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**Induction therapy in elderly kidney transplant recipients with low immunological risk**

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**Abbreviations :** 95% Confidence Interval (95%CI); Anti-Thymocyte Globulins (ATG); Bk Virus (BkV); Body Mass Index (BMI); Biopsy Proven Acute Rejection (BPAR); Basiliximab (BSX); Calcineurin Inhibitors (CNI); Cold Ischemia Time (CIT); Cytomegalovirus (CMV); Delayed Graft Function (DGF); Donor-Specific Antibodies (DSA); Expanded Criteria Donor (ECD); estimated Glomerular Filtration Rate (eGFR); Hazard Ratio (HR); Human Leucocyte Antigen (HLA); Kidney Disease Improving Global Outcomes (KDIGO); Mycophenolate Mofétil (*MMF*); Mycophenolic Acid (MPA); Odds-Ratio (OR); Post Transplant Lymphoproliferative Disorder (PTLD); Restricted Mean Survival Time (RMST); Restricted Mean Time Lost (RMTL); Serum Creatinine (SCr); Static Cold Storage (SCS); Standard Error of the Mean (SEM); Standard deviation (sd).

## ABSTRACT

**Background.** In nonimmunized patients, similar rejection rates are observed for patients who have undergone Thymoglobulin (ATG) or Basiliximab (BSX) therapy. Whilst ATG may improve Delayed Graft Function (DGF), it may also be associated with higher infection rates and malignancy risk. We compared survival and clinical outcomes in elderly recipients with low immunological risk according to their induction therapy. **Methods.** We conducted a multicentric study on nonimmunized  $\geq 65$  years patients receiving a first kidney transplant between 2010 and 2017. The principal outcome was patient and graft survival. Secondary outcomes were cumulative probabilities of infection, first acute rejection episode, malignancy, de novo DSA, Post Transplant Diabetes (PTD), cardiac complications, eGFR, occurrence of DGF. Cox, logistic or linear statistical models were used depending on the outcome studied, and models were weighted on the propensity scores. **Results.** 204 patients were included in the BSX group and 179 in the ATG group, with the average age 71.0 and 70.5 years respectively. Patient and graft survival at 3 years posttransplantation were 74% (95%CI from 65% to 84%) and 68% (95%CI from 60% to 78%) in ATG and BSX group respectively, without significant difference. Occurrence of PTD was significantly higher in BSX group (23% vs 15%,  $p = 0.04$ ) due to higher trough levels of Tacrolimus on month 3 (9.48 vs 7.30 ng/ml,  $p = 0.023$ ). There was no difference in other evaluated outcomes. **Conclusion.** In elderly recipients, ATG does not lead to poorer outcomes compared to BSX and could permit lower trough levels of Tacrolimus, thus reducing occurrence of PTD.

## INTRODUCTION

The choice of induction therapy that target graft recipient immune T cells remains controversial in kidney transplantation. Thymoglobulin® or ATG is a polyclonal antibody directed primarily against T cells, which also depletes other immunological cellular subsets.<sup>1</sup> Whilst it is known that ATG provides better outcomes for high immunological risk patients,<sup>2,3</sup> there is no consensus on whether it is beneficial in low immunological risk patients. Basiliximab (BSX) is a chimeric mouse-human monoclonal antibody (Simulect®) that targets the CD25  $\alpha$  chain (CD25) of the IL-2 receptor of T cells.

Furthermore there is no difference between ATG and BSX regarding biopsy-proven acute rejection in patients following a corticosteroid withdrawal or corticosteroid free therapy regimen.<sup>4</sup> Nevertheless, ATG is associated with side effects especially a higher risk of infections due to prolonged lymphopenia induced by its depleting effect.<sup>5-7</sup> In addition, ATG use has been associated with cytokine release syndrome,<sup>8</sup> fever, chills, pain, serum sickness, headache, diarrhea hypertension, thrombocytopenia, dyspnea and has been shown to increase malignancy, especially Post Transplant Lymphoproliferative Disorder (PTLD).<sup>9-11</sup> Based on a meta-analysis and regarding the side effects of ATG,<sup>12</sup> the International KDIGO consensus recommends using BSX as induction therapy in low immunological risk patients.

Despite these guidelines, ATG is still the primary induction therapy used in North American countries<sup>13</sup> and in many others. This may be in order to avoid steroid use and to delay the introduction of calcineurin inhibitors (CNI) without increasing rejection rates.<sup>14,15</sup> The protective effect of ATG could be coupled to low dose CNI therapy, especially in recipients receiving an Expanded Criteria Donor (ECD) graft who are more susceptible to CNI-induced nephrotoxicity. Finally, data showed that ATG may prevent Delayed Graft Function (DGF),<sup>16</sup> and that DGF of greater than 6 days is strongly associated with reduced long-term survival of kidney allografts.<sup>17</sup>

Elderly patients are more likely than others to receive ECD grafts,<sup>18</sup> which may therefore support the use of ATG in this population. However, they also have a higher risk for malignancy and infections due to reduction in immune system function that occurs with age, supporting the use of BSX, especially if they are at low immunological risk. The best induction therapy in these patients therefore remains unclear.<sup>19–21</sup> While kidney transplants for elderly recipients (> 65 years) represents around 20% of the total kidney transplants performed today in France,<sup>22</sup> few studies have compared patient and graft outcomes based on ATG or BSX in this population.<sup>23–25</sup> Clinical trials investigating the choice of induction therapy have not considered elderly recipients,<sup>26,27</sup> nor reflected routine settings.<sup>28,29</sup> We thus need observational studies in this population cohort to evaluate the most relevant induction therapy. Using a French multicentric cohort, we compared a range of posttransplantation outcomes between ATG and BSX in elderly kidney recipients at low immunological risk.

## **MATERIAL AND METHODS**

### **Studied population**

The included patients were adults  $\geq 65$  years receiving a first kidney transplantation from brain-dead donors, treated with either ATG or BSX as induction therapy between 2010 and 2017. Multiple organ transplants were not considered. We only included patients with nondetectable anti-HLA class I and II antibodies using Luminex® assay (Donor Specific and Nonspecific Antibodies) at the time of transplantation.

Data were extracted from the French DIVAT cohort ([www.divat.fr](http://www.divat.fr), approved by the CNIL, n°914184) consisting of recipients monitored in Nantes, Lyon, Montpellier, Nancy and Nice. The quality of the DIVAT data bank is validated by an annual cross-center audit. All participants gave informed consent.

The patients in the BSX group received 20mg of Simulect® at day 0 and day 4. The patients in the ATG group received Thymoglobulin®, administered at 1.5mg/kg/day (maximum 75 to 100mg per day, depending of centers). All patients received initial corticotherapy during the first days of transplantation, followed by maintenance immunosuppressive therapy.

### **Available data**

Donor features included age, gender, creatinine level, allograft status (Extended Criteria Donor or Standard Criteria Donor), cause of death, and CMV and EBV status. Recipient characteristics included age, gender, BMI, comorbidities (diabetes, hypertension, dyslipidaemia, neoplasia, vascular, cardiovascular history), duration on waiting list, preemptive transplantation, CMV serology status and initial renal disease. Transplantation parameters were the Cold Ischemia Time (CIT) and the number of HLA-A-B-DR incompatibilities. Patients lost of follow-up were right-censored for mid- or long-term time-to-event. We assumed that the corresponding information were noninformative. For missing data, we excluded patients for which value spread too much from the initial date (> 3 months for one-year analysis).

### **Outcomes**

The principal outcome was the patient and graft survival, defined by the time between the transplant and the first event requiring return to dialysis, preemptive retransplantation or death with a functioning graft. Secondly, we studied cumulative probabilities of all infectious causes (bacterial, BkV viremia or BkV Nephropathy, CMV or mycotic), specific infections (bacterial, BkV and CMV), first acute rejection episode (all Biopsy Proven Acute Rejections including borderline lesions), malignancy, Post Transplant Diabetes (PTD) (patients with a diabetes diagnosed before transplantation were excluded), and cardiovascular complications (defined as occurrence of cardiac insufficiency, coronaropathy disease, rhythmic complication or valvopathy). For patients with Bk Virus infection, we considered only viremia and BkV

Nephropathy, (all patients with viremia underwent a diagnosis biopsy or were considered as BkV Nephropathy on clinical elements if biopsy were not possible).

Achievement of protocol biopsies were performed according to center practice policy. According to KDIGO recommendations, we considered indicated biopsies for rejection by the occurrence of one criterion among: increasing of creatininemia ( $> 25\%$ ) without any explanation, Delayed Graft Function  $> 10$  days, occurrence of de novo Donor Specific Anti HLA-Antibody, new onset of proteinuria, or unexplained proteinuria  $> 3$  g per day. Occurrence of de novo DSA detected by Luminex® assay, and the eGFR (estimated by MDRD) at 1-year post transplantation were evaluated (patients that died or lost-of follow-up before the first anniversary were excluded). We also studied the DGF occurrence, defined as the need for at least one dialysis session within the 7 days following the transplant. For this last outcome, the preemptive transplant patients and patients treated by peritoneal dialysis were excluded because the DGF definition was not evaluable.

### **Statistical analysis**

The characteristics at the time of transplantation between ATG and BSX groups were compared using Chi-square tests or Fisher's exact test for categorical variables and using Student t-tests for continuous variables. Except for eGFR, to consider possible confounding variables, we weighted the models on the propensity scores,<sup>30</sup> which were obtained by a multivariable logistic regression, including the center among the explicative variables. The variables significantly associated with the outcome were retained ( $p < 0.2$ ). Splines on continuous covariates were used to ensure the log-linearity assumption. Stabilized weights were computed for estimating the average treatment effect on the entire population.<sup>31</sup> Diagnostics of the models were assessed graphically for the positivity assumption and from the standardized differences for the correct model specification. Influential values were detected by a Cook distance greater than 1 in absolute value.

For the time-to-event outcomes, cause-specific Cox models were estimated by maximizing the partial weighted likelihood with robust estimator for the variance.<sup>32</sup> Proportionalities of hazards were checked graphically with log minus log survival curves. The adjusted survival curves were obtained using the weighted Kaplan-Meier estimator and compared using the adjusted log-rank test.<sup>33,34</sup> We estimated the restricted mean survival time (RMST) at 3 years, i.e. the mean time without the event for a cohort followed up to 3 years, by the area under the survival curve up to 3 years posttransplantation. The corresponding Restricted Mean Time Lost (RMTL) at 3 years was obtained by 3 minus the RMST.

For the DGF and de novo DSA outcomes, logistic models were also performed by maximizing the partial weighted likelihood with robust estimator for the variance. For the 1-year eGFR, a multiple linear model was performed.

Statistical analyses were performed using Plug-Stat® software ([www.labcom-risca.com/plug-stat](http://www.labcom-risca.com/plug-stat)) based on the R software.<sup>35</sup> **Tables S1-S14 (SDC, <http://links.lww.com/TP/B752>)** describe the variables significantly associated with the outcomes in the pseudo-samples after weighting on propensity scores. Note that respecting the methodology in causal inference, we did not consider the corticotherapy and tacrolimus residual dosages in the propensity scores. Obviously, physicians adapt the maintenance therapies according to the initial induction therapy. **Figures S1-S4 (SDC, <http://links.lww.com/TP/B752>)** represent the positivity assumption validations.

### **Ethics Statement**

All patients were included and extracted from the DIVAT database, after informed consent. In order to respect confidential medical information, all data were anonymized before analysis.

## RESULTS

### Description of the cohort

The study flow-chart is presented in **Figure 1**. The characteristics of the 383 studied patients at transplantation are described in **Table 1**. 204 patients were in the BSX group (53.3%) versus 179 in the ATG group (46.7%). 19 patients were lost of follow-up (8 in ATG group and 11 in BSX group). Use of ATG between the centers was heterogeneous, ranging from 16.2% to 73.2%. The average administration of ATG was 6.8 days (from 2 to 18 days) and average total dose of Thymoglobulin was 5.22 mg/kg (standard deviation =  $\pm 1.81$ ); ranging from 1.1mg/kg to 12mg/kg (n=146 patients). The mean recipient age was 71.0 years in the BSX group versus 70.5 years in the ATG group ( $p = 0.373$ ). About 25% of patients had a history of neoplasia before transplantation in both groups (25.5% vs 23.5%,  $p = 0.646$ ). Among the characteristics significantly different between the two groups, was the higher percentage of preemptive transplants in the BSX group (21.1% vs. 10.1%,  $p = 0.004$ ). Patients in the BSX group also had more frequent histories of dyslipidemia (62.7% vs. 51.4%,  $p = 0.025$ ) but less frequent CMV positive serologies (54.7% vs 68.0%,  $p = 0.008$ ). Almost all grafts resulted from Extended Criteria Donor, with a similar CIT in both groups (15.3 hours vs 15.9 hours,  $p = 0.282$ ). Finally, we noticed a trend to a larger use of Machine Perfusion in the BSX group (59.8% vs 48%,  $p = 0.068$ ).

In the BSX group, 57.3% of patients received corticotherapy by month 3, whereas this was 82.9% for the ATG patient group ( $p < 0.001$ ) with average dosage of 9.4mg/day and 8.4mg/day respectively ( $p = 0.376$ ). By one year, 62.5% and 78.2% of patients ( $p = 0.005$ ) received a corticotherapy with average dosages of 6.5mg/day and 5.8mg/day ( $p = 0.078$ ) in the BSX and ATG groups respectively. In both groups, there was a large use of CNI during the first year post transplantation (n = 319 patients, 96.4% vs 99.4%,  $p = 0.091$  on month 3; n = 289 patients, 89.2% vs 90.6%,  $p = 0.841$  on month 12 on ATG and BSX groups

respectively). Trough levels of Tacrolimus were similar at one year post transplantation whereas we observed a higher mean value on month 3 in the BSX group compared to the ATG group (9.48 vs 7.30 ng/ml,  $p = 0.023$ ). Finally, patients underwent a maintenance therapy by MMF or MPA were similar between groups during the first year whereas their average dosage was higher in the ATG group on month 3 (1378 vs 1166 mg,  $p = 0.001$  in the ATG and BSX groups respectively, expressed on equivalent MMF). All these results are summarized in **Figure 2**.

The median follow-up time in the cohort was 2.0 years (ranging from 0.0 to 8.2 years). During follow-up, 42 deaths with a functioning graft (28 in the BSX group) and 43 returns to dialysis (24 in the BSX group) were observed. We observed 197 patients with at least one infection, 63 patients with at least one acute rejection and 64 patients with a de novo malignancy.

### **Patient and graft survival**

The confounder-adjusted patient and graft survival curves are presented in **Figure 3A**. The patient and graft survival at 1 and 3 years posttransplantation were 87% (95%CI from 82% to 93%) and 74% (95%CI from 65% to 84%) in the ATG group, versus 89% (95%CI from 84% to 94%) and 68% (95%CI from 60% to 78%) in the BSX group.

The confounder-adjusted RMST at 3 years posttransplantation was 2.47 years (95%CI from 2.29 to 2.66) in the ATG group, i.e. the mean time of patient and graft survival was 2.47 years for the ATG cohort followed up 3 years posttransplantation. Comparatively, the RMST was 2.51 years (95%CI from 2.37 to 2.65) for the patients treated by BSX. This means that patients receiving ATG had a life expectancy with a functioning graft decreased by 0.48 months (0.04 year) compared to similar patients receiving BSX, for an observation period of 3 years. Overall, the corresponding HR (Hazard Ratio) equalled 0.96 between the BSX group compared to the ATG group (95%CI from 0.58 to 1.60).

### **Cumulative probability of acute rejection episode**

Two centers practiced protocol biopsy and three don't. Totally, 234 protocol biopsies were performed, on 136 patients (54 in the ATG group and 82 in the BSX group). The confounder-adjusted cumulative probabilities of acute rejection episodes are presented in **Figure 3B**. The probability at 1-year posttransplantation were 17% (95%CI from 11% to 23%) in the BSX group versus 16% (95%CI from 9% to 22%) in the ATG group. Among them, 22.5 % were diagnosed on protocol biopsies (25% in ATG group, and 20% in BSX group), and about 65% of these protocol biopsies revealed Borderline Rejection. The confounder-adjusted RMTL at 3 years posttransplantation was 0.55 years (95%CI from 0.36 to 0.75) in the ATG group, i.e. patients treated by ATG lost 0.55 years on average during the first 3 years of follow-up. Comparatively, the RMTL was 0.56 years (95%CI from 0.40 to 0.72) for the patients treated by BSX. Overall, the corresponding HR equalled 1.03 between the BSX group compared to the ATG group (95%CI from 0.61 to 1.76).

In addition, most of patients that presented a rejection episode during first year had Borderline lesions (15 in the ATG group and 10 in the BSX group), some had T Cell Mediated Rejection (4 in the ATG group and 12 in the BSX group), some had Antibody Mediated Rejection (3 in the ATG group and 1 the in BSX group), and a few ones Mixed Rejections (4 in the ATG group and 1 in the BSX group).

### **Cumulative probability of infection**

The confounder-adjusted cumulative probabilities of infection are presented in **Figure 3C**. The probability at 1-year posttransplantation was 52% (95%CI from 44% to 59%) in the BSX group versus 51% (95%CI from 42% to 59%) in the ATG group. The HR for time to first infection equalled 1.08 between the BSX group compared to the ATG group (95%CI from 0.76 to 1.54). Regarding different causes of infections, the related cause-specific HRs were 0.64 (95%CI from 0.33 to 1.26) for BkV infection, 0.69 (95%CI from 0.43 to 1.12) for CMV

infection, and 1.22 (95%CI from 0.81 to 1.85) for bacterial infection. Among all patients that presented BkV viremia, only 2 patients (1 in the ATG group, and 1 in the BSX group) had histological lesions of BkV Nephropathy on biopsy. All others patients presented a nonsymptomatic BkV viremia.

For one center, we had access to the evolution of immune cells during the first year following induction therapy. As illustrated in **Figure 4**, we observed a trend to higher white cells count in the BSX group during the first 3 months post transplantation compared to ATG (6800 vs 5800/mm<sup>3</sup> on month 3). Moreover, regarding total lymphocytes, as expected, there was a lower count in patients receiving ATG than BSX, remaining during the first 6 months (1070 vs 490/mm<sup>3</sup> on month 1; 1230 vs 580/mm<sup>3</sup> on month 6). Unfortunately, the small number of patients having a follow-up of immune cells after transplantation did not permit a statistical analysis.

#### **Cumulative probability of malignancy**

During our cohort follow up, we observed a total of 38 malignancies among BSX group (3 PTLD, 9 cutaneous squamous cell carcinoma, 16 basal cell carcinoma, and 15 other malignancies). In the ATG group, there was 26 malignancy events (4 PTLD, 8 cutaneous squamous cell carcinoma, 11 basal cell carcinoma, 6 others malignancies). Among the 94 patients with cancer history at the time of their transplantation, 23 malignancies were diagnosed during the first 3 years (10 were treated by ATG and 13 by BSX) and none of these where recurrence.

The confounder-adjusted cumulative probabilities of malignancy are presented in **Figure 3D**. The probability at 1-year posttransplantation were 7% (95%CI from 3% to 11%) in the BSX group versus 6% (95%CI from 2% to 10%) in the ATG group. The probability at 3-year posttransplantation were 23% (95%CI from 14% to 31%) in the BSX group versus 16% (95%CI from 6% to 26%) in the ATG group. The confounder-adjusted RMTL at 3 years

posttransplantation was 0.28 years (95%CI from 0.13 to 0.44) in the ATG group versus 0.33 years (95%CI from 0.21 to 0.45) for the patients treated by BSX. Overall, the corresponding HR equalled 1.13 between the BSX group compared to the ATG group (95%CI from 0.58 to 2.19). The small number of neoplasia subtypes did not allow a robust confounder-adjusted statistical analyses

### **De novo DSA at 1-year posttransplantation**

The observed number of patients with de novo DSA at 1-year were 4 in the ATG group (5,8%) and 7 in the BSX group (4,8%). The small number of events did not permit an evaluation of the adjusted Odds-Ratio (OR).

### **Renal function at 1-year posttransplantation**

For this study, among the 383 included patients, 20 were excluded because of a follow-up shorter than 1-year posttransplantation, 8 were excluded due to death before 1-year posttransplantation and 76 patients were excluded because of missing data, (**Table S13, SDC, <http://links.lww.com/TP/B752>**). Before 1-year posttransplantation, 27 returns to dialysis were observed and the corresponding eGFR levels were fixed at 5 ml/min/1.73m<sup>2</sup>. As illustrated in **Figure S5 (SDC, <http://links.lww.com/TP/B752>)**, the observed means of the 1-year eGFR were 36.8 ml/min/1.73m<sup>2</sup> (95%CI from 33.5 to 40.0) in the ATG group versus 38.1 ml/min/1.73m<sup>2</sup> (95%CI from 35.4 to 40.8) in the BSX group. After adjustment, the corresponding difference in the eGFR level at 1-year posttransplantation was 0.67 ml/min/1.73m<sup>2</sup> (95% CI from -4.61 to 5.95; p=0.804).

### **Delayed Graft Function**

For this study, among the 383 included patients, 61 patients were excluded because of preemptive transplantations, 33 because they were treated by peritoneal dialysis, and 9 because of missing data (**Table S14, SDC, <http://links.lww.com/TP/B752>**). The observed DGF percentages were 32% (95%CI from 25% to 40%) in the ATG group versus 29%

(95%CI from 21% to 36%) in the BSX group. The corresponding confounder-adjusted percentages were 28% (95%CI from 20% to 36%) versus 29% (95%CI from 21% to 39%) respectively. The adjusted OR for the BSX group compared to the ATG group equalled 1.06 (95%CI from 0.58 to 1.95).

### **Cumulative probability of Posttransplant Diabetes**

We excluded 123 patients with a history of diabetes at transplantation from this analysis (**Table S12, SDC, <http://links.lww.com/TP/B752>**). The confounder-adjusted cumulative probabilities of PTD are presented in **Figure 3E**. The probability at 1-year posttransplantation were 23% (95%CI from 16% to 30%) in the BSX group versus 15% (95%CI from 7% to 21%) in the ATG group. The confounder-adjusted RMTL at 3 years posttransplantation was 0.41 years (95%CI from 0.21 to 0.60) in the ATG group. Comparatively, the RMTL was 0.71 years (95%CI from 0.51 to 0.92) for the patients treated by BSX. Overall, the corresponding HR equalled 1.88 between the BSX group compared to the ATG group (95%CI from 1.00 to 3.56).

### **Cumulative probability of cardiac complication**

The confounder-adjusted cumulative probabilities of cardiac complications are presented in **Figure 3F**. The probability at 1-year posttransplantation was 22% (95%CI from 16% to 28%) in the BSX group versus 34% (95%CI from 25% to 42%) in the ATG group. The confounder-adjusted RMTL at 3 years posttransplantation was 1.03 years (95%CI from 0.79 to 1.27) in the ATG group. Comparatively, the RMTL was 0.81 years (95%CI from 0.63 to 0.98) for the patients treated by BSX. Overall, the corresponding HR equalled 0.72 between the BSX group compared to the ATG group (95%CI from 0.47 to 1.11). **Figure 5** summarize all the relative risks for studied outcomes comparing ATG and BSX in elderly recipients with low immunological risk.

## DISCUSSION

With populations ageing, more and more kidney transplants are performed for elderly recipients. The choice of induction therapy for nonimmunized elderly kidney transplant recipients remains an important consideration. In our study, the use of ATG was very heterogeneous between the different centers, ranging from 16.2% to 73.2% in this population, suggesting a lack of clinical consensus on best practices for nonimmunized elderly recipients.

We did not observe any statistically significant differences between patients treated by ATG and BSX in terms of patient and graft survival, infections, acute rejection, malignancy, 1-year de novo DSA, 1-year eGFR and DGF, in agreement with other studies.<sup>36</sup> In the Harmony Trial,<sup>4</sup> the investigators included patients with low immunological risk with a rapid corticosteroid withdrawal and demonstrated the absence of superiority of ATG in comparison to BSX for patient/graft survival, rejection or infection rates. Other studies have reported similar findings for induction therapy among patients with low immunological risk.<sup>6,15</sup>

However, all of these studies were conducted on the entire population of transplanted patients, and the average age was 50 years-old or less. In a retrospective study conducted in elderly recipients,<sup>23</sup> no statistically significant difference between ATG and BSX were reported. The authors hypothesized that this result may be explained by a lower ATG dose compared to the planned one. However, our data showed that even with a complete ATG dose (average of 5.2 mg/kg total dose), there was no difference between ATG and BSX in terms of patient and graft survival.

Several studies reported a higher rate of viral infections, especially CMV<sup>3,37</sup> with full depletant induction. In our study, ATG did not induced significantly more CMV infections, but this can be related to an insufficient number of patients. Likewise, there was a minor trend to higher cardiovascular complications in ATG group, that could be linked to CMV status.<sup>38</sup>

We didn't observe any difference between groups regarding the occurrence of malignancy, whilst a previous study reported that ATG can be associated with an increased risk of PTLD.<sup>9</sup> This is very valuable data especially in the context of an elderly population: about 25% of our patients had a history of malignancy and ATG did not only lead to similar neoplasia outcomes, but also didn't seem to increase the recurrence of a pretransplant neoplasia.

Although we had very few data on lymphocytes following induction therapy our data suggest a persistent lymphopenia during the first year after ATG therapy comparing to BSX. Moreover, these data were from a center that practiced low doses of ATG (75mg a day during 2 days) so we suggest that this is valid for most of patients that underwent an ATG induction in our cohort.

We observed similar infectious and neoplasia outcomes following ATG and BSX, whilst a prolonged lymphopenia in the ATG group. This may be linked to functionality of lymphocytes, that is known to decrease in elderly population, added to the maintenance therapy.<sup>39</sup> This is concordant with recent data which tend to show that depleting induction therapy has no effect on PTLD risk with current maintenance immunosuppressive regimens.<sup>11</sup> Rejection rates at 1-year were also similar for both groups (16% and 11% for ATG and BSX respectively), but were slightly higher than previously described,<sup>12</sup> possibly due to the fact that we considered all rejection episodes including borderline episodes (representing about 50% in each group). It is noteworthy that if ATG is commonly used to decrease rejection rates, its impact on T cells subsets is not fully understood and some have demonstrated that the homeostatic repopulation observed after ATG induction influence negatively CD4<sup>+</sup>FoxP3<sup>+</sup> Treg cells and also could favor the up-regulation of markers associated with rejection.<sup>40-42</sup> As expected, we observed a low occurrence of de novo DSA at 1-year (5.8% and 4.8% in ATG and BSX groups respectively, without significant difference). This rate is lower than

described by others,<sup>43</sup> confirming a low immunological risk population with possibly a less effective immune system linked to the age.

In our cohort, we detected more PTD in the BSX group than in the ATG group, whereas the number of patients which underwent a steroid therapy was smaller in BSX group. Nevertheless, steroid dosages were rather low in both groups (less than 10 mg per day) and the increased number of PTD in the BSX group could be more related to the use of higher dosages of Calcineurin Inhibitors, traduced by significant higher trough levels.

Finally, the occurrence of DGF was similar in our study, whereas other have shown a benefit by using ATG.<sup>16</sup> Kyllonen et al. concluded that intraoperative administration of ATG lead to lower DGF than BSX, probably because of the avoidance of leukocyte sequestration in the allograft by reducing adherence to the antigen-presenting endothelial cells.<sup>44,45</sup> Thus, it has been found that intraoperative ATG results in better reduction in DGF than postoperative administration.<sup>46</sup> In our study, the lack of data on the exact time of ATG administration precludes any conclusion on the effects of ATG on DGF. Moreover, we observed a trend to a higher use of machine of perfusion in the BSX group, that could have minimized the potential difference of DGF between groups. The low observed eGFR at one year in both groups is probably related to the fact that all patients received a graft from an ECD (mean donor age was 72.7 years) because of their age and that they underwent a CNI therapy.<sup>47</sup>

Our study suffers from some limitations. First, one cannot exclude possible unobserved confounders. The randomized clinical trial remains the design of reference for evaluating the theoretical effect of ATG versus BSX. But because elderly recipients are often poorly represented in such trials, cohort-based studies with representative patients as in real-life practices are the most relevant studies to evaluate the effect of ATG versus BSX. Second, the sample size of our study is relatively small, which might explain the statistically nonsignificant trends for a higher incidence of CMV and BkV infections related to ATG

induction therapy, and the lack of difference between rejection rates between groups (added to nonsystematic surveillance biopsies). In addition, the potential effect of the center as a confounder has been considered. Indeed, the center was an explicative variable of the propensity scores, ensuring the balanced repartition of centers between both the ATG and Basiliximab. Finally, regarding our mid-term results, we still need further long-term studies to validate the robustness of our conclusion, especially for late related outcomes.

In conclusion, whereas elderly kidney transplant recipients have weaker immune reconstitution following ATG induction therapy, there is no difference for patient and graft outcomes between ATG and BSX as induction therapy, neither for infections and neoplasia outcomes. This highlight the fact that with most recent maintenance therapies permitting lower doses of ATG, added to prophylaxis and preventive strategies of infections (especially the CMV), induction therapy wouldn't be the leading factor linked to infectious nor neoplasia complications. However, using a nondepletant therapy could require higher CNI trough levels and thus increase occurrence of PTD.

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## FIGURES

**Figure 1.** Flow-chart of the study (eGFR: estimated Glomerular Filtration Rate; PTD: Post Transplant Diabetes; DGF: Delayed Graft Function).

**Figure 2.** Representation of maintenance therapies used among groups. **A.** CNI use (cyclosporin in respectively grey and white and tacrolimus in black and striped) and levels of residuals dosages of tacrolimus (with corresponding Standard Error of the Mean, SEM) among ATG and BSX groups. **B.** MPA and MMF use (MPA in respectively grey and white and MMF in black and striped) and average of equivalent MMF dosages among groups (with corresponding SEM). **C.** Percentage of patients underwent a steroid therapy among groups, and their average dosage (with corresponding SEM). \* represents a p value < 0,05; \*\* represents a p value < 0,01; \*\*\* represents a p value < 0,001; n = 319 patients on month 3, 302 patients on month 6 and 289 patients on month 12.

**Figure 3.** Confounder-adjusted probabilities of events according to the time post-transplantation and the induction therapy (ATG: Anti-Thymocyte Globulins; BSX: Basiliximab). **A-** Patient and graft survival. **B-** Cumulative probability of acute rejection episode. **C-** Cumulative probability of infection. **D-** Cumulative probability of malignancy. **E-** Cumulative probability of PTD. **F-** Cumulative probability of cardiac complications.

**Figure 4.** Evolution of White Cells count (**A**) and Lymphocytes count (**B**) depending of the induction therapy (based on the analysis of available data)

**Figure 5.** Summary of the relative risks of using Basiliximab instead of Thymoglobulin for the studied outcomes (OR: Odds-Ration; HR: Hazard-Ratio).

## TABLES

**Table 1.** Description of the entire cohort according to induction therapy (Abbreviations: BMI, body mass index; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ECD, Expanded criteria donor; HLA, human leucocyte antigen; NA, not available (missing); sd, standard deviation).

	Global (n=383)			ATG (n=179)			BSX (n=204)			p-value
	NA	n	%	NA	n	%	NA	n	%	
Male recipient	0	284	74.2	0	137	76.5	0	147	72.1	0.3180
Recurrent causal nephropathy	0	63	16.4	0	29	16.2	0	34	16.7	0.9024
Preemptive transplantation	1	61	16.0	1	18	10.1	0	43	21.1	0.0035
History of diabetes	0	123	32.1	0	64	35.8	0	59	28.9	0.1530
History of hypertension	0	327	85.4	0	150	83.8	0	177	86.8	0.4124
History of vascular disease	0	109	28.5	0	53	29.6	0	56	27.5	0.6405
History of cardiac disease	0	153	39.9	0	75	41.9	0	78	38.2	0.4651
History of cardiovascular disease	0	203	53.0	0	99	55.3	0	104	51.0	0.3973
History of malignancy	0	94	24.5	0	42	23.5	0	52	25.5	0.6457
History of dyslipidemia	0	220	57.4	0	92	51.4	0	128	62.7	0.0250
Positive recipient CMV serology	5	230	60.8	4	119	68.0	1	111	54.7	0.0082
Male donor	0	187	48.8	0	93	52.0	0	94	46.1	0.2510
ECD donor	1	372	97.4	1	172	96.6	0	200	98.0	0.5244
Use of machine perfusion	12	208	54.3	6	86	48.0	6	122	59.8	0.0684
Vascular cause of donor death	0	275	71.8	0	126	70.4	0	149	73.0	0.5655
Donor hypertension	11	224	60.2	9	103	60.6	2	121	59.9	0.8927
Positive donor CMV serology	0	240	62.7	0	115	64.2	0	125	61.3	0.5486
Positive donor EBV serology	1	370	96.9	1	172	96.6	0	198	97.1	0.8102
HLA-A-B-DR incompatibilities >4	5	97	25.7	3	41	23.3	2	56	27.7	0.3256
	NA	m	sd	NA	m	sd	NA	m	sd	
Recipient age (years)	0	70.8	4.8	0	70.5	4.8	0	71.0	4.8	0.3733
Recipient BMI (kg.m-2)	3	26.7	4.0	3	26.9	4.2	0	26.5	3.9	0.2796
Duration on waiting list (months)	16	16.5	19.0	11	17.9	18.9	5	15.4	19.1	0.2082
Donor age (years)	1	72.7	8.8	1	72.1	10.0	0	73.1	7.5	0.2739
Donor creatininemia (µmol/l)	1	82.9	39.5	0	85.5	41.0	1	80.7	38.0	0.2331
Cold ischemia time (hours)	3	15.6	5.0	1	15.9	5.2	2	15.3	4.8	0.2820

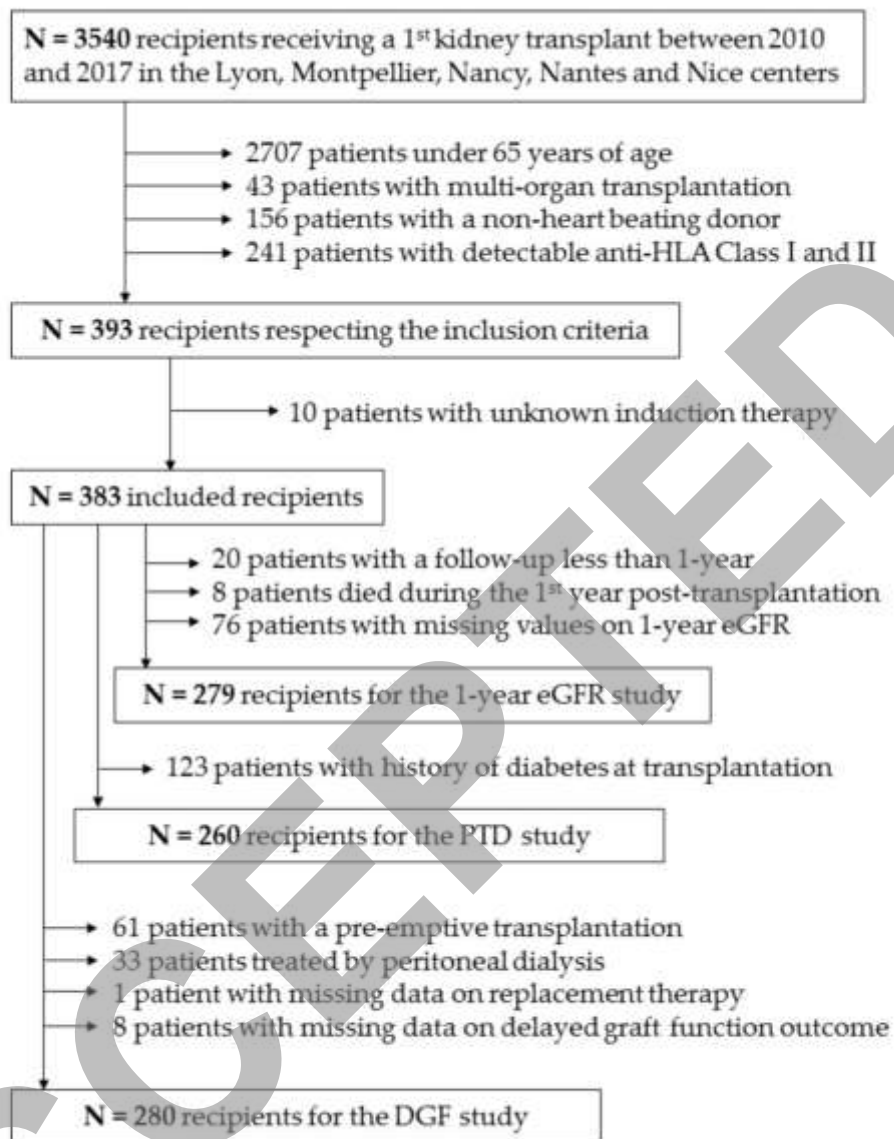


Figure 1

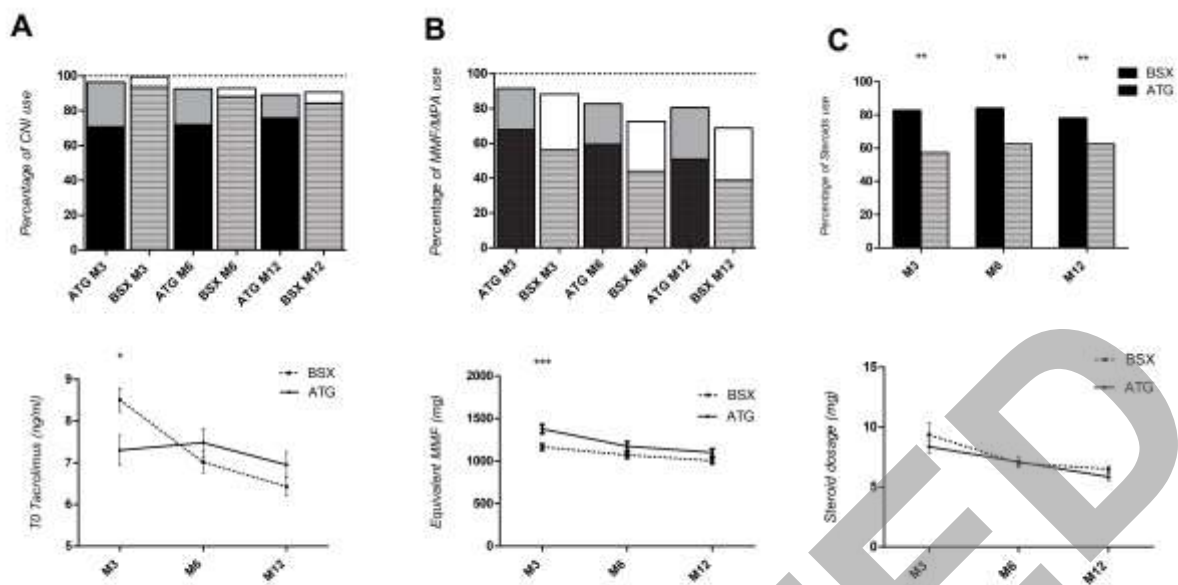
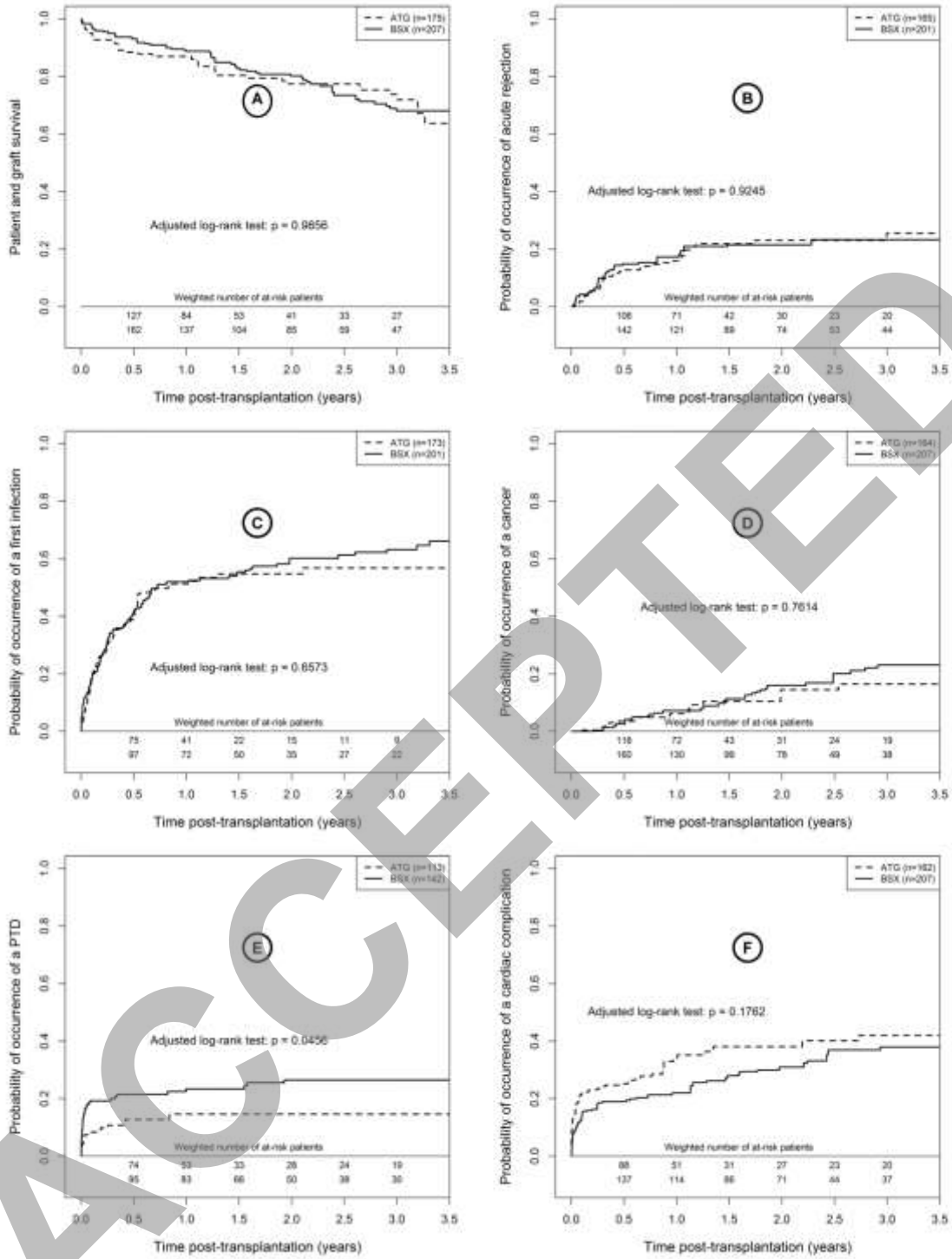


Figure 2



**Figure 3**

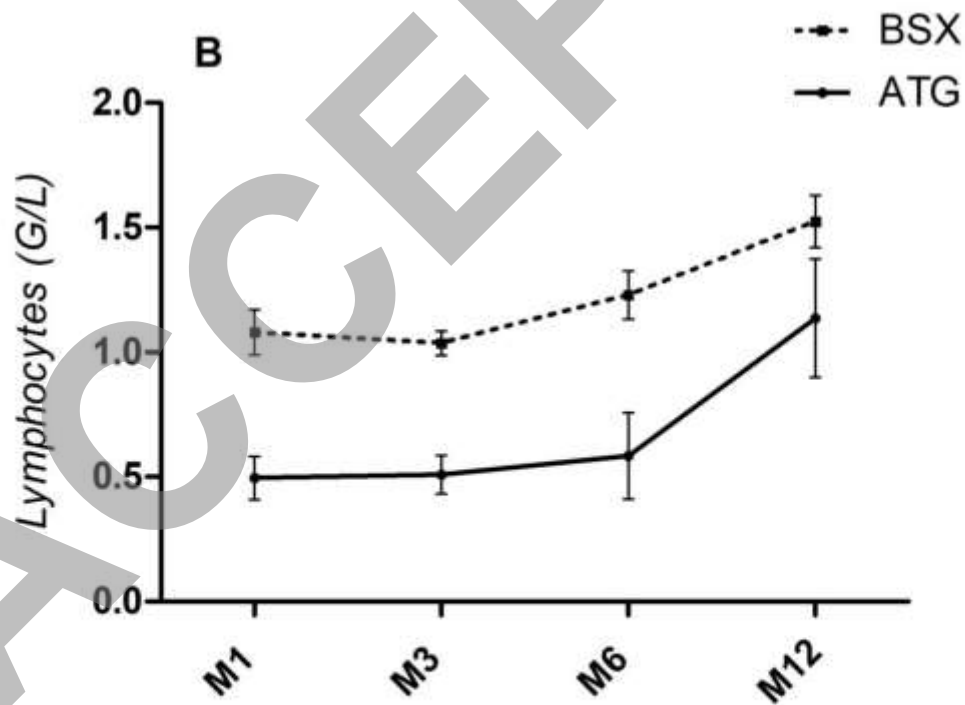
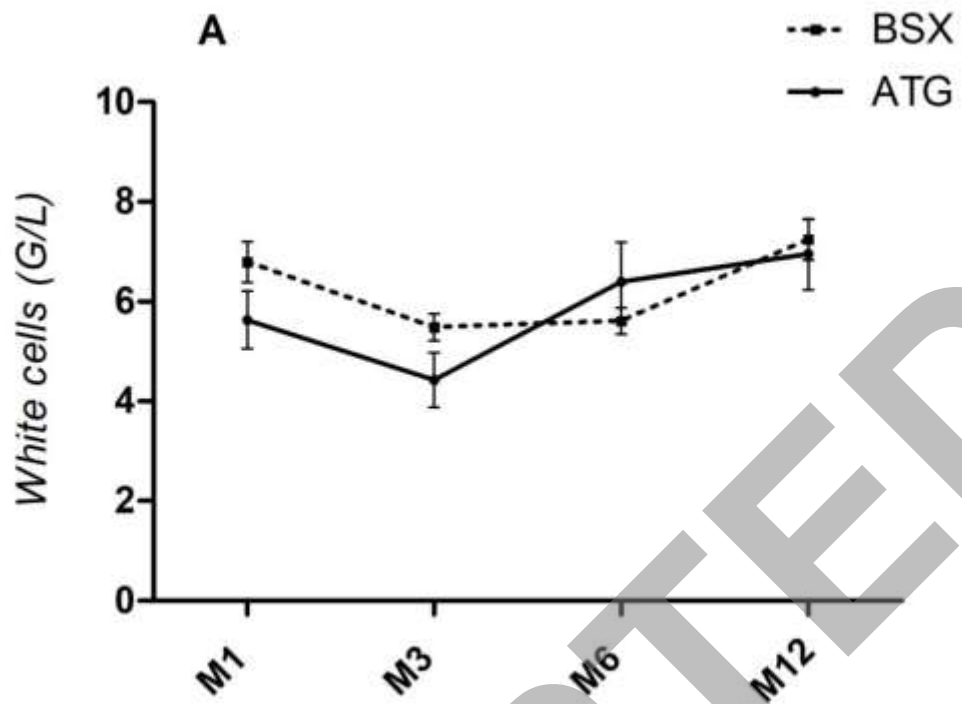
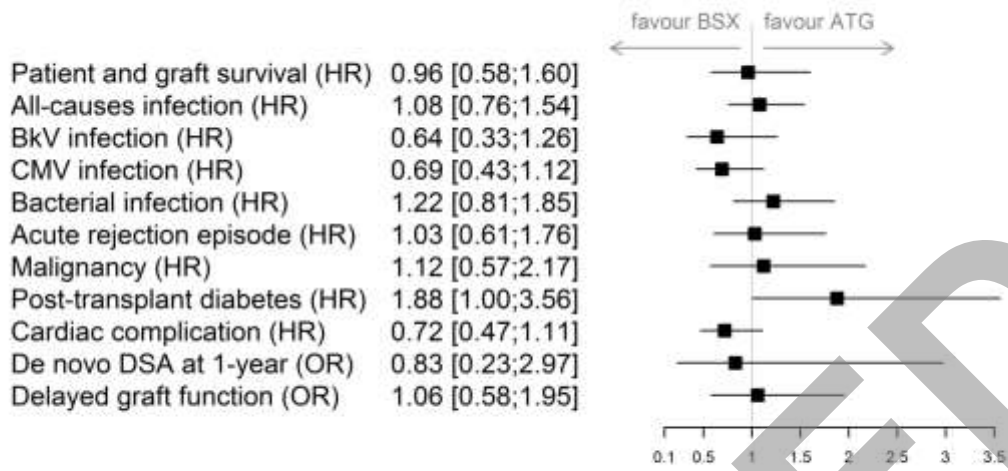


Figure 4.



**Figure 5**