ORIGINAL ARTICLE

Increased risk of rejection after basiliximab induction in sensitized kidney transplant recipients without pre-existing donor-specific antibodies – a retrospective study

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SUMMARY

Depleting induction therapy is recommended in sensitized kidney transplant recipients (KTRs), though the detrimental effect of nondonor-specific anti-HLA antibodies is not undeniable. We compared the efficacy and safety of basiliximab and rabbit anti-thymocyte globulin (rATG) in sensitized KTRs without pre-existing donor-specific antibodies (DSAs). This monocentric retrospective study involved all sensitized KTR adults without pre-existing DSAs ($n = 218$) who underwent transplantation after June 2007. Patients with basiliximab and rATG therapy were compared for risk of biopsy-proven acute rejection (BPAR) and a composite endpoint (BPAR, graft loss and death) by univariate and multivariate analysis. Patients with basiliximab ($n = 60$) had lower mean calculated panel reactive antibody than those with rATG ($n = 158$; 23.7 ± 24.2 vs. 63.8 ± 32.3, $P < 0.0001$) and more often received a first graft (88% vs. 54%, $P < 0.0001$) and a transplant from a living donor (13% vs. 2%, $P = 0.002$). Risks of BPAR and of reaching the composite endpoint were greater with basiliximab than rATG [HR = 3.63 (1.70–7.77), $P = 0.0009$ and HR = 1.60 (0.99–2.59), $P = 0.050$, respectively]. Several adjustments did not change those risks [BPAR: 3.36 (1.23–9.16), $P = 0.018$; composite endpoint: 1.83 (0.99–3.39), $P = 0.053$]. Infections and malignancies were similar in both groups. rATG remains the first-line treatment in sensitized KTR, even in the absence of pre-existing DSAs.

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Introduction

Induction treatment has decreased the rate of acute allograft rejection in kidney transplant recipients (KTRs) [1] and is strongly recommended [2]. Most transplant centres therefore use lymphocyte-depleting polyclonal antibodies such as rabbit anti-thymocyte globulin (rATG, Thymoglobulin®) or interleukin-2 receptor (IL2R) antagonists, especially basiliximab (Simulect®). As two randomized clinical trials [3,4] showed a lower incidence of rejection in KTRs who received rATG versus an IL2R antagonist, prescription of rATG has been

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increasing in several countries [5,6]. In the United States, 60% of KTRs received rATG in 2013, compared with 39% in 2006 [5]. In Australia, the use of basiliximab therapy decreased by 17% between 2011 and 2015 [7].

The KDIGO recommendations encourage the use of lymphocyte-depleting agents in high-immunological KTRs, while IL2R antagonists are considered as the first-line induction therapy [2]. Immunological risk, as defined in previous studies, depends on the number of previous transplantations, recipient age, donor age, African-American ethnicity, number of human leucocyte antigen (HLA) mismatches and panel-reactive antibodies (PRAs) >30% [2–4]. Individual immunological risk assessment in clinical practice, however, is now mainly based on the potential presence of donor-specific antibodies (DSAs) detected before transplantation by single HLA-antigen flow bead (SAB) assay, that has gradually replaced former cytotoxic techniques and solid-phase assays. Indeed, the presence of pretransplant DSAs increases the risk of antibody-mediated rejection (ABMR) and graft loss [8,9].

Thus, advances in techniques for detecting anti-HLA antibodies have deeply reshuffled the cards in terms of indications for induction therapy. On the one hand, some patients waiting for a first transplantation without historical immunological exposure have natural anti-HLA antibodies that can cause graft rejection if specifically directed against a donor's antigen [10,11]. In this setting, patients historically considered at low immunological risk should benefit from depleting induction therapy. On the other hand, the actual immunological risk in sensitized recipients (previously considered at high risk) is debated when no DSAs are identified before transplantation, since several recent reports have suggested that the risk of rejection may not be affected by nondonor-specific anti-HLA antibodies [8,12–14]. With the increased risk of opportunistic infections and malignancies after rATG induction therapy, we have used basiliximab in sensitized patients without DSAs [1,15].

In this study, we compared the efficacy and safety of basiliximab and rATG in sensitized KTRs without preexisting DSAs (i.e. KTR with nondonor-specific anti-HLA antibodies).

Patients and methods

Study population

We performed a cohort study with KTRs in Tours who underwent transplantation between June 2007 and June 2016. The selection of sensitized patients without preformed DSAs is described in Fig. 1. As since June 2007, the presence of circulating DSAs has been systematically assessed in our centre with SAB assays (One Lambda, Canoga Park, CA, USA), we ruled out all patients who received a transplant before June 2007. In addition, we excluded KTRs with transplantation after June 2017 in order to obtain a follow-up greater than 1 year in all patients. All patients included in this study provided their consent to a prospective collection and use of their data in our hospital's institutional database of transplant patients and the ASTRE database (DR-2012-518). We retrospectively assessed 1030 patients with transplantation during this period and identified 218 with at least one anti-HLA antibody before or the day of transplantation without preformed DSAs (anti-A, anti-B, anti-Cw, anti-DR, anti-DQ, anti-DP antibodies) who received induction therapy with rATG (Thymoglobulin®; Genzyme, Cambridge, MA, USA) or basiliximab (Simulect®; Novartis, Rueil-Malmaison, France).

Initial immunosuppression therapy involved methylprednisolone (250 mg pre- and postoperatively) and basiliximab (20 mg on days 0 and 4) or rATG before transplantation (100 mg on days 0 and 1, next daily dose adapted to maintain CD3-lymphocyte count at $<20/mm^3$ trough until tacrolimus level >8 μg/l). Before 2007, we exclusively used rATG in all sensitized recipients. We then used basiliximab in slightly (PRA < 50%) sensitized recipients without known pretransplant DSA. The maintenance immunosuppressive regimen was mycophenolate mofetil or enteric-coated mycophenolate sodium, calcineurin inhibitor (mainly tacrolimus after 2009) and prednisone in most patients. Target tacrolimus trough levels were 8–12 μg/l before month 3 and 5–8 μg/l thereafter. Prednisone at 1 mg/kg/day for the first 2 weeks was gradually decreased and finally withdrawn during the first year post-transplantation in the absence of clinical or subclinical rejection (protocol graft biopsy at month 3 performed since 2009), *de novo* DSAs and according to the PRA and risk of kidney disease recurrence. Anti-HLA antibodies were screened using the LABScreen Luminex technique (LABScreen Mixed 1 2; One Lambda, Canoga Park, CA, USA) before and after transplantation. In addition, we systematically assessed anti-HLA antibodies at the time of biopsy for cause. For LABScreen Mixed, positive Normalized Background Ratio cut-off was set at >2.5, calculated as the following: NBG ratio = (S#N − SNC bead)/(BG#N − BGNC bead). S#N: sample-specific fluorescent value for bead #N; SNC bead: sample-specific fluorescent value for Negative Control bead; BG#N:

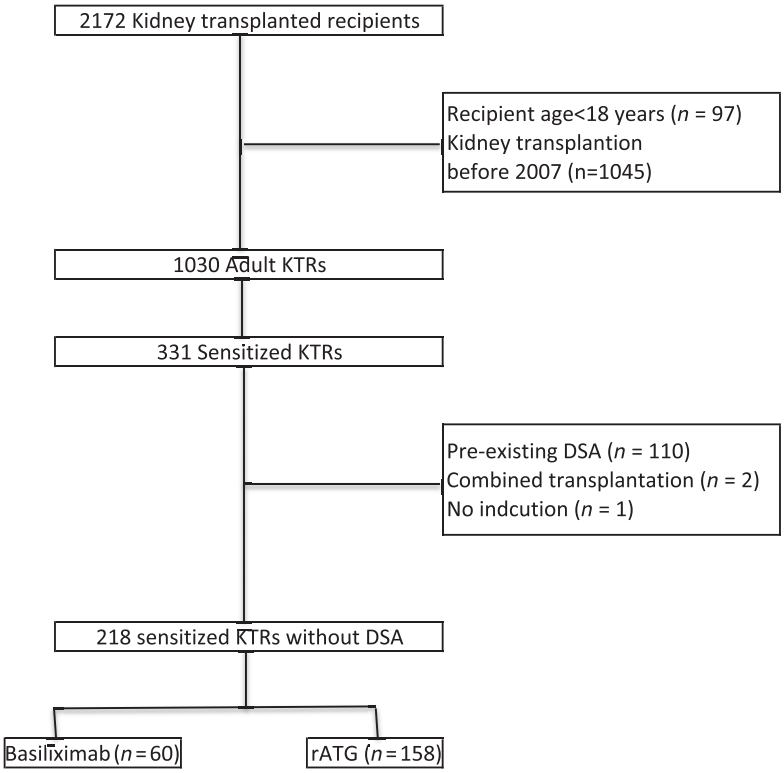

Figure 1 Flow chart of patients in the study. DSA, donor-specific antibody; KTR, kidney transplant recipient; rATG, rabbit anti-thymocyte globulin.

background negative control serum fluorescent value for bead #N; BGNC bead: background negative control serum fluorescent value for Negative Control bead. NC serum: Negative Control Serum validated for a given lot of LABScreen beads. In case of positivity, LABScreen Single Antigen was performed for class I and/or II according to the profile in LABScreen Mixed. The results were expressed as median fluorescence intensity (MFI). MFI > 1000 was considered positive. The absence of pretransplant DSA identification was validated after analysis of each file.

Pneumocystis jirovecii pneumonia prophylaxis with trimethoprim/sulphamethoxazole was administered for 3 months to all patients and continued while CD4 T-cell count <200/mm^3. Recipients with the highest risk of cytomegalovirus (CMV) disease D+R+ and D+R− received prophylaxis with valganciclovir for 3 or 6 months, whatever the induction treatment. All CMV-positive recipients (R+) received prophylaxis with valganciclovir for 3 months after 2009, except D−R+ recipients who received basiliximab (pre-emptive strategy). CMV load was monitored every month after withdrawal of prophylaxis or every 2 weeks until 3 months, then every month until 12 months in pre-emptive strategy. BK virus (BKV) viraemia was assessed twice a month for 3 months, once a month between months 3 and 6 after transplantation, then at months 8, 10–12, 18 and 24. Maintenance immunosuppressive regimen was reduced with BKV viraemia load >3 log.

Variables studied

Baseline data collected included donor and recipient age, donor and recipient sex, type of donor (living or deceased standard or with extended criteria), recipient ethnicity, cause of end-stage renal disease, duration of dialysis, transplantation rank, donor and recipient CMV serology, number of class I and II HLA mismatches, cold ischaemia time and immunosuppressive medications. We retrieved each medical file to determine pretransplant allogeneic exposures (pregnancy, blood transfusions) and post-transplant infections and malignancies. Particular attention was paid to *de novo* DSAs that were systematically assessed at months 3, 6 and 12 during the first year, then every year after transplantation and at the time of any biopsy. Biopsy-proven acute rejection (BPAR) was individually analysed. A biopsy was oriented by increased serum creatinine value (>20% serum creatinine value) or proteinuria (>1 g/day) or systematically performed for protocol graft biopsy at month 3 or in case of *de novo* DSA appearance. The Banff international classification was used to

discriminate biopsy-proven T-cell–mediated rejection (TCMR; i.e. grade ≥1) and ABMR.

Statistical analysis

Continuous and categorical variables are expressed with means (±SD) and number (%) respectively. Continuous data were compared by the Mann–Whitney test and categorical data by the chi-square or Fisher exact test. We compared the incidence of BPAR (all, TCMR and ABMR), overall graft survival, patient survival (end-stage renal disease censored) and death-censored graft survival between patients who received rATG and basiliximab with log-rank tests. Univariate Cox analyses were performed to identify the factors associated with BPAR. We then performed a step-by-step multivariate Cox regression analysis including acknowledged BPAR risk factors, risk factors previously identified ($P < 0.1$) and clinically relevant variables, estimating hazard ratios (HRs) and 95% confidence intervals (CIs). In addition, we compared the incidence of *de novo* DSAs in the two groups, with threshold MFI > 1000. Finally, we analysed a composite risk including BPAR, patient death and graft loss by univariate and multivariate Cox analysis. Statistical analyses involved use of XL-STAT and R v3.3.3 (R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org/). $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

The baseline characteristics of the whole cohort ($n = 218$) and KTRs with rATG ($n = 158$) and basiliximab ($n = 60$) are shown in Table 1. Overall, 53.2% of KTRs were women and the incidence of retransplantation was high (56.7%). Most donors (94.9%; mean age 55.5 ± 14.8 years) were deceased.

We therefore compared the basiliximab and rATG group, in which patients had received a mean dose of 5.05 ± 1.65 mg/kg of rATG for a mean of 4.90 ± 1.43 days. Some differences must be highlighted with regard to the risk of rejection in the two groups. The number of HLA class II mismatches was higher with basiliximab than rATG (2.2 ± 1.1 vs. 1.7 ± 1.2, $P < 0.01$), conferring a theoretically higher risk of rejection in those patients. Other differences rather conferred a higher immunological risk in KTR who received rATG, such as broader sensitization (cPRA 63.8 ± 32.3% vs. 23.7 ± 24.2%, $P < 0.0001$), a higher proportion of retransplantation (12.7% vs. 0%, $P = 0.001$) and a lower daily dose of mycophenolate mofetil (MMF) at 3 (1207 ± 326 vs. 1380 ± 388 mg/day, $P = 0.003$), 6 (1243 ± 803 vs. 1319 ± 359 mg/day, $P = 0.008$ and 12 (1126 ± 289 vs. 1267 ± 353 mg/day, $P = 0.004$) months after the transplantation. Importantly, most patients (91.3%) who received a calcineurin inhibitor (CNI) received tacrolimus, without any differences concerning daily dose and trough level throughout the first post-transplant year (data not shown). The duration and daily dose of prednisone were also similar in the two groups (data not shown).

Graft and patient survival with basiliximab and rATG

Nineteen patients died [12 (7.6%) and 7 (11.7%) with rATG and basiliximab, $P = 0.105$]. Malignancies ($n = 9$) and cardiovascular diseases ($n = 5$) were the main causes of death and were similarly observed in both groups. In total, 30 patients experienced graft loss [25 (15.8%) and 5 (8.3%) with rATG and basiliximab], without difference in death-censored graft survival, overall graft survival and patient survival between the two groups ($P = 0.423$, $P = 0.715$ and $P = 0.105$; Fig. 2). The first cause of graft loss was chronic dysfunction of undetermined cause (47%) followed by venous thrombosis (16%, all in rATG group), infection (13%), initial nephropathy recurrence (13%) and ABMR (10%).

Graft rejection by induction therapy

In total, 348 renal biopsies were performed in 172 KTRs (78.9%), similarly in both treatment groups [124 (78.5%) and 48 (80%) with rATG and basiliximab, $P = 0.81$]. Considering only the first confirmed TCMR and ABMR episode in each patient, we recorded 28 cases of BPAR (16 TCMR and 12 ABMR) in 27 (12%) patients; one KTR experienced both kinds of rejections. BPAR occurred more frequently with basiliximab than rATG ($P = 0.0004$, Fig. 3a). This difference was observed for biopsy-proven TCMR (i.e. grade ≥I; Fig. 3c, $P = 0.028$) and for ABMR (Fig. 3b, $P = 0.014$), while the incidence of *de novo* DSAs was numerically higher with basiliximab than rATG [Fig. 4: 13 (21.7%) vs. 25 (15.8%), $P = 0.167$]. *De novo* DSAs were predominantly directed against HLA class II (23 patients: 60.5%), especially against DQ antigens (16 patients: 69.6%), despite a lower rate of patients with class II anti-HLA antibodies before transplantation in both treatment groups.

We next considered ABMR and biopsy-proven TCMR in a multivariate analysis. We found that

Table 1. Baseline characteristics of kidney transplant recipients by induction therapy with basiliximab or rabbit anti-thymocyte globulin.

	Total (n = 218)	rATG (n = 158)	Basiliximab (n = 60)	P-value
Donor characteristics				
Age (years)	55.5 ± 14.8	55.5 ± 14.8	55.5 ± 16.1	0.26
Male sex	123 (58.3)	91 (57.6)	32 (53.3)	0.68
Expanded criteria donor	104 (47.7)	74 (46.8)	30 (50.0)	0.79
Recipient characteristics				
Age (years)	53.5 ± 12.5	52.5 ± 12.0	56.0 ± 13.6	0.08
Male sex	102 (46.8)	69 (43.7)	33 (55.0)	0.14
Caucasian	183 (83.9)	133 (84.2)	50 (83.3)	0.96
Diabetes mellitus	41 (18.8)	31 (19.6)	10 (16.7)	0.76
BMI (kg per m^2)	25.7 ± 5.6	25.7 ± 5.6	25.6 ± 4.4	0.75
Cause of ESRD				
Glomerulonephritis	53 (24.3)	38 (24.1)	15 (25.0)	0.88
Polycystic kidney	34 (15.6)	24 (15.2)	10 (16.7)	0.79
Diabetes	20 (9.2)	16 (10.1)	4 (6.7)	0.60
Vascular	15 (6.9)	12 (7.6)	3 (5.0)	0.76
Other	50 (22.9)	40 (25.3)	10 (16.7)	0.17
Unknown	46 (21.1)	28 (17.7)	18 (30.0)	0.07
Duration of dialysis (months)	43.9 ± 43.8	45.4 ± 46.0	39.5 ± 37.0	0.58
Transplantation characteristics				
First transplantation	138 (63.3)	85 (53.8)	53 (88.3)	**<0.0001**
Living donor	11 (5.1)	3 (1.9)	8 (13.3)	**0.002**
Mean cold ischaemia time (h)	17.0 ± 7.0	18.2 ± 6.7	13.8 ± 6.5	**<0.001**
cPRA	53.0 ± 35.2	63.8 ± 32.3	23.7 ± 24.2	**<0.001**
Number of class I HLA mismatches	2.5 ± 1.3	2.5 ± 1.2	2.6 ± 1.2	0.37
HLA A	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	0.77
HLA B	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	0.24
Number of class II HLA mismatches	1.8 ± 1.2	1.7 ± 1.2	2.2 ± 1.1	**<0.01**
HLA DR	0.9 ± 0.7	0.9 ± 0.7	1.1 ± 0.7	**0.02**
HLA DQ	0.9 ± 0.7	0.8 ± 0.6	1.1 ± 0.7	**<0.01**
DGF	41 (18.8)	32 (20.3)	9 (15.0)	0.49
Serum creatinine level at 6 months (μmol/l)	133.7 ± 55.3	136.2 ± 57.6	127.3 ± 48.8	0.31
eGFR on MDRD at 6 months (ml/min/1.73 m^2)	51.9 ± 18.9	51.0 ± 19.4	54.2 ± 17.7	0.19
Urine protein value at 6 months (g/24 h)	0.45 ± 0.61	0.41 ± 0.41	0.56 ± 0.93	0.35
CMV serologic status D+/R−	49 (22.5)	34 (21.5)	15 (25.0)	0.71
CMV prophylaxis	132 (60.6)	100 (63.3)	32 (53.3)	0.18
Follow-up (years)	4.0 [0–10.0]	4.5 [0–9.6]	3.4 [0.5–10.0]	**0.004**
Initial immunosuppressive treatment				
CNI + MMF + steroids	212 (97.2)	157 (99.4)	55 (91.7)	**0.001**
CNI + mTORi + steroids	5 (2.3)	0	5 (8.3)	**0.001**
Immunosuppressive treatment 3 months after transplantation (n = 212)				
CNI + MMF ± steroids	189 (89.2)	142 (93.4)	47 (78.3)	**0.003**
CNI + mTORi ± steroids	17 (8.0)	9 (5.9)	8 (13.3)	0.090

BMI, body mass index; CNI, calcineurin inhibitor; cPRA, calculated population-reactive antibodies; D, donor CMV status; DFG, delayed function graft; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HLA, human leucocyte antigen; MDRD, modification of diet in renal disease; MMF, mycophenolate mofetil; mTORi, mechanistic target of rapamycin inhibitor; R, recipient CMV status.

Data are mean ± SD, n (%) or median [min–max]. Bold values have a significant P value <0.05.

basiliximab remained significantly associated with an increased risk of rejection in different models including rank of transplantation, recipient age, number of HLA mismatches and cPRA (Table 2A). We additionally adjusted the risk of any rejection for maintenance immunosuppressive regimen in 198 free-rejection patients at month 3 and confirmed a higher risk of rejection with basiliximab [HR = 5.17 (1.66–16.07),

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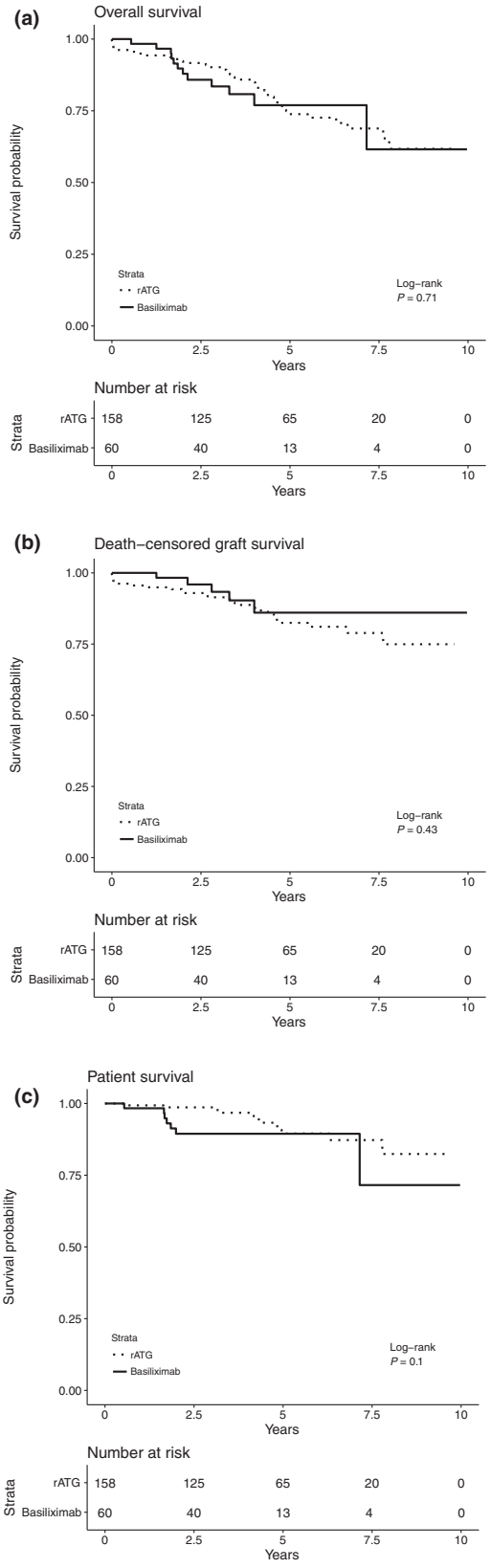

Figure 2 Estimated overall survival (a), death-censored graft survival (b) and patient survival (Fig. c) by induction therapy with basiliximab and rabbit anti-thymocyte globulin.

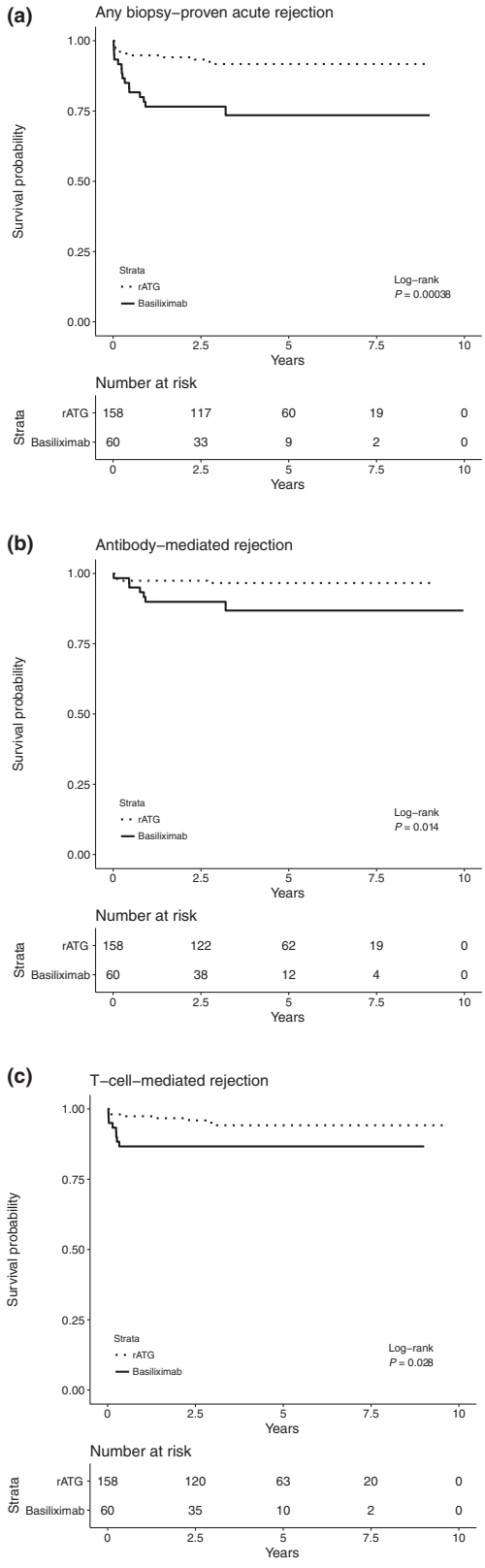

Figure 3 Incidence of acute rejection by induction therapy with basiliximab and rabbit anti-thymocyte globulin: any biopsy-proven acute rejection (a), antibody-mediated rejection (b), T-cell–mediated rejection (c).

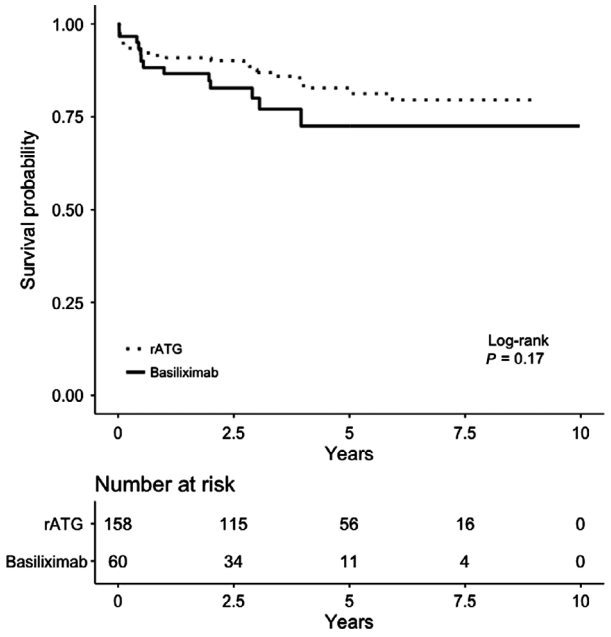

Figure 4 Survival without *de novo* donor-specific antibodies by induction therapy with basiliximab and rabbit anti-thymocyte globulin.

$P = 0.005$]. Finally, we did not identify a phenotype of patients with rejection risk after treatment with basiliximab by different allogeneic exposure (Table 3).

The composite endpoint, including any confirmed rejection episode, graft loss or death, occurred more frequently in patients with basiliximab than rATG (Fig. 5, $P = 0.05$). This increased composite risk remained after adjustment in different models (Table 2B), except after adjustment for cPRA with a hazard ratio remaining stable. We therefore carried out a similar analysis in patients who did not receive a combination of mTOR inhibitor and tacrolimus after an induction with basiliximab (Table S1). We found that patients who received basiliximab had still a higher risk of BPAR [HR: 3.27 (1.20–8.91), $P = 0.021$].

Characteristics of BPAR by induction treatment

The characteristics of BPAR with each treatment are presented in Table 4. Overall, 15 (25%) and 13 (8.2%) cases of BPAR were diagnosed with basiliximab (TCMR: 8, ABMR: 7) and rATG (TCMR: 8, ABMR: 5) treatment respectively. Most cases of BPAR occurred before 12 months [23 (82.1%): 14 (93.3%) and 9 (69.2%) with basiliximab and rATG, $P = 0.244$]. More than 50% of BPAR cases were clinical rejection, with one resistant steroid TCMR in the basiliximab group. The mean time to onset of ABMR was 9.7 ± 12.6 months. During the first post-transplant year, 6 (10%) and 4 (2.5%) cases of ABMR were observed, respectively, with basiliximab and rATG ($P = 0.028$). We did not observe any obvious differences regarding the severity of TCMR and ABMR. Finally, only one patient showed *de novo* DSA after a TCMR episode in rATG group.

Table 2. Multivariate analysis of basiliximab as a risk factor of graft rejection (A) and a composite endpoint including biopsy-proven acute rejection (BPAR), graft loss and death (B).

	HR	95% CI	*P*-value
(A) BPAR ($n = 27$)			
Crude risk	3.63	1.70–7.77	0.0009
Model 1	3.02	1.27–7.18	0.013
Model 2	3.05	1.27–7.34	0.013
Model 3	3.36	1.23–9.16	0.018
(B) Composite end-point ($n = 78$)			
Crude risk	1.60	0.99–2.59	0.050
Model 1	1.81	1.05–3.14	0.033
Model 2	1.80	1.04–3.12	0.036
Model 3	1.83	0.99–3.39	0.053

95% CI, 95% confidence interval; HR, hazard ratio.

(A) Model 1: adjusted for recipient age, type of donor (deceased vs. alive), rank of transplantation and initial immunosuppressive strategy.

Model 2: model 1 + adjusted for number of HLA mismatches.

Model 3: model 2 + adjusted for cPRA.

(B) Model 1: adjusted for recipient age and sex, donor age, type of donor (deceased vs. alive), rank of transplantation, cold ischaemia time and initial immunosuppressive strategy.

Model 2: model 1 + adjusted for number of HLA mismatches.

Model 3: model 2 + adjusted for cPRA.

Safety

The incidence of infection and malignancies did not differ with rATG and basiliximab at the end of follow-up (Table 5). Thus, despite a higher incidence of lymphopaenia with rATG than basiliximab, CMV viraemia, symptomatic CMV reactivation and BKV viraemia were similar in the two groups; only five BKV nephropathies (2.3%) were reported. Likewise, the incidence of all bacterial infections, graft pyelonephritis and pneumonitis did not differ. The frequency of malignancies was similar.

Discussion

Our study is the first to specifically assess the impact of immunosuppressive induction therapy with basiliximab

Table 3. Sensitization factors by the presence of biopsy-proven acute rejection (BPAR) with rabbit anti-thymocyte globulin (rATG) and basiliximab induction therapy.

	BPAR with rATG	*P*-value	BPAR with basiliximab	*P*-value
cPRA				
<20%	2/22 (9.1)	0.74	8/34 (23.5)	0.92
20–50%	3/34 (8.8)		5/18 (27.8)	
>50%	7/102 (6.8)*		2/8 (25.0)	
No. of HLA class II mismatches				
<1	1/33 (3.0)*	0.66	1/5 (20.0)	0.90
1–2	8/87 (9.2)		7/30 (23.3)	
>2	3/37 (8.1)		7/25 (28.0)	
Rank of transplantation				
First	8/85 (9.4)*	0.39	14/53 (26.4)	0.67
≥2	4/73 (5.5)		1/7 (14.3)	
Sex				
Female	9/89 (10.1)*	0.23	6/27 (22.2)	0.77
Male	3/69 (4.3)		9/33 (27.3)	
Pregnancy (males excluded)				
None	2/7 (28.6)	0.15	2/6 (33.3)	0.59
≥1	7/81 (8.6)*		4/21 (19.0)	
History of blood transfusion				
No	10/108 (9.3)*	0.34	14/55 (25.5)	1.00
Yes	2/50 (4.0)		1/5 (20.0)	
History of alloimmune exposure				
No	1/20 (5.0)	0.61	10/33 (30.3)	0.38
Yes	11/138 (8.0)*		5/27 (18.5)	

cPRA, calculated panel reactive antibodies; HLA, human leucocyte antigen.

Data are *n* (%).

*One patient with rATG experienced T-cell–mediated rejection and antibody-mediated rejection.

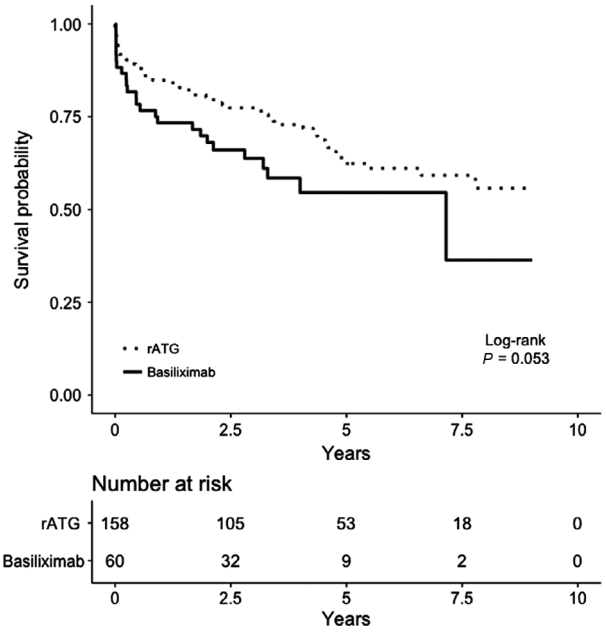

Figure 5 Survival without composite endpoint including biopsy-proven acute rejection, death and graft loss by induction therapy with basiliximab and rabbit anti-thymocyte globulin.

and rATG on outcome for sensitized KTRs without pre-formed DSAs. Risk of BPAR, TCMR and ABMR was greater with basiliximab than with rATG. This result remained significant after adjustment for numerous variables including classical risk factors of rejection or in a composite endpoint including BPAR, graft loss and death. As basiliximab was not more safe than rATG, our results argue for an advantageous efficacy – safety balance in favour of rATG for these patients.

Risk of BPAR is highly variable depending on patient characteristics, maintenance immunosuppressive treatment and recipient ethnicity. The largest studies that enrolled KTRs with low immunological risk and receiving tacrolimus reported a confirmed BPAR rate generally at almost 10% [16,17], regardless of induction therapy. Sensitized patients represented less than 20% of patients in these studies and were not specifically assessed. In our study, we found a BPAR rate of 8.2% in patients receiving rATG, fairly close to these previous results. By contrast, the rejection rate of our patients who received basiliximab was 25%, similar to rates reported for patients with pre-existing DSAs [18,19] and with high immunological risk defined by the previous parameters [3,4]. Hence, our results suggest that only rATG prevents BPAR as effectively as in non-sensitized recipients. This assumption is in agreement with previous studies that failed to find any difference in risk of rejection between non-sensitized and sensitized patients without pre-existing DSAs who received intensive rATG therapy [8,9]. To the best of our knowledge, only one study gave reliable data concerning the risk of rejection in sensitized recipients without DSAs by SAB assay [20]. In this study, Wehmeier *et al.* [20] found a confirmed rejection rate of 25.8%; 93.6% of patients had received basiliximab induction therapy. Our results suggest that sensitized KTRs without pre-existing DSAs should be considered at moderate rather than low immunological risk concerning the choice of immunosuppressive induction.

Both TCMR and ABMR were more frequent with basiliximab therapy. Approximately half of these rejections were subclinical, discovered by protocol biopsies. Systematic biopsy is important in this population. We did not include borderline changes because both their impact and their actual significance are sometimes

Table 4. Biopsy-proven acute rejection characteristics by rabbit anti-thymocyte globulin and basiliximab induction therapy.

	Total (*n* = 28)	rATG (*n* = 13)	Basiliximab (*n* = 15)	*P* value
Clinical rejection	15 (53.6)	9 (69.2)	6 (40.0)	0.15
TCMR	(*n* = 16)	(*n* = 8)	(*n* = 8)	
First year	13 (81.3)	5 (62.5)	8 (100.0)	0.20
Grade				
I	13 (81.0)	7 (87.5)	6 (75.0)	1.00
II	3 (19.0)	1 (12.5)	2 (25.0)	
ABMR	(*n* = 12)	(*n* = 5)	(*n* = 7)	
First year	10 (83.3)	4 (80.0)	6 (85.7)	1.00
Elementary lesions				
g	2.17 ± 0.58	1.80 ± 0.45	2.4 ± 0.53	0.08
ptc	1.58 ± 0.67	1.60 ± 0.55	1.57 ± 0.79	0.86
g + ptc	3.75 ± 0.75	3.4 ± 0.55	4 ± 0.82	0.14
v > 0	1 (8.0)	0	1 (14.0)	1.00
c4d+	4 (33.3)	1 (20.0)	3 (42.9)	0.58
cg > 0	3 (25.0)	1 (20.0)	2 (29.0)	1.00
IFTA (ci + ct)	2 ± 1.86	1.20 ± 1.64	2.57 ± 1.90	0.35

ABMR, antibody-mediated rejection; cg, chronic glomerulopathy score; g, Banff glomerulitis score; IFTA, interstitial fibrosis and tubular atrophy; MVI (g + ptc), microvascular inflammation; ptc, Banff peritubular capillaritis score, TCMR, T-cell–mediated rejection; v, vasculitis score.

Data are mean ± SD or *n* (%).

Table 5. Infections and malignancies by basiliximab and rabbit anti-thymocyte globulin induction therapy.

	Total	rATG	Basiliximab	*P* value
Lymphopaenia				
At 3 months	122 (56.0)	113 (71.5)	9 (15.0)	**<0.0001**
At 6 months	114 (52.3)	101 (63.9)	13 (21.7)	**<0.0001**
At 12 months	84 (38.5)	75 (47.5)	9 (15.0)	**<0.0001**
Infection				
Positive CMV viraemia	44 (20.2)	32 (20.3)	12 (20.0)	0.97
CMV syndrome/disease	24 (11.0)	18 (11.4)	6 (10.0)	0.77
BKV viraemia	20 (9.2)	16 (10.1)	4 (6.7)	0.60
BKV nephropathy	5 (2.3)	4 (2.3)	1 (1.7)	1.00
Opportunist infection	6 (2.8)	5 (3.2)	1 (1.0)	1.00
Bacterial infection	71 (32.6)	52 (32.9)	19 (31.0)	0.86
Graft pyelonephritis	56 (25.7)	41 (25.9)	15 (25.0)	0.89
Pneumonitis	21 (9.6)	15 (9.5)	6 (10.0)	0.91
Cancer				
Solid cancer	13 (5.9)	9 (5.7)	4 (6.7)	0.76
Skin cancer	20 (9.2)	16 (10.1)	4 (6.7)	0.60
PTLD	4 (1.8)	2 (1.3)	2 (3.3)	0.30

BKV, BK virus; CMV, cytomegalovirus; PTLD, post-transplant lymphoproliferative disorders.

Data are *n* (%). Lymphopaenia defined by lymphocyte counts <0.80 G/l. Bold values have a significant *P* value <0.05.

questioned [21–23], and therefore, we opted to consider only biopsy-proven TCMR, a well-known risk factor of death-censored graft loss [24,25] and also associated with subsequent *de novo* DSA appearance [26,27]. More importantly, we found a fourfold greater risk of ABMR with basiliximab than rATG; ABMR is known as the

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first cause of kidney graft loss [28,29]. We found similar graft survival in the two treatment groups. The deleterious effects of rejection would be apparent after a longer follow-up, but basiliximab has mostly been used in sensitized patients in our centre only since 2013 (Fig. S1). Finally, the deleterious impact of the increased risk of rejection with basiliximab remains to be confirmed.

Unexpectedly, we did not find a lower incidence of infections with basiliximab versus rATG. Concerning CMV, it is usually accepted that risk of viraemia depends on donor and recipient CMV status, which were well balanced in our study. The numerically less frequent use of valganciclovir prophylaxis in D−R+ recipients who received basiliximab may explain our results, because the incidence of CMV viraemia in D−R+ patients can reach 37% with a pre-emptive strategy [30]. Also, we used a total dose of <7 mg/kg of rATG based on T-cell monitoring, which could contribute to a lower incidence of CMV [31]. The BKV viraemia incidence was similar in the two groups and quite low in our patients versus others [32]. We observed a slight numerical difference in favour of basiliximab, but a few patients had received a combination of mechanistic target of rapamycin inhibitor–tacrolimus–steroids that seems less associated with BKV viraemia than mycophenolate mofetil–tacrolimus–steroids [33].

Of course, several limitations need to be discussed. First, our study is not powerful enough to reliably assess the impact of rejection in the two groups, because of a too low number of patients with follow-up >5 years (4 and 5 in basiliximab and rATG group respectively). Second, the two groups differed in several characteristics. Indeed, because sensitized patients with low cPRA levels were frequently first transplantation recipients and could be considered at reduced risk of BPAR with a low risk of anamnestic response, basiliximab was more often preferred to rATG for these recipients. Moreover, the deeper immunological explorations performed with living donors, such as systematic flow-cytometry crossmatching using several recipients' sera, might also encourage clinicians to use basiliximab. Hence, the two groups were effectively different but with a theoretically lower risk of rejection with basiliximab than rATG, which indirectly strengthens our results. Third, this was a single-centre retrospective analysis including mainly Caucasian recipients. The results should be confirmed in a multicentre study and in a clinical trial specifically assessing sensitized recipients without pre-existing DSAs. Our study contributes important data to the design of such a prospective study. In particular, we failed to identify a subgroup of patients with a specific risk of rejection after basiliximab induction therapy.

In conclusion, we report a higher incidence of both TCMR and ABMR in Caucasian-sensitized KTRs without pre-existing DSAs who received basiliximab versus rATG induction therapy, while safety data did not suggest a safer profile for basiliximab. The use of rATG in these patients should remain the first-line induction unless a prospective randomized clinical trial challenges our results.

Authorship

AG performed the study, collected data, analysed data and wrote the paper. PG designed the study, performed the study, collected data, analysed data and wrote the paper. BS performed the study, collected data, analysed data and wrote the paper. EB collected data and contributed to writing. EM-S collected data and contributed to writing. BP collected data and contributed to writing. LB collected data and contributed to writing. HL collected data and contributed to writing. CB contributed to writing. J-MH contributed to writing and analysed data. MB contributed to writing, collected data and analysed data.

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SUPPORTING INFORMATION

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

Figure S1. Evolution of induction therapy use in kidney transplantation in Tours.

Table S1. Multivariate analysis of basiliximab as a risk factor of graft rejection (A) and a composite endpoint including biopsy-proven acute rejection (BPAR), graft loss and death (B) in 212 patients initially receiving a calcineurin inhibitor-based immunosuppressive treatment.

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