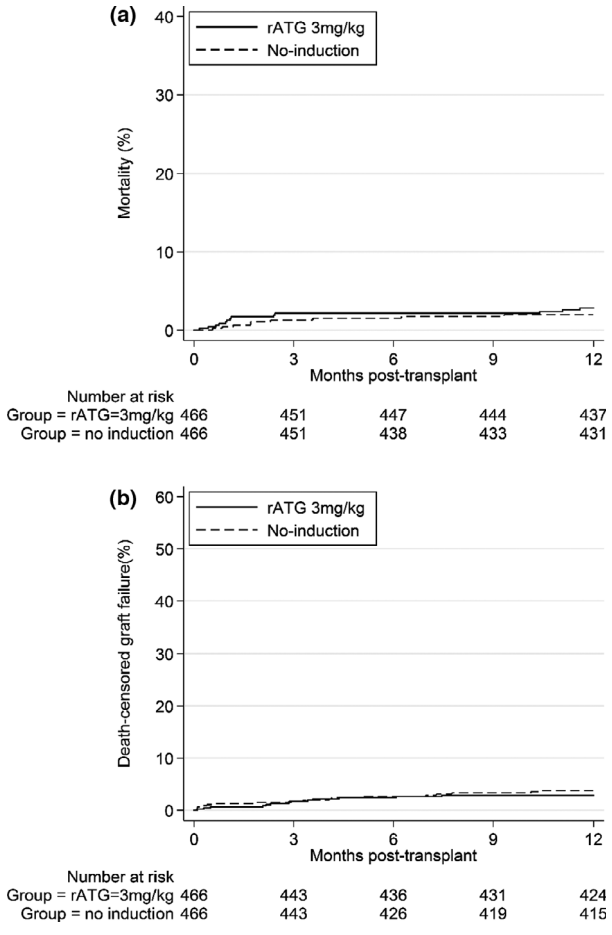

Figure 4 Mortality (a) and death-censored graft failure (b) in low-risk recipients who received rATG 3 m/kg compared with those who received no induction.

This immunosuppressive protocol also proved to be safe, as illustrated by comparable rates of CMV infection, regardless of the lack of CMV prophylaxis and the use of targeted preemptive therapy. The ideal preventive strategy, universal prophylaxis or preemptive therapy, is still open for debate according to the last international consensus [31]. We opted for preemptive therapy for several reasons, including our low proportion of high-risk D+/R− patients (6%), the relatively low incidence and short duration of the treatment of CMV infection with ganciclovir, and the high cost and the high incidence of neutropenia associated with the use of valganciclovir. A recent study showed that among 40 D+/R− kidney transplant recipients receiving pharmacological prophylaxis for 200 days, the incidence of neutropenia was 53%, 30% had to reduce/discontinue MPA and 35% valganciclovir, with still 15% late CMV infection. In the same study, among 92 R+ patients receiving preemptive therapy, 40% were treated for CMV, with a mean duration of 21 days, and only 5% developed neutropenia [32]. A recent multicenter, open-label, randomized clinical study showed that the incidence of CMV infection or disease was 11.5% among patients receiving prophylaxis and 39.7% in those receiving preemptive therapy. Interestingly, both strategies were similarly effective in preventing graft loss and death after a follow-up of up to 84 months [33], a finding also observed in an independent cohort study [34]. Therefore, the incidence of CMV infection/disease in our cohort is

lower than that observed in contemporaneous studies using preemptive strategies.

Accounting for baseline characteristics, the risk of CMV infection was similar among recipients receiving 3 mg/kg rATG compared with no induction (aHR $_{0.86}1.10_{1.40}$, $P = 0.5$). Several factors might be associated with this observation, including the use of a lower rATG dose, the use of AZA instead of mycophenolate, the 80% reduction in the incidence and severity of AR, and the 74% reduction in severe AR treated with rATG.

Furthermore, we have found that the CMV D+/R− was a risk factor for CMV infection, whereas 3 mg/kg rATG was not, suggesting that CMV infection was driven by pretransplant CMV serologic status. In line with this observation, a recent study confirmed that D+R− KTRs have the highest risk of CMV DNAemia, which is not increased by the use of rATG [35]. We confirmed by effect modification analysis that the association between CMV infection and rATG was modified by recipient pretransplant CMV serostatus. However, after adjustments for baseline characteristics, CMV infection was no longer statistically significant in either group (R+ and R−), suggesting that the use of 3 mg/kg of ATG was not associated with an increased risk of CMV infections, regardless of the pretransplant CMV serologic status.

There were no differences in the incidence of CMV infection/disease in patients receiving preemptive therapy, both in the CMV D+/R− high-risk group and after treatment for AR. In one previous cohort analysis, we showed that 47% of patients treated for acute rejection during the first year after transplantation developed CMV infection/disease [10]. The significant reduction in the incidence of acute rejection in patients receiving rATG induction is therefore associated with lower incidence of CMV infection/disease. On the other hand, in patients monitored by physician discretion the incidence of CMV infection/disease was higher in the rATG group compared with the no-induction group, perhaps because of the known higher perceived risk. Also, the proportion of patients with CMV infection was higher in the CMV D+/R− high-risk group and in those patients monitored by physician discretion, suggesting that the use of rATG was associated with earlier diagnosis, before development of any signals and symptoms. These data suggest that the higher incidence of CMV infection/disease in patients monitored at the discretion of the physician is compensated by a significant reduction in the number of CMV infection/disease observed after treatment of AR, confirming our initial hypothesis.

The use of 3 mg/kg of rATG was associated with shorter initial hospital stay and lower number of readmission because of AR. Yet, consistent with previous studies, we found that despite the reduction in AR incidence, there was no difference in death-censored graft survival. Also, the use of 3 mg/kg of rATG was not associated with increased mortality at 12 months.

Our study has several limitations that merit consideration. The single-center nature of the study, retrospective design, demographic characteristics of the population, and lack of CMV prophylaxis, even for high-risk D+/R− KT, cannot exclude the potential for intervention bias or the role of unmeasured confounding variables. The unique targeted preemptive strategy prevents extrapolation of the data to other populations. However, we accounted for confounding and treatment selection bias using adjusted logistic regression models. We also feel that the disadvantages of a retrospective study are mitigated by our ability to harness a natural experiment created by the introduction of an induction protocol change. Furthermore, to our knowledge, this is the first report of the impact of a single 3 mg/kg dose of ATG on the incidence of AR and CMV infection in low-risk KT recipients with no pharmacological CMV prophylaxis.

This cohort study highlights that the use of a single 3 mg/kg dose of rATG in combination with tacrolimus and azathioprine, a drug that currently is not standard, combined with a preemptive approach for the prevention of CMV infection is safe, effective, and undoubtedly associated with significant cost savings. This inference is based on simple direct cost analysis. First, the annual cost of the combination of tacrolimus and mycophenolate is 2.8 times higher compared with tacrolimus and azathioprine (US$ 1048.00 vs. US$ 368.00) for each patient receiving standard doses of each drug combination, based on the unitary cost paid by the national public unified health system (https://paineldeprecos.planejamento.gov.br/). Secondly, the incidence of CMV infection/disease is higher among patients receiving rATG 3 mg/kg in combination with tacrolimus and mycophenolate (62.5%) [5] than in those receiving tacrolimus and azathioprine in this analysis (33.7%). Finally, additional US$ 7654.00 would be necessary only to provide CMV prophylaxis for 90 days with valganciclovir, regardless of the immunosuppressive drug combination.

Clearly, tacrolimus and azathioprine without induction therapy and with preemptive CMV strategy were associated with higher incidence of acute rejection and CMV infection compared with the standard of care in combination with universal CMV pharmacological prophylaxis. Yet, the addition of rATG 3 mg/kg induction therapy is now providing low incidence of acute rejection comparable to the standard of care. Lastly, instead of exposing all

patients to at least 90 days of CMV pharmacological prophylaxis, we have the option to treat only 35% of them in the outpatient setting with intravenous ganciclovir for a mean time of 20 days at an overall cost of U$ 112.00.

These findings are in agreement with a previous prospective randomized trial supporting the choice of azathioprine over mycophenolate in renal transplant patients receiving cyclosporine microemulsion [36]. This immunosuppressive strategy is an alternative to the current standard of care therapy and seems to be cost-effective, especially when access to mycophenolate and valganciclovir is limited because of cost constraints [37]. Nonetheless, longer follow-up is required to provide efficacy data for the prevention of de novo DSA formation and development of late acute and chronic antibody-mediated rejection.

In summary, among low-risk KTR receiving azathioprine and preemptive CMV therapy, a single 3 mg/kg dose of ATG was associated with a significant reduction in the AR without increasing the risk of CMV infection, regardless of recipient pretransplant CMV serostatus.

Authorship

MIP: contributed to study design, data acquisition, statistical analysis, and writing and revision of the manuscript. MGB, AAS, JGW and ABM: performed statistical analysis, and wrote and revised the manuscript. ABB: performed data acquisition. CRF, MPC and JMP: designed the study and revised the manuscript. DLS and HTS: designed the study, and wrote and revised the manuscript.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors thank the Epidemiology Research Group in Organ Transplantation for supporting the development of this study. The analyses described here are the responsibility of the authors alone and do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the U.S. Government. Funding for this study was provided in part by the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) grant numbers: F30DK116658 (PI: Shaffer) and K24DK101828 (PI: Segev).

Conflict of interest

The following authors of this manuscript have conflicts of interest to disclose: Helio Tedesco-Silva has received speaker's fees and travel or accommodation expenses for development of educational presentations and scientific advice from Novartis, Pfizer, and Roche. Jose Medina Pestana has received speaker's fees and travel or accommodation expenses for development of educational presentations and scientific advice from Bristol-Myers Squibb, Novartis, Pfizer, and Roche. Claudia Felipe has received speaker's fees for development of educational presentations and travel or accommodation expenses from Novartis and Pfizer. Marina Cristelli has received speaker's fees for development of educational presentations and travel or accommodation expenses from Novartis and Pfizer. Dorry Segev receives speaking honoraria from Sanofi. The other authors of this manuscript have no conflicts of interest to disclose.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Institutional immunosuppressive drug regimens according to immunological risk stratification.

Figure S2. Box plot of the tacrolimus whole blood trough concentrations at 1, 3, 6, and 12 months.

Table S1. Risk factors associated with CMV infection after kidney transplantation using treated acute rejection as time-varying covariate.

Table S2. Adjusted risk for treated acute rejection and biopsy-confirmed acute rejection censored for graft loss, lost to follow-up, death, and administrative censoring at 1 year.

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