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Poor Patient and Graft Outcome After Induction Treatment by Antithymocyte Globulin in Recipients of a Kidney Graft After Nonrenal Organ Transplantation

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Background. End-stage renal failure occurs in a substantial number of patients having received a nonrenal transplantation (NRT), for whom a kidney transplantation is needed. The medical strategy regarding the use of immunosuppression (IS) for a kidney graft in patients after an NRT is not well established. The prekidney grafts long-term IS advocates for a mild induction, such as using anti-IL-2R antibodies, whereas addition of new incompatibilities and anti-HLA preimmunization may suggest using stronger IS such as induction by polyclonal antithymocyte globulins (ATG). **Methods.** We performed Cox multivariate and propensity score analysis of our validated transplant database to study the impact of the type of induction therapy on kidney graft survival of recipients of a kidney graft after NRT. **Results.** We report here that kidney transplantation after NRT treated with an ATG induction has a poorer outcome (kidney and recipient survival) than that with an anti-IL-2R induction. After accounting for potential baseline differences with a multivariate Cox model, or by adjusting on a propensity score, we found that despite patients having received ATG cumulate more risk factors, ATG appears independently involved. As animal-derived biotherapeutics induce antiglycan antibodies and particularly anti-N-glycolylneuraminic acid (Neu5Gc) IgGs which may activate endothelial cells in patients and grafts, we also investigated the magnitude and the nature of the anti-Neu5Gc elicited by the induction and showed that induction was associated with a shift in anti-Neu5Gc IgG repertoire. Possible reasons and mechanisms of a deleterious ATG usage in these patients are discussed. **Conclusions.** Our study suggests that ATG induction after a kidney transplantation in recipients already under maintenance IS for a NRT should be used cautiously.

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Transplantation of nonrenal solid organs, such as the heart, lung, or liver, is vital for patients with end-stage failure of the respective organs, but may be complicated by end-stage renal disease (ESRD) due to multifactorial causes. Ojo et al¹ have shown that 16.5% of nonrenal transplantation (NRT) recipients developed chronic renal failure (CRF) over a median follow-up of 3 years, and that nearly one third of these CRF patients already reached ESRD necessitating dialysis or transplantation. Occurrence of CRF in NRT patients increased 4.5 times the risk of death and kidney transplantation for ESRD was associated with a significantly lower 5-year risk of death than dialysis. Thanks to the improved care, NRT recipients now live longer, as a result, they have more time to develop ESRD and to become an emerging subpopulation of kidney transplant patients.²

However, little is known about the suitable treatment and particularly the optimal type of induction for NRT patients at the time of kidney transplantation. Because all these recipients are receiving combined immunosuppressive maintenance treatment for their NRT, it is unclear whether they also need a superimposed induction therapy for kidney transplantation and, if so, which type of induction therapy would be suitable. Some centers prefer induction with lympho-depleting antibodies, such as antithymocyte globulin (ATG),³ or alemtuzumab⁴ to ensure sufficient immunosuppression (IS) for the kidney allografts, whereas others opt for a nondepleting anti-IL-2R monoclonal antibody. A single-center retrospective study reported that induction therapy by alemtuzumab in kidney transplantation recipients after NRT was associated with lower acute rejection rate.⁴ However, another study analyzing the United Network for Organ Sharing database showed that induction therapy with either depleting or non-depleting antibodies was not associated with improved patient survival and even yielded inferior survival of kidney recipients after liver transplantation.⁵ In fact, there is an extreme paucity of literature regarding the impact of induction treatment on kidney graft outcome after NRT.

In this report, based on our multicenter validated données Informatisées et Validées en Transplantation (DIVAT) registry database (www.divat.fr), we identified 128 recipients of kidney transplant after NRT. The aim of our study was to determine whether induction therapy with ATG or anti-IL-2R at the time of kidney transplantation affects the kidney graft and patients survival. After accounting for potential baseline differences with a multivariate Cox model, or by adjusting on a propensity score we found that, despite patients having received ATG cumulate more risk factors, ATG appears independently involved in the lower graft and patient survival after ATG induction. In addition, because animal-derived bio-therapeutics such as ATG are immunogenic and can induce anti-anti-N-glycolylneuraminic acid (Neu5Gc) antibodies^{6,7} which are able to react with patient and graft endothelial cells,⁸ we also measured the levels and repertoire of anti-Neu5Gc IgG antibodies in the serum of a small subgroup of patients after induction after the kidney transplantation.

PATIENTS AND METHODS

Patients

Study Design

This is a retrospective analysis of the outcome of kidney grafts after NRT using data extracted from the DIVAT

database (see <http://www.divat.fr>), a registered and validated database of solid organ transplant recipients from French university hospitals including Lyon, Montpellier, Nancy, Nantes, and Necker (Paris). We included 140 adult patients (≥ 18 years old) who underwent a kidney transplant between January 1, 1997, and December 31, 2013, after having previously received at least one of the after NRT: heart, liver, or lung. Twelve patients without induction therapy at the time of kidney transplant were excluded from the study due to their small number. Of the remaining 128 patients (Figure 1), 72 received ATG (thymoglobulin, Genzyme) and 56 received anti-IL-2R (54 basiliximab and 2 daclizumab). The indication of ATG in this specific clinical situation differed among the participating groups: 1 center gave ATG in all such patients and other centers used ATG only when the recipients were preimmunized. However, as shown latter in Table 1, the protocols could not be follow in a substantial number of patients indicating that other clinical criteria were taken into account. The endpoint of our study was kidney graft survival noncensored for death (hereinafter referred to as graft survival), calculated from the date of kidney transplantation to the date of return to chronic dialysis or death with a functioning kidney graft, the latter was thus also considered as graft loss.⁹

Statistical Comparison of Graft Outcome

Continuous variables were compared using the *t* test. Categorical variables were compared using the χ^2 test or Fisher exact test whenever appropriate. Kaplan-Meier survival curves were created and the log-rank test was used to study the impact of each of the following variables on kidney graft survival: donor age, donor type, cold ischemia time, recipient age and sex, year of kidney graft, time from NRT to kidney graft, time on dialysis before kidney graft, anticlass I and/or class II HLA antibodies (Abs), recipient cytomegalovirus (CMV) and hepatitis C virus (HCV) status, type of NRT, induction type (ATG or anti-IL-2R) for NRT and the kidney graft, type of maintenance treatment (calcineurin inhibitors, mycophenolate mofetil or azathioprine, and corticosteroids), and occurrence of acute rejection (intended to treat). A Cox univariate analysis was done for each of the aforementioned variables to calculate the hazard ratio (HR) as well as the 95% confidence interval (95% CI). Next, a Cox regression

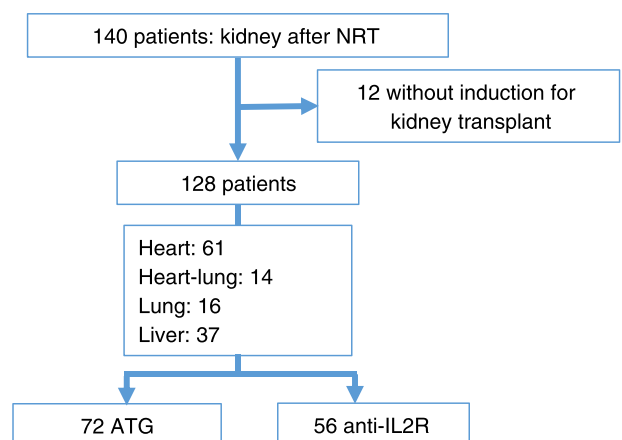


FIGURE 1. Patient flowchart.

TABLE 1.**Recipient and donor characteristics of kidney after NRT according to type of induction therapy**

Variables	Total (n = 128)	ATG (n = 72)	Anti-IL-2R (n = 56)	P
Kidney donor				
Age ^a	49.2 (3-79)	49.6 (3-76)	48.6 (4-79)	0.73
Deceased donor, n (%)	111 (87)	66 (92)	45 (80)	0.071
Cold ischemia time, ^a h	18.1 (0.2-41.2)	18.7 (0.2-41.2)	17.3 (0.3-39)	0.41
Recipient				
NRT type				
Heart, n (%)	61 (47.7)	37 (51.4)	24 (42.9)	0.37
Heart-lung, n (%)	14 (10.9)	3 (4.2)	11 (19.6)	0.008
Lung, n (%)	16 (12.5)	3 (4.2)	13 (23.2)	0.002
Liver, n (%)	37 (28.9)	29 (40.3)	8 (14.3)	0.0015
Age at kidney graft ^a	49.8 (18-74)	52.3 (22-71)	46.7 (18-74)	0.034
Male, n (%)	91 (71)	51 (71)	40 (71)	1.0
Kidney graft ≤ 2008, n (%)	67 (52)	39 (54)	28 (50)	0.72
Time from NRT to kidney graft, ^a y	10.3 (1-28)	10.6 (1-24)	9.9 (1-28)	0.49
Time on dialysis, ^a y	2.1 (0-16.4)	2.5 (0.01-10.6)	2.5 (0.03-16.4)	0.98
Anti-HLA class I pos, n (%)	33 (26)	20 (28)	13 (23)	0.68
Anti-HLA class II ^b pos, n (%)	41 (34)	30 (43)	11 (21)	0.012
Combined anti-HLA class I/II ^b				
Either class I or II pos, n (%)	53 (43.4)	36 (52)	17 (32)	
Both class I and II neg, n (%)	69 (56.6)	33 (48)	36 (68)	
CMV pos, n (%)	78 (61)	52 (72)	26 (46)	0.004
HCV pos, n (%)	11 (9)	7 (10)	4 (7)	0.75
Maintenance treatment				
CNIs, n (%)	125 (98)	71 (99)	54 (96)	0.58
Tacrolimus, n (%)	72 (56)	41 (57)	31 (55)	0.86
MMF, n (%)	114 (89)	63 (88)	51 (91)	0.58
Azathioprine, n (%)	9 (7)	6 (8)	3 (5)	0.73
Corticosteroids, n (%)	122 (95)	72 (100)	50 (89)	0.006

^a Continuous variables (age and time) are reported as mean (range).

^b Missing data for anti-class II in 6 patients (3 in each group).

CNIs, calcineurin inhibitors; MMF, mycophenolate mofetil; neg, negative; pos, positive.

multivariate analysis was performed including predefined clinically relevant variables: type of induction, recipient and donor age, donor type, type of NRT and of their induction, anti-class I and/or class II-HLA Abs, year of kidney graft, presence of acute rejection and of other variables potentially associated with kidney graft survival in the univariate analysis ($P \leq 0.20$ in the log-rank test of Kaplan-Meier estimates). Then, a reduced model containing only significantly and independently variables associated with kidney graft survival was obtained using a backward procedure. Finally, to better assess baseline differences between ATG and anti-IL-2R subjects, a multivariate logistic regression model was generated to estimate a propensity score to receive ATG for each patient. All covariates were predefined clinically relevant variables as well as variables associated with ATG in the univariate analysis remaining significantly and independently associated with ATG after a backward analysis. The model performance was appreciated with the χ^2 -Hosmer-Lemeshow and the c-statistic tests. P values less than 0.05 were considered to be statistically significant. Analyses were performed using R version 3.2.0. The statistical analysis was subcontracted to Methodomics, Toulouse, France.

Measurement of Anti-Neu5Gc and Anti- Gal IgG by ELISA

anti-Neu5Gc IgG levels were measured using mouse serum proteins as coating antigens as previously described.¹⁰

Details are given in **supplementary materials and methods**, <http://links.lww.com/TXD/A75>.

Sialoglycan Microarray

Arrays were prepared on epoxy slides, then developed and analyzed as previously described.¹⁰ Details of the assay are also described in **supplementary material and methods**, <http://links.lww.com/TXD/A75>.

Analysis of Neu5Gc in Basiliximab (Simulect) and ATG by Mass Spectrometry

Simulect and ATG (100 µg each) was reduced with dithiothreitol (Sigma-Aldrich), alkylated with iodoacetamide (Sigma-Aldrich), and digested with trypsin (4 µg, Promega).¹¹ Glycopeptides were enriched from the digestion mixtures using ProteoExtract kits (EMD Millipore). Mass spectrometry analyses of the glycopeptides were performed on an UltrafleXtreme mass spectrometer (Bruker Daltonics) with dihydroxy benzoic acid (DHB; Sigma-Aldrich) as the matrix.

RESULTS

Patient Characteristics

Compared with the anti-IL-2R group, the ATG group recipients were older and more patients had anti-HLA class II

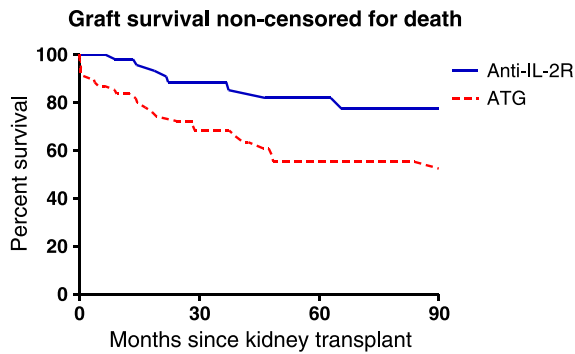
antibodies and a CMV-positive serology, and had received a corticosteroid maintenance treatment. There were more liver transplant in the ATG group and more lung and heart-lung transplant in the anti-IL-2R group. Other recipient and donor characteristics were not different between the 2 groups (Table 1). To further evaluate the differences in demographics between the 2 groups, Cohen *d* was also calculated for all the continuous variables. Only recipient age at kidney graft showed a small effect size with a Cohen *d* of 0.38, for other continuous variables (donor age, cold ischemia time, time from NRT to kidney graft, and time from dialysis), the Cohen *d* coefficients were very small (0.06, 0.15, 0.12, and 0.003, respectively), which means that there were no major differences in these demographic characteristics. As indicated above, 4 of 5 participating centers were supposed to use ATG only in kidney recipients with immunological risks, particularly those with positive anti-HLA Abs. However, this general rule was not strictly applied, and the preestablished protocols of the induction therapy were often modified by the transplant physician at the time of kidney transplantation. Consequently, as shown in Table 1, half of patients who received ATG had no anti-HLA Abs. On the contrary, one-third patients who received anti-IL-2R had either anti-class I or anti-class II Abs. As shown below, the impact of anti-HLA Abs on graft survival was analyzed by univariate as well as multivariate Cox model.

Graft and Patient Outcome

During the follow-up period of the study, 39 patients reached the endpoint, including 12 who returned to chronic dialysis and 27 deaths. Univariate analysis of variables in relation to graft survival was performed by log-rank test of Kaplan Meier estimates as well as by Cox univariate analysis. As shown in Table 2, donors of 55 years or older and receiving ATG induction therapy (vs anti-IL-2R induction) were significantly associated with an increased risk of return to chronic dialysis or death (HR, 2.18 and 3.27; 95% CI, 1.14-4.17 and 1.49-7.17, and *P* Cox = 0.018 and 0.003, respectively). Figure 2 shows the Kaplan-Meier survival curve comparing the groups with ATG and anti-IL-2R induction (*P* = 0.002, log rank test). As some kidney recipients received ATG induction years before anti-IL-2R antibodies were available, a comparison restricted to the grafts performed on overlapping years was also done and showed a significant difference (*P* < 0.032) of roughly the same magnitude (Figure S1 <http://links.lww.com/TXD/A69>). A separate assessment of death and graft survival censored for death (Figure S2A and S2B <http://links.lww.com/TXD/A70>) also showed that ATG treatment was significantly associated with poor survival. The type of induction treatment (ATG, anti-IL-2R, or no induction) or the previous NRT/NRT type (heart or liver) had no significant influence on graft survival. Next, the multivariate Cox regression model showed that ATG induction treatment was the only variable

TABLE 2.
Univariate analysis of kidney graft loss

Variables	HR	95% CI	<i>P</i> , Cox	<i>P</i> , log-rank
Kidney donor				
Age ≥55 y	2.18	1.14-4.17	0.018	0.016
Deceased donor	5.6	0.77-40.9	0.089	0.055
Cold ischemia time ≥ 20 h	0.7	0.35-1.39	0.303	0.3
Recipient				
NRT type				0.605
Heart	1			
Heart-lung (vs heart)	0.49	0.15-1.67	0.255	
Lung (vs heart)	1.07	0.36-3.15	0.907	
Liver (vs heart)	1.18	0.55-2.54	0.67	
ATG induction (vs no ATG) for NRT				0.94
Age at kidney graft	1.01	0.99-1.04	0.179	
Male	1.21	0.55-2.67	0.644	0.643
Kidney graft > 2008	1.09	0.5-2.38	0.832	0.832
Time from NRT to kidney graft ≥ 10 y	1.16	0.62-2.19	0.641	0.641
Time on dialysis ≥ 2 y	1.88	0.97-3.66	0.063	0.059
Anti-HLA class I pos	1.71	0.86-3.39	0.127	0.122
Anti-HLA class II pos	1.21	0.6-2.47	0.593	0.593
Either class I or II pos (vs both class I and II neg)	1.49	0.76-2.91	0.242	0.239
CMV pos	1.46	0.76-2.83	0.26	0.257
HCV pos	0.75	0.26-2.15	0.59	0.589
ATG induction (vs anti-IL-2R induction) for kidney graft	3.27	1.49-7.17	0.003	0.002
Maintenance treatment				
CNI	0.18	0.02-1.46	0.109	0.072
Tacrolimus	1.1	0.57-2.11	0.778	0.778
MMF	1.48	0.56-3.87	0.427	0.424
Azathioprine	0.95	0.33-2.7	0.92	0.92
Corticosteroids	1.95	0.27-14.25	0.511	0.503
Acute rejection	1.25	0.52-3.02	0.616	0.615



Anti-IL-2R 56 32 22 10
ATG 72 36 19 14

FIGURE 2. Kaplan-Meier estimate of graft survival (nonsensored for death). ATG induction was associated with significantly lower kidney graft survival compared with anti-IL-2R induction ($P = 0.0017$, log-rank test).

remaining significantly and independently associated with kidney graft survival (Table 3). Antithymocyte globulin induction was indeed associated with a 3.33-fold increase in the risk of return to dialysis or death compared to anti-IL-2R induction (95% CI, 1.44-7.70; $P = 0.005$). Donors 55 years or older and deceased donors showed a nonsignificant tendency to be associated with a lower graft survival ($P = 0.067$ and 0.057 , respectively). The presence of either anti-class I or anti-class II HLA Abs (vs the absence of both) and the presence of at least 1 acute rejection episode were not associated with lower graft survival ($P = 0.40$ and 0.38 , respectively).

Because of the difference in distribution of variables in the 2 groups, a propensity score to receive ATG (vs anti-IL-2R), including recipient age, year of kidney graft, presence of class II anti-HLA antibodies and recipient CMV status was also applied. The performance of the propensity score was satisfactory: the c-statistic for the logistic regression model was 0.77 (95% CI, 0.69-0.85) and the χ^2 -Hosmer-Lemeshow

statistic model reached 0.85. The ATG treatment was confirmed to associate with poor prognosis in terms of return to dialysis or death by the propensity score method (HR, 3.22; 95% CI, 1.30-7.95, $P = 0.011$), showing that despite differences in giving ATG instead of anti-IL-2R at baseline, there is a significant and independent effect of the induction treatment contributing to the graft survival.

Complications and Causes of Death

To investigate whether patients having received ATG were clinically overimmunosuppressed compared with those treated by anti-IL-2R, we analyzed the incidence of infectious and neoplastic complications after kidney transplantation and the frequency of acute rejection. We found no difference in global incidence of CMV, HSV, and VZV viral infections, in severe infections (including sepsis, urinary tract infection, and pneumonia/lung abscess), nor malignancies, between the 2 groups (Figure S3A, S3B, and S3C <http://links.lww.com/TXD/A71>). Acute rejection episodes presented the nonsignificant trend to be higher in the ATG group: 21% in the ATG, versus 9% in the anti-IL-2R induction group ($P = 0.068$) (Figure S2C, <http://links.lww.com/TXD/A70>).

The main causes of death (Table 4) were as follows: infection (7), cancer (3), chronic rejection of the NRT (3), cardiac diseases (5), stroke (2), mesenteric infarction (1), hepatic failure (2), and others (4). Despite a trend, there was no significant difference in cardiovascular complications leading or not to death in the ATG group (Figure S3D, <http://links.lww.com/TXD/A71>). In addition, compared with other NRTs, a prior heart transplant was not associated with an increase in cardiovascular complications after kidney transplantation ($P = 0.49$, log-rank test). A substantial number of early failures (6 kidney transplant failures and 2 deaths) occurred within 4 months in the group ATG. Four recipients presented a vascular complication (without rejection), one had an acute pyelonephritis, and the last one, a multifactorial

TABLE 3.

Cox multivariate analysis of kidney graft loss

Variable	Reference category	Adjusted HR	95% CI	P
ATG induction (for kidney graft)	Anti-IL-2R induction	3.1	1.22-7.85	0.017
Recipient age		1	0.97-1.03	0.927
Donor age ≥ 55 yr	<55 yr	2.02	0.88-4.66	0.097
Deceased donor	Live donor	6.05	0.74-49.44	0.093
NRT: heart-lung	Heart	0.85	0.23-3.12	0.804
NRT: lung	Heart	1.48	0.45-4.9	0.517
NRT: liver	Heart	0.63	0.24-1.65	0.351
Anti-HLA: either class I or II pos	Both class I and II neg	1.36	0.66-2.82	0.405
Year of kidney graft > 2008	≤ 2008	1.07	0.43-2.64	0.891
Acute rejection	No	1.56	0.58-4.22	0.38
Dialysis time ≥ 2 yr	<2 yr	1.58	0.73-3.42	0.242
CNI treatment	No	0.17	0.02-1.46	0.107
Reduced Cox model ^a				
ATG induction	Anti-IL-2R induction	3.33	1.44-7.7	0.005
Donor age ≥ 55 yr	<55 yr	1.88	0.96-3.68	0.067
CNI treatment	No	0.13	0.02-1.06	0.057
Cox model adjusted on the propensity score ^b				
ATG induction	Anti-IL-2R induction	3.22	1.3-7.95	0.011

^aThe reduced model containing only significantly and independently variables associated with kidney graft survival was obtained using a backward procedure.

^bPropensity score to receive ATG (vs anti-IL-2R) included recipient age, year of kidney graft, the presence of class II anti-HLA antibodies and recipient CMV status.

TABLE 4.**Cause of death according to type of induction therapy**

	Anti-IL-2R (n = 56)	ATG (n = 72)	Total (128)
Infections	2	4	6
Cardiovascular diseases	1	11	12
Stroke	1	2	3
Myocardial infarction or ischemia	0	2	2
Mesenteric infarction	0	1	1
Cardiac failure	0	3	3
Cardiac arrest (undetermined cause)	0	3	3
Cancer	1	2	3
Others	3	3	6
All causes	7	20	27

cause with a posttransplantation serum creatinine always above 300 $\mu\text{mol/L}$.

Anti-Neu5Gc and Anti-Gal antibodies

Antithymocyte globulin can induce anti-Neu5Gc⁷ that is able to interact with diet-derived Neu5Gc present on graft and patient endothelial cells.^{6,8} In contrast to ATG,⁶ mass spectrometry analysis did not show evidence of the presence of Neu5Gc on Basilimax (Table S1 <http://links.lww.com/TXD/A74>). The levels of anti-Neu5Gc were thus measured in a subgroup of patients with available sera at 3 different time points: at the day of the kidney graft before induction (designated as month 0, M0), as well as at 12 (M12) and 36 (M36) months after the kidney graft (time points chosen in agreement with previous data showing a late response with roughly similar IS⁶). Eleven of them received ATG and 14 received anti-IL-2R as induction of their kidney graft. None of the patients in the ATG group and 2 in the anti-IL-2R group developed antidonor antibodies after kidney graft. Figure S4 <http://links.lww.com/TXD/A72> shows no difference in anti-Neu5Gc and anti-Gal IgG antibodies compared at M0, M12, and M36 between the patients having received ATG or anti-IL-2R induction. Also, there was no difference in the changes of the level of antibodies before and after induction of each patient (paired test) at each time point, whatever the nature of the kidney graft induction.

Finally, despite similar levels of overall anti-Neu5Gc IgG after induction, we examined a possible shift in the repertoire of elicited anti-Neu5Gc IgG antibodies compared with the patterns observed for such preexisting antibodies using a unique sialoglycan microarray. Figure S5 <http://links.lww.com/TXD/A73> provides a comprehensive overview of the shift in recognition patterns of anti-Neu5Gc IgG after induction treatment (see also **Supplementary File on Array Data** <http://links.lww.com/TXD/A76>). Most ATG-treated patients (8/11) had an increased fold-change in antibodies levels against some array glycans, whereas in anti-IL-2R-treated patients, antibodies levels increased (7/14) or decreased (7/14) (Figure S5A-B <http://links.lww.com/TXD/A73>). Details of the new-Neu5Gc epitope recognized are given in the legend of Figure S5 <http://links.lww.com/TXD/A73>. Despite all being oriented toward a more vigorous and diversified response against Neu5Gc in ATG induction, none of the trends between the ATG and the anti-IL-2R groups were significant.

DISCUSSION

To the best of our knowledge, so far, there has been no study sustaining a clear recommendation as to the immunosuppressive treatment strategy for such recipients, that is, already exposed to long-term IS treatment at the time of their kidney transplantation. In one study on the outcome of kidney transplantation after NRT,⁵ induction therapy was not associated with improved patient survival and was even associated with inferior patient survival in the subset of patients with kidney graft after liver transplantation. Similarly, in a recent article aimed at assessing the impact of repeat mismatches in kidney transplantations, induction therapy was also not found to affect kidney graft survival.¹² However, the cohort analyzed in this article markedly differs from our own because it included up to 25% of patients who had received a simultaneous kidney/NRT (kidney/pancreas, kidney/liver, etc) as the first graft. In another study showing no difference in kidney graft survival between primary, repeat, and kidney after NRT, induction therapy was also not found to be a significant determinant of graft outcome.¹³ However, in these aforementioned studies, there was no direct comparison of the impact of ATG versus anti-IL-2R induction therapy on graft survival. The 2 groups of patients in our study differed by several variables classically associated with lower survival due to the policy of preferential indication of ATG induction in patients at high immunological risks. However, the multivariate analysis has taken into account those variables and confirms that patients having received induction by ATG for their kidney transplantation have higher risk of graft loss or of death compared with those who had an anti-IL-2R induction. The difference remains similar when the patients having received ATG before the availability of anti-IL-2R Abs were excluded and only patients grafted in the same period were compared (Figure S1 <http://links.lww.com/TXD/A69>). Moreover, this conclusion was emphasized by the propensity score analysis, which strongly suggests that the lower survival of ATG treated patients was not only due to baseline differences and that ATG itself also contributed to the difference observed. Finally, the poor outcome associated with ATG induction therapy appears to be limited to kidney recipients after NRT. In one of our studies based on our DIVAT database, we analyzed a large cohort of patients having received first and second kidney grafts and found that induction therapy with depleting antibodies (ATG or anti-CD3) was not associated with different risk of graft failure compared with nondepleting antibodies (anti-IL-2R).¹⁴ Unlike recipients of kidney graft after a NRT, patients with a second kidney graft have rejected their first kidney graft and are no longer under maintenance IS at the time of the second graft, we hypothesize that stronger induction with ATG might be useful for these patients.

The analysis of possible causes of patient and kidney graft losses did not provide a direct answer to explain the lower graft survival in the ATG group. We did not find a significant increase in the frequencies of severe infectious diseases and malignancies in the ATG group, perhaps larger cohort studies are needed to determine whether ATG causes overimmunosuppression in this type of patients. Similarly, cardiovascular complications were also not different between the 2 groups (Figure S3D <http://links.lww.com/TXD/A71>), despite more frequent early cardiovascular complications in the ATG group. Diet-derived

Neu5Gc can accumulate in endothelial cells of humans despite the loss mutation of the *CMAH* gene^{15,16} and thus patients having been exposed to a higher or different response against Neu5Gc may suffer from vascular inflammation.^{8,17} Furthermore, if basal preexisting anti-Neu5Gc IgGs (diet-induced) are likely not detrimental to human ECs owing to their presence in the sera of most of healthy individuals, ATG-elicited antibodies are able to recognize new epitopes of Neu5Gc at higher levels, as evidenced here and in other clinical contexts.¹⁸ Indeed, although ATG contains antigenic Neu5Gc and Gal. In contrast, the chimeric anti-IL-2R used in our patients (basiliximab) is prepared in Chinese hamster ovary cells and did not exhibit detectable Neu5Gc by mass spectrometry (Table S1 <http://links.lww.com/TXD/A74>). A detailed array study of the immune response to a variety of Neu5Gc epitopes after ATG and Basiliximab induction and the shift of anti-Neu5Gc IgG repertoire for new epitopes were thus analyzed in a subgroup of patients with enough prekidney and postkidney transplantation sera available for the tests. The fine analysis of the anti-Neu5Gc IgG repertoire on the sialoglycan arrays also showed that some newly recognized specificities (red score in Figure S5A <http://links.lww.com/TXD/A73>) were indeed strongly increased, whereas the global ELISA-based assessment of the anti-Neu5Gc IgGs levels could not detect these changes. However, whether a shift in the antibody repertoire against Neu5Gc epitopes after ATG induction contributes to the difference in clinical outcome, particularly to the incidence of early cardiovascular complications, remains speculative and requires similar investigations using sialoglycan microarray in a much larger cohort.

Despite several limitations, especially the small number of patients and the heterogeneity of the study population, our study suggests that ATG induction after a kidney transplantation in recipients already under maintenance immunosuppressive treatment for a previous nonrenal graft should be used cautiously. Our report also urges randomized prospective studies not only to compare anti-IL-2R to ATG treatment but also to compare induction with no induction to determine whether induction therapies are beneficial to these patients.

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