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Induction and Donor Specific Antibodies in Low Immunologic Risk Kidney Transplant Recipients

Natalie M. Bath[1], Arjang Djamali[2], Sandesh Parajuli[2], Didier Mandelbrot[2], Glen Leverson[3], Luis Hidalgo[1], Thomas Ellis[4], Jillian L Descourouez[1], Margaret R. Jorgenson[1], Dave Hager[1], Dixon B. Kaufman[1], Robert R. Redfield III[1]

[1] Department of Surgery, Division of Transplant, University of Wisconsin Hospital and Clinics

[2] Department of Medicine, Division of Nephrology, University of Wisconsin Hospital and Clinics

[3] Department of Surgery, University of Wisconsin Hospital and Clinics

[4] Department of Pathology and Laboratory Medicine, University of Wisconsin Hospital and Clinics

*Corresponding author

Robert R Redfield III, MD

H4/761 Clinical Science Center

600 Highland Avenue

Madison, WI 53792

Phone: (608) 265-6471

E-mail: redfield@surgery.wisc.edu

Abstract

Background: Optimal induction for patients without pre-transplant donor specific antibodies (DSA) is poorly defined. The goal of this study was to compare the incidence of de novo DSA (dnDSA) and graft outcomes between induction therapies in patients with a negative virtual cross match (VXM).

Methods: A retrospective chart review was performed which identified 782 patients with a negative VXM who underwent kidney transplantation at a single high-volume institution between January 2013 and May 2017. Kaplan-Meier analysis was used to assess the incidence of dnDSA and allograft survival between induction therapies in this group. DnDSA is defined as the development of new post-transplant DSA, at any MFI level.

Results: Induction therapy included alemtuzumab (N=87, 11.1%), basiliximab (N=522, 66.8%), and anti-thymocyte globulin (ATG) (N=173, 22.1%). One-year graft survival was similar between groups (alemtuzumab 100%, basiliximab 98.2%, ATG 98.8%). Incidence of acute rejection at one year was less than 2% and not different between the three groups. Alemtuzumab was associated with the highest incidence of dnDSA at 13.8% compared to 5.2% and 8.1% in basiliximab and ATG groups at 1 year, respectively (p=0.009). In multivariate regression analyses, alemtuzumab retained its significant association with dnDSA HR 2.5 (95% CI 1.51-4.25, p=0.0004).

Conclusions: In summary, alemtuzumab was associated with a higher rate of dnDSA development in patients with a negative VXM; however, this finding was not associated with rejection or graft failure.

Introduction

The use of immunosuppression medications as induction therapy is routine in the majority of kidney transplants with the ultimate goal of reducing the risk of early acute rejection.[1] In addition to decreased risk of early acute rejection, induction therapy can potentially confer the benefit of reducing the intensity of subsequent immunosuppression regimens including steroid use, improving graft survival, and decreasing delayed graft function when compared to no induction therapy.[2-4] Induction therapy is of particular benefit to those at high risk of rejection including sensitized patients. However, as immunosuppression therapies in transplant have evolved, the appropriate induction agent for a specific patient population has not always been clear, particularly for patients with varying immunologic risks. As with any immunosuppression regimen, inappropriate use of induction therapy can also result in infectious complications or post-transplant malignancy.[5,6]

Multiple previous studies have described the benefits and risks associated with various induction regimens; however, there remains a lack of data demonstrating which induction therapy is superior in patients with or without donor specific antibody (DSA).[2,7,8] Patients with a high risk of rejection often have pre-formed DSA prior to transplant and as a result will undergo desensitization or will receive a more aggressive immunosuppression regimen following transplant. At the same time, patients without pre-transplant DSA who are deemed low-risk for rejection can subsequently develop de novo donor specific antibody (dnDSA) post-transplant. The presence of DSA post-transplant has already been demonstrated as having a significantly deleterious effect on graft function and survival.[9,10] Therefore, it is critical to better understand the relationship between induction therapies and the development of dnDSA post-transplant. Our

institution has implemented induction protocols that stratify patients based on the intensity of pre-transplant virtual cross match (VXM).[11,12] The most recent protocol divides patients into 3 groups: (1) negative (absence of pre-transplant DSA); (2) VXM borderline positive (<1000 mean fluorescence intensity sum (MFI_{sum})); and (3) VXM positive (≥1000 MFI_{sum}). The goal of this study is to compare the incidence of dnDSA and graft outcomes between induction therapies in patients with no pre-transplant DSA.

Materials and methods

Data source and patient population

This was a single center, longitudinal cohort study of patients undergoing kidney transplantation at our institution with alemtuzumab, anti-thymocyte globulin (ATG) or basiliximab induction between January 2013 and May 2017. This study was approved by the University of Wisconsin-Madison Minimal Risk Institutional Review Board (Health Sciences) under the title "Outcomes of Kidney Transplant Recipients." IRB number is 2014-1072-CR004. Data were obtained from the prospectively collected Wisconsin Allograft Recipient Database (WisARD) and electronic medical records at the University of Wisconsin (UW) Hospital. Patients were excluded if they did not receive induction with alemtuzumab, rATG, basiliximab or if they received any of the above agents in combination, if they were not tested for DSA pre-transplant, and if they were < 18 years old. Of the patients who met inclusion criteria, we further identified those with a negative VXM, which indicated they did not have pre-transplant DSA level, to be included in this study. Patients were then grouped according to their induction therapy (alemtuzumab, basiliximab, ATG). The choice of induction therapy in this low-risk patient population is typically based on patient specific variables including age, primary cause of ESRD, and a

compelling indication for early steroid withdrawal. In general, basiliximab with long-term steroid use is the induction therapy of choice for low-risk patients at our institution. Alemtuzumab with early steroid withdrawal is reserved for patients under 60 years old who are deemed to benefit from limited steroid use. These patients include those with sensitivities to steroids such as diabetics or those with steroid psychosis-related symptoms. ATG with long-term steroid use is typically given to those who have autoimmune disorders as the primary cause of renal failure and are at risk of recurrence such as IgA nephropathy or focal segmental glomerulosclerosis. During the study period, alemtuzumab was given as a single intra-operative 30 mg dose for induction. Dosing of ATG for induction at our institution involves an intraoperative dose of 1.5 mg/kg followed by daily post-operative dosing to a goal of 4.5-6 mg/kg based on compelling conditions. Basiliximab is given as a single intraoperative 20 mg dose with an optional additional 20 mg dose given on post-operative day 3, per surgeon discretion. In the study presented here, 67.0% (N=350) of patients who received basilixiamb induction received two doses. The post-transplant protocolized maintenance immunosuppressive regimen at our center is a triple drug regimen consisting of tacrolimus, mycophenolate (MPA) and corticosteroids. Institutional protocol dictates tacrolimus troughs range between 8-12 ng/mL for the first year after transplant. No significant differences in mean trough levels were found at 1-month, 3-month, 6-month, or 1 year. Mean 3-month trough levels between induction groups were as follows: alemtuzumab 7.8 ± 2.8 ng/mL, basiliximab 8.2 ± 2.9 ng/mL, and ATG 8.3 ± 3.3 ($p=0.51$). At our institution, tacrolimus trough levels are not run any differently based on steroid use in the patient. Post-transplant biopsies are not routinely performed in pre-transplant negative VXM patients. Instead, for-cause biopsies are performed in cases of elevated creatinine or the development of DSA in patients. Changes in maintenance immunosuppression were made if

dnDSA was detected in a patient. After undergoing for-cause biopsy, patients who were identified as having rejection were treated for rejection as previously described.[13] Patients who had a negative biopsy following the development of dnDSA underwent optimization of tacrolimus and MPA doses. Belatacept is infrequently used as a calcineurin substitute in the setting of compelling scenarios.

Data collection and outcomes

Primary outcomes included the development of dnDSA, graft survival, and incidence of biopsy-proven rejection. DnDSA was defined as the development of new post-transplant DSA, at any MFI level. Graft failure was defined as a return to dialysis, re-transplantation, patient death, transplant nephrectomy, or primary non-function. Secondary outcomes included the incidence of cytomegalovirus viremia (CMV), BK viremia, antibody mediated rejection (AMR), acute cellular rejection (ACR), delayed graft function (DGF), and length of hospital stay. CMV infection, defined as viremia via molecular diagnostic testing (positive PCR) or biopsy proven end organ disease via diagnosis code, within the study period. Molecular diagnostic methodology was consistent throughout the study period with the exception of the adoption of the WHO international standard in 2015, which resulted in a conversion from copies/mL to IU/mL. BK viremia was defined as borderline positive (>1,000 copies/mL) and positive (>10,000 copies/mL). AMR and ACR were both identified as biopsy proven rejection per pathology reports. Data on organ donors and recipients were collected including ethnicity, gender, age, and BMI (body mass index). KDPI (kidney donor profile index), donor type (live, donor after cardiac death (DCD), donor after brain death (DBD)), and cold ischemia time (CIT) data were also collected on organ donors. CIT was calculated in deceased donors only and in all donors

combined. Additional data collected on transplant recipients included the following: blood transfusion, calculated panel reactive antibodies (cPRA), pre-transplant dialysis, and HLA (human leukocyte antigen) mismatch.

Anti-HLA antibody screening by Solid-Phase Fluorescent Beads

DSA were detected pre- and post-transplant using Luminex single antigen beads (One Lambda, Canoga Park, CA) performed according to the manufacturer's instructions with a reduced volume of beads (3 vs. 5 μl).[14] In our program, we do not rely on strict MFI cutoffs to assign HLA antibody specificities. Instead, antibodies were identified using multiple criteria including patterns of epitope reactivity, MFI value, specific bead behaviors, and assay background, as described previously.[15] All DSA detected in this study had MFI values greater than 100. DSAs were classified as de novo if they appeared after transplantation and were not detected in pre-transplant samples. Since pre-transplant antibodies did not need to meet a minimum MFI threshold to be "identified", de novo antibody identified in this study is less likely to be due to increases in weak pre-transplant DSA. Previous studies have established that low levels of DSA (MFI <1000) can result in AMR, which indicates that low levels of DSA are clinically significant and should be followed.[13]

The strength of dnDSA were represented as the sum of the mean fluorescence intensity value (MFI_{sum}) of all DSA. Since 2014, routine post-transplant monitoring of DSA was performed on all transplant recipients at 6 and 12 months, and annually thereafter. Patients with a pre-transplant cPRA >0 were tested at an additional three-week time point. Patients with dnDSA underwent transplant biopsy. All patients undergoing renal transplant biopsy for other reasons

had DSA testing done as a part of the biopsy visit. The yearly DSA monitoring included patients transplanted before 2014.[16] Median sum MFI of dnDSA and 25^{th}-75^{th} interquartile range was calculated using the first values that were found to be positive for HLA class I and class II. Immunodominant DSA was determined as the specificity with the highest MFI value when first detected as positive.

Statistical analysis

Statistical analysis was performed with SAS software, and P-values less than 0.05 were considered statistically significant. Differences between induction groups were assessed with ANOVA for continuous variables and Fisher's exact tests for nominal variables. The methods of Kaplan and Meier were employed to estimate the incidence of dnDSA, graft survival, patient survival, rejection, CMV viremia, and BK viremia and rates were compared between induction groups using log-rank tests. Multivariable analyses were carried out using Cox proportional hazards regression models. After initial multivariable analyses were run, significant variables were included in an additional multivariable analysis in order to determine the relative impact of each variable on dnDSA development. Chi-square was used for nominal variables.

A propensity-score matching analysis was also performed to help control for clinical differences between groups. Due to the size of each population and the need to control for many variables, the alemtuzumab cohort (N=87) was matched to a combined basiliximab/ATG cohort (N=348) on a 1:4 basis.

Results

One thousand one hundred forty-seven (1,147) patients underwent kidney transplantation from January 2013 to May 2017. One hundred ninety-five (195) of these patients were excluded based on the criteria listed above. Of the 952 included patients, 782 patients were identified as having a negative VXM and were included in this study. The majority of patients received basiliximab at 66.8% (N=522); 11.1% (N=87) received alemtuzumab and 22.1% (N=173) received ATG (Fig 1).

Patients who received alemtuzumab were significantly younger and less sensitized

Demographic data and baseline characteristics for kidney transplant recipients are presented in Table 1. Patients who received alemtuzumab were significantly younger with a mean age of 47.9 ± 12.3 compared to 54.9 ± 12.9 and 51.2 ± 12.8 in the basiliximab and ATG groups, respectively ($p<0.0001$). Patients who received alemtuzumab were more likely to be white at 81.6% compared to 75.7% of basiliximab and 71.1% of ATG patients ($p<0.01$). No difference was seen in gender or BMI between groups. Notably, patients who received alemtuzumab were less sensitized compared to both basiliximab and ATG groups as evidenced by fewer blood transfusions ($p<0.02$) and a lower cPRA ($p<0.0001$). Additionally, there were significantly more patients in the ATG induction group who had undergone a previous kidney transplant at 22.0% (N=38) compared to 8.0% (N=42) in the basiliximab and 9.2% (N=8) in the alemtuzumab induction groups ($p<0.0001$).

Patients who received alemtuzumab were more likely recipients of live donor transplants

Demographic data and baseline characteristics of organ donors are listed in Table 2. Patients who received alemtuzumab were more likely to receive a live donor kidney and low KDPI compared to basiliximab and ATG groups ($p<0.0006$). Mean KDPI in the alemtuzumab group was 41.3% ± 28.2, although this was only significantly lower than the basiliximab group at 50.7% ± 28.2. Overall the mean CIT in the alemtuzumab group was significantly lower at 12.9 ± 7.1 hours when compared to basiliximab (14.9 ± 6.4 hours) and ATG (15.6 ± 7.4 hours), which is likely a reflection of the alemtuzumab group being more likely to receive a live donor kidney ($p<0.05$). When live donors were excluded, no significant CIT difference existed between induction groups. The mean age at donation, gender, or BMI were not significantly different between groups.

Highest incidence of de novo DSA seen in patients who received alemtuzumab

The overall incidence of dnDSA at 1 year in kidney transplant patients with no pre-transplant DSA during this period was 6.8%. At 1-year post-transplant, 13.8% of patients who received alemtuzumab as induction therapy developed dnDSA (Table 3). This incidence of dnDSA is significantly higher than the incidence seen in the basiliximab and ATG induction groups ($p=0.0009$). At 1-year, the basiliximab group had the lowest incidence of dnDSA at 5.2%; ATG demonstrated an incidence of 8.1% (Fig 2). Fifty percent of the dnDSA that developed in the alemtuzumab induction group was HLA class I alone, 25% class II alone, and 25% both class I

and II. Basiliximab induction group primarily developed class II dnDSA (44.4%) whereas the ATG induction group primarily developed class I dnDSA (42.9%) (Fig 3) (p=0.36). An additional analysis was performed comparing rates of dnDSA development in patients who received early steroid withdrawal (ESW) in the alemtuzumab (N=76) and ATG (N=39) induction groups. When controlling for ESW, the alemtuzumab induction group still developed dnDSA at a greater rate compared to ATG at 1 year (14.7% versus 5.3%) ($p<0.02$) (Fig 4). Tacrolimus trough levels were not significantly different between groups at 3 months. The mean 3 month trough levels for each group were as follows: alemtuzumab 7.8 ± 2.8, basiliximab 8.2 ± 2.9, and ATG 8.3 ± 3.3 ($p=0.51$).

When analyzed in a multivariate analysis including baseline characteristics, steroid withdrawal, and belatacept use, alemtuzumab demonstrated a 4.2-increased risk of dnDSA development relative to ATG (HR 4.2; 95% CI, 1.57-11.04; p=0.0042). Basiliximab was not associated with an increased risk of dnDSA development. Black patients demonstrated a 2.4 increased risk of dnDSA development relative to white patients (HR 2.4; 95% CI 1.42-4.02; p=0.001) and older age at transplant demonstrated a decrease in risk of dnDSA development (HR 0.98; 95% CI 0.96-0.99; p=0.01) (Table 4). In a multivariate model containing alemtuzumab, black race, age at transplant, and hospital length of stay, alemtuzumab retained its strong association with the incidence of dnDSA (HR 2.5; 95% CI, 1.51-4.25; p=0.0004) (Table 5).

Alemtuzumab not associated with inferior rejection or graft survival rates

Despite the significant difference of dnDSA incidence, there was no association between induction agent and the incidence of biopsy-proven rejection, overall actuarial graft survival or patient survival. Patients were followed for a mean of 2.4 years. Episodes of rejection were further characterized as AMR or ACR based on biopsy results. No episodes of AMR at 1 year occurred in patients receiving alemtuzumab; low incidences of 0.4% and 1.8% were seen in the basiliximab and ATG groups, respectively. The incidence of ACR was greater than AMR across all groups; however, these results were still not statistically significant (Fig 5). Importantly, the development of dnDSA and occurrence of ACR and AMR did not correspond with a higher rate of graft loss. Patients who received alemtuzumab had 100% graft survival at 1 year; similarly, basiliximab and ATG groups demonstrated graft survival rates of 98.2% and 98.8%, respectively (Table 3). In multivariate analyses, induction therapy had no significant effect on graft survival, patient survival, or any type of rejection (Tables S1-5).

We performed a propensity-score matching analysis in order to control for clinical differences seen among groups. Graft survival, patient survival, rejection incidence, and dnDSA development were measured outcomes. Groups were matched on a 1:4 basis between alemtuzumab cohort (N=87) and combined basiliximab/ATG cohort (N=348). Alemtuzumab group had a significantly higher 1-year rate of dnDSA development at 14.6% compared to 5.4% in the combined basiliximab/ATG group (p=0.0004). No significant difference was seen for all other outcomes.

ATG associated with significantly greater incidence of CMV viremia but not BK viremia

Incidence of CMV and BK viral infection were examined between induction groups. The depletion induction agents had significantly higher incidence of CMV viremia on univariate analysis than that seen in the basiliximab group (ATG 38.4%, alemtuzumab 36.6%,and basiliximab 22.3%; p<0.0003) (Table 3). There was no difference in incidence of CMV viremia on univariate analysis between ATG and alemtuzumab groups (p=0.83). In a multivariate analysis, basiliximab conferred a protective benefit against CMV viremia (HR 0.6; 95% CI 0.41-0.78; p=0.0004), alemtuzumab was not significantly different from the referent ATG in CMV risk (HR 0.9, CI 0.54-1.67, p=0.85) (Tables S6-8). The incidence of BK viremia was not statistically significantly different at either borderline positivity (BK >1000 copies/mL; alemtuzumab 20.9%, basiliximab 25.3%, ATG 23.6%, p=0.45)) or positive (BK >10,000 copies/mL; alemtuzumab 15.3%, basiliximab 16.7%, ATG 12.4%, p=0.24) between groups on univariate analysis. On multivariate analysis where ATG was the referent, there was also no difference in risk of BK virus based on induction type (alemtuzumab HR 1.6 CI (0.68-3.98, p=0.27, basiliximab HR 1.3, CI 0.76-2.13, p=0.36) (Fig 6).

Other outcome measurements include DGF and length of hospital stay. Overall rates of DGF were low at <10%. Basiliximab induction group had the highest DGF incidence among groups at 8.4%; however, this was not statistically significant (alemtuzumab 3.5% vs basiliximab 8.4% vs ATG 5.8% p=0.18). The mean length of hospital stay was lowest in the alemtuzumab group at 4.3 days compared to 5.1 days in both the basiliximab and ATG groups (p<0.02) (Table 3).

Among those who developed dnDSA, graft survival and rejection rates are equivalent between induction groups

Lastly, we further characterized outcomes among those who developed dnDSA at 1 year between the induction groups (Table 6). Alemtuzumab induction group had the lowest median sum MFI of 1179 (interquartile range 640.3-2335) for de novo DSA compared to basiliximab (2264 (1231-8252)) and ATG (2138 (752.3-5642)). However, no statistically significant difference of median sum MFI was found between groups (p=0.27). The average number of DSA contributing to the sum MFI was similar between groups (alemtuzumab 1.6 ± 0.9, basiliximab 2.1 ± 1.9, thymoglobulin 2.0 ± 1.6) (p=0.70). Mean time to development of dnDSA post-transplant was longest in the basiliximab group at 198.6 ± 95.3 days but was not different from the other induction groups (Alemtuzumab 172.1 ± 112 days; ATG 163.2 ± 104.9 days; P=0.53). The majority of patients who developed dnDSA in the alemtuzumab induction group were on the early steroid withdrawal protocol (83.3%, N=10) whereas 100% (N=27) of the basiliximab and 85.7% (N=12) of the ATG induction groups who developed dnDSA were on maintenance steroids (p<0.0001). Graft survival at 1 year was excellent between all 3 induction groups. Alemtuzumab induction group had the lowest overall graft survival at 75.0% (N=9); however, this was not significantly different from other groups. The highest rate of AMR at 1 year was seen in the alemtuzumab induction group (16.7%; N=2) with the highest overall AMR rate demonstrated in the basiliximab induction group (18.5%; N=5). At 1 year, 41.7% (N=5) of the alemtuzumab patients with dnDSA developed ACR compared to 29.6% (N=8) and 21.4% (N=3) in the basiliximab and ATG groups, respectively (P=0.53). Changes in MFI levels of the immunodominant dnDSA are represented in Fig 7 for each patient who developed rejection during the study period.

Discussion:

Here we present the results of a retrospective study examining the development of de novo donor specific antibody and kidney allograft outcomes between the induction therapies alemtuzumab, basiliximab and ATG in low immunologic risk patients. Our results suggest that patients with no pre-transplant DSA who receive alemtuzumab induction therapy are more likely to form dnDSA than those who receive basiliximab or ATG. This association was independent of other risk factors including black race, age at transplant, and hospital length of stay. Length of hospital stay was likely lowest in the alemtuzumab group due to the significantly higher rate of living donors, which has previously been shown to be associated with shorter hospital stays.[17] Notably, the increased incidence of dnDSA in those receiving alemtuzumab was not associated with a significantly higher incidence of biopsy-proven rejection compared to other induction groups at 1 year. Although basiliximab demonstrated a lower rate of dnDSA development compared to ATG at 1 year, the rates between these two groups were not significantly different when evaluated in the controlled analysis. ATG was found to have a significantly higher incidence of CMV viremia. Despite these differences in the development of dnDSA and CMV viremia, no single induction therapy was associated with a superior overall graft survival rate.

Current induction regimens commonly include the use of T lymphocyte depleting agents ATG or alemtuzumab or non-depleting agents such as basiliximab. Each of these agents carries its own set of risks and benefits. ATG, a polyclonal T lymphocyte depleting antibody made in rabbits, has been associated with a decreased risk of acute rejection and increased survival particularly among high immunologic risk patients but has significant side effects including increased

opportunistic infections, thrombocytopenia, and leukopenia.[2,18-21] Alemtuzumab is a humanized monoclonal antibody that targets the cell surface marker CD52 resulting in the long-term depletion of T lymphocytes and a more transient depletion of B lymphocytes and monocytes.[22] Alemtuzumab has been shown to be effective when used as an induction agent in a steroid-free maintenance regimen.[23] In contrast to ATG and alemtuzumab, basiliximab is a non-depleting monoclonal antibody that inhibits T lymphocyte activation through the blockade of the cell surface receptor IL-2. Single-dose basiliximab has been shown to be an effective induction agent for low immunologic risk patients and has previously been associated with fewer infectious complications; however, it was associated with a higher incidence of acute rejection in moderate to high-risk recipients compared to ATG.[24-27]

Despite the fact that alemtuzumab was not associated with inferior graft outcomes or increased risk of rejection at 1 year, the increased incidence of dnDSA associated with alemtuzumab use in patients with no pre-transplant DSA remains a significant finding. Previous studies have demonstrated a strong association between dnDSA development and graft failure.[9,28] The delay between dnDSA production and resulting clinical manifestations such as proteinuria, elevated creatinine, or biopsy-proven rejection is likely a result of antibody damage through chronic repetitive injury to the allograft.[10,28-30] Therefore, sufficient time following antibody production is required prior to the manifestation of allograft injury or failure. The presence of dnDSA prior to clinical detection represents one explanation for no difference in outcomes among those who received alemtuzumab induction in our study.

Additionally, it is important to note the HLA class-specific antibodies that developed in each induction group due to the fact that not all dnDSA are equal. The development of HLA class II antibodies have previously been associated with a greater risk of AMR compared to HLA class I antibodies. In our cohort, 75% of patients who developed dnDSA in the alemtuzumab group formed HLA class I dnDSA compared to 55.5% of basiliximab and 78.6% of anti-thymocyte globulin groups. One explanation for preserved outcomes between groups may be that the increase in dnDSA in the Alemtuzumab group was primarily HLA class I. In addition to having the highest rate of dnDSA development, median sum MFI was not significantly lower in the alemtuzumab induction group. Although no difference in patient or graft survival was noted at 1 year, it is important to note that the HLA class I dnDSA that developed regardless of induction group could potentially impact long-term graft outcomes.

Our findings are supported by Todeschini et al. In this single-center matched-cohort study comparing alemtuzumab to combined low dose anti-thymocyte globulin/basiliximab, Todeschini et al. found alemtuzumab to be associated with a higher incidence of dnDSA, inferior graft function, and B lymphocyte phenotypic changes that correlated with dnDSA development. The authors hypothesized the B cell depletion with alemtuzumab led to a dysregulated re-population in the post-transplant follow-up period that was not seen in the anti-thymocyte globulin/basiliximab cohort and may be due to elevated BAFF levels in alemtuzumab treated patients. In this study, the development of dnDSA ultimately was found to be associated with worse long-term graft function. This association between dnDSA development and worse long-term graft function supports the concept that an extended period is required before the clinical

effects (ie graft failure) of dnDSA are evident.[31] Therefore, patients who develop dnDSA in the post-transplant period may require extended surveillance for graft injury or failure.[7]

Alemtuzumab has also frequently been associated with secondary autoimmune disease when given to patients with multiple sclerosis. The mechanism underlying this phenomenon is thought to be related to faster B lymphocyte than T lymphocyte recovery after alemtuzumab administration. The recovered B lymphocytes then allow for unregulated B lymphocyte expansion and antibody production in response to self-antigens. B lymphocyte re-population also coincided with increased serum BAFF, which has been seen in both transplant populations and in other B lymphocyte-related autoimmune disorders.[32-34] This mechanism in addition to the findings by Todeschini et al. further support the association of alemtuzumab and dnDSA development seen in our study.

It is important to note that alemtuzumab is often given with the intention of limiting maintenance immunosuppression that is administered. Specifically, steroid use is associated with numerous complications. In our study population, 87.4% of patients who received alemtuzumab and 22.5% of patients who received ATG as induction therapy were placed on an ESW protocol and therefore did not receive steroids as part of their maintenance immunosuppression regimen. When controlling for maintenance steroid use, the alemtuzumab group still developed dnDSA at the significantly higher rate of 13.7% compared to 5.3% in the ATG group at 1 year ($p<0.02$). No significant difference in tacrolimus trough levels were seen between groups, which remains an important finding and requires further investigation. These findings indicate that dnDSA

development in the alemtuzumab group likely cannot be attributed to lack of maintenance steroid use but may be impacted by tacrolimus trough levels.

Limitations

There are several limitations to address in this study. Although several patient factors are taken into consideration when choosing induction therapy in this low-risk group, the choice of induction therapy is ultimately up to the treating physician, which allows for varying degrees of selection bias. Although this study did not find Alemtuzumab to be associated with a significantly higher incidence of biopsy-proven rejection compared to other groups at 1 year, subclinical rejection and chronic AMR could still develop at varying rates between induction groups beyond this time period. Our current practice is for patients who develop dnDSA to undergo a protocol kidney biopsy; therefore, patients who have developed rejection after the development of dnDSA in this cohort have been captured. Lastly, this study is limited by the inherent biases associated with retrospective studies.

Conclusions

The widespread use of induction therapy has resulted in significantly reduced rates of rejection in kidney transplant, which ultimately is associated with improved graft survival. Various induction therapies and subsequent maintenance regimens are each associated with potential risks and benefits when used in particular patient populations. Specifically, special attention must be given to patients with low and high immunologic risk for rejection. In a low risk patient population, we have demonstrated an increased risk of de novo donor specific antibody production without an association of inferior graft outcomes including biopsy-proven rejection and graft failure with

alemtuzumab when compared to basiliximab and anti-thymocyte globulin. Additionally, anti-thymocyte globulin was associated with increased risk of CMV viremia. Despite the overall equivalent outcomes between induction groups, the association of dnDSA development with alemtuzumab induction therapy may warrant increased surveillance in this patient population. Further studies examining long-term follow-up are required.

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Author Contributions:

N Bath: Data curation; Formal analysis; Investigation; Writing - original draft; Writing - review and editing

A Djamali: Conceptualization; Methodology; Resources; Supervision; Writing - review and editing

S Parajuli: Methodology; Supervision; Writing - original draft; Writing - review and editing

D Mandelbrot: Conceptualization; Formal analysis; Methodology; Writing - review and editing

G Leverson: Formal analysis; Methodology; Writing - review and editing

LG Hidalgo: Formal analysis; Validation; Writing - review and editing

T Ellis: Investigation; Writing - review and editing

J Descourouez: Investigation; Methodology; Writing - review and editing

M Jorgenson: Data curation; Investigation; Writing - review and editing

D Hager: Data curation; Investigation; Writing - review and editing

D Kaufman: Conceptualization; Investigation; Methodology; Supervision; Writing - review and editing

R Redfield: Conceptualization; Investigation; Methodology; Supervision; Writing - original draft; Writing - review and editing

Supplemental Material

Supplemental Table 1. Risk of kidney graft survival. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 2. Risk of patient survival. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 3. Risk of rejection. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 4. Risk of antibody mediated rejection. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 5. Risk of acute cellular rejection. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 6. Risk of CMV viremia. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 7. Risk of BK >1,000 copies/mL. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

Supplemental Table 8. Risk of BK >10,000 copies/mL. DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody.

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Tables

Table 1. Baseline characteristics of recipients.

Characteristic, recipient (Total N = 782)	Alemtuzumab, N = 87 (%)	Basiliximab, N = 522 (%)	ATG, N = 173 (%)	*P value*
Gender • Male • Female	 59 (67.8) 28 (32.2)	 356 (68.2) 166 (31.8)	 109 (63.0) 64 (37.0)	0.45
Race • White • Black • Other	 71 (81.6) 7 (8.1) 9 (10.3)	 395 (75.7) 65 (12.4) 62 (11.9)	 123 (71.1) 34 (19.7) 16 (9.2)	0.01
Age at transplant, mean (range) years	47.9 (18.6-69.6)	54.9 (20.8-81.4)	51.2 (18.8-73.0)	<0.0001
BMI, mean (range)	29.2 (19.2-40.4)	28.6 (16.3-47.6)	28.3 (16.3-40.8)	0.44
Blood transfusion	25 (28.7)	229 (44.0)	86 (50.0)	<0.02
Previous kidney transplant	8 (9.2)	42 (8.0)	38 (22.0)	<0.0001
Pre-transplant dialysis, months	21.6	26.6	26.0	0.75
End cPRA, mean ± SD	7.7 ± 22.6	12.6 ± 27.0	24.7 ± 37.6	<0.0001
HLA mismatch, mean ± SD	4.0 ± 1.5	3.9 ± 1.5	3.8 ± 1.7	0.69
Belatacept	21 (24.1)	1 (0.2)	17 (9.8)	<0.0001
Early steroid withdrawal	76 (87.4)	5 (1.0)	39 (22.5)	<0.0001

BMI, body mass index; PRA, panel reactive antibody; HLA, human leukocyte antigen

Table 2. Baseline characteristics of donors.

Characteristic, donor (Total N = 782)	Alemtuzumab, N = 87 (%)	Basiliximab, N = 522 (%)	ATG, N = 173 (%)	*P* value
Gender				0.26
• Male	41 (47.1)	294 (56.3)	92 (53.2)	
• Female	46 (52.9)	228 (43.7)	81 (46.8)	
Race				0.86
• White	80 (92.0)	475 (91.0)	154 (89.0)	
• Black	2 (2.3)	19 (3.6)	5 (2.9)	
• Other	5 (5.7)	28 (5.4)	14 (8.1)	
Donor type				0.0006
• Live	47 (54.0)	177 (33.9)	57 (33.0)	
• DBD	26 (29.9)	250 (47.9)	71 (41.0)	
• DCD	14 (16.1)	95 (18.2)	45 (26.0)	
Age at donation, mean (range) years	43.3 (6.0-69.0)	44.4 (1.0-76.0)	42.7 (4.0-74.0)	0.34
BMI, mean (range)	27.8 (15.1-59.9)	28.0 (12.2-60.6)	28.7 (12.7-63.3)	0.46
KDPI, % ± SD	41.3 ± 28.2	50.7 ± 25.9	48.7 ± 27.6	0.10
CIT, mean (range) hours	12.9 (1.0-27.2)	14.9 (0.5-34.7)	15.6 (1.0-40.9)	0.06
Deceased donor only CIT, mean (range) hours	15.6 (6.7-27.2)	15.6 (4.5-34.7)	16.7 (2.5-40.9)	0.25

BMI, body mass index; KDPI, kidney donor profile index; CIT, cold ischemia time.

Table 3. Comparison of outcomes by induction group.

Variable	Alemtuzumab, % (N)	Basiliximab, % (N)	ATG, % (N)	*P value*
Development of dnDSA at 1 year	13.8 (12)	5.2 (27)	8.1 (14)	0.0009
Graft survival at 1 year	100	98.2	98.8	0.81
Rejection at 1 year				
• AMR	0.0	0.4	1.8	0.64
• ACR	5.9	4.5	4.6	0.66
CMV viremia at 1 year	36.6	22.3	38.4	0.0003
BK viremia at 1 year				
• BK >1000	20.9	25.3	23.6	0.45
• BK >10000	15.3	16.7	12.4	0.24
Delayed graft function	3.5	8.4	5.8	0.18
Length of hospital stay, mean ± SD, days	4.3 ± 1.7	5.1 ± 3.0	5.1 ± 2.1	0.02

dnDSA, de novo donor specific antibody; AMR, antibody mediated rejection; ACR, acute cellular rejection; DGF, delayed graft function

Table 4. Risk of development of dnDSA.

Variable	Multivariate Analysis	
	HR (95% CI)	***P value***
Induction group		
• Anti-thymocyte globulin	1	
• Alemtuzumab	4.2 (1.57-11.04)	0.004
• Basiliximab	1.2 (0.67-2.26)	0.50
Race		
• White	1	
• Black	2.4 (1.42-4.02)	0.001
• Other	1.6 (0.89-3.02)	0.12
Donor type		
• Live	1	
• DBD	0.9 (0.53-1.58)	0.75
• DCD	1.7 (0.95-3.02)	0.07
Age at transplant, years	0.98 (0.96-0.99)	0.01
End cPRA	1.0 (0.99-1.01)	0.94
Transplant to discharge, days	1.1 (1.00-1.14)	0.045
Maintenance steroids	1.7 (0.59-4.59)	0.34
Belatacept	1.6 (0.61-4.19)	0.34

DBD, donation by brainstem death. DCD, donation by cardiac death. cPRA, calculated panel reactive antibody

Table 5. Alemtuzumab is associated with greatest risk of dnDSA development.

Variable	Multivariate Analysis	
	HR (95% CI)	***P value***
Alemtuzumab	2.5 (1.51-4.25)	0.0004
Black race	2.3 (1.44-3.77)	0.0006
Age at transplant (years)	0.98 (0.96-0.99)	0.0043
Transplant to discharge (days)	1.1 (1.01-1.14)	0.0332

Table 6. Comparison of outcomes in patients who developed dnDSA by induction group.

Variable	Alemtuzumab, % (N=12)	Basiliximab,% (N=27)	ATG, % (N=14)	*P value*
Development of dnDSA at 1 year	13.8 (12)	5.2 (27)	8.1 (14)	0.0009
Sum MFI of dnDSA at first test, median (interquartile range)	1179 (640.3-2335)	2264 (1231-8252)	2138 (752.3-5642)	0.27
Number of DSA contributing to sum MFI, mean ± SD	1.6 ± 0.9	2.1 ± 1.9	2.0 ± 1.6	0.70
Days to development of dnDSA, mean ± SD	172.1 ± 112	198.6 ± 95.3	163.2 ± 104.9	0.53
Steroid status • Early steroid withdrawal • Maintenance steroids	 83.3 (10) 16.7 (2)	 0.0 (0) 100.0 (27)	 14.3 (2) 85.7 (12)	 <0.0001
Graft survival at 1 year	100.0 (12)	96.3 (26)	100.0 (14)	0.61
Graft survival overall	75.0 (9)	92.6 (25)	92.9 (13)	0.24
Rejection at 1 year • AMR • ACR	 16.7 (2) 41.7 (5)	 14.8 (4) 29.6 (8)	 14.3 (2) 21.4 (3)	 0.98 0.53
Rejection overall • AMR • ACR	 16.7 (2) 41.7 (5)	 18.5 (5) 33.3 (9)	 14.3 (2) 21.4 (3)	 0.94 0.53

Figures and legends

Fig 1. Study flowchart of kidney transplant population. Patients were excluded if DSA was not tested for pre-transplant or no induction agents were used. DSA, donor specific antibody. ESW, early steroid withdrawal

Fig 2. Kaplan-Meier curves comparing the development of de novo DSA by induction group. Table under the graphic indicates the number of patients remaining in each induction group over time. DSA, donor specific antibody

Fig 3. Development of dnDSA by class in induction group.

Fig 4. Kaplan-Meier curves comparing the development of de novo DSA in patients who received early steroid withdrawal. Table under the graphic indicates the number of patients remaining in each induction group over time.

Fig 5. Kaplan-Meier curves comparing the incidence of (A) AMR and (B) ACR by induction group. AMR, antibody mediated rejection. ACR, acute cellular rejection. Table under the graphic indicates the number of patients remaining in each induction group over time.

Fig 6. Kaplan Meier curves comparing the development of (A) CMV viremia, (B) BK viral load >1,000 copies/mL, and (C) BK viral load >10,000 copies/mL by induction group.

Table under the graphic indicates the number of patients remaining in each induction group over time.

Fig 7. MFI trend of immunodominant DSA in patients who developed rejection. Immunodominant DSA was defined as the DSA with the highest MFI value when first detected as positive. Each line represents one patient who developed rejection. (A) Alemtuzumab (N=7), (B) Basiliximab (N=10), (C) ATG (N=4).

Figure 1. Flow chart of kidney transplant population

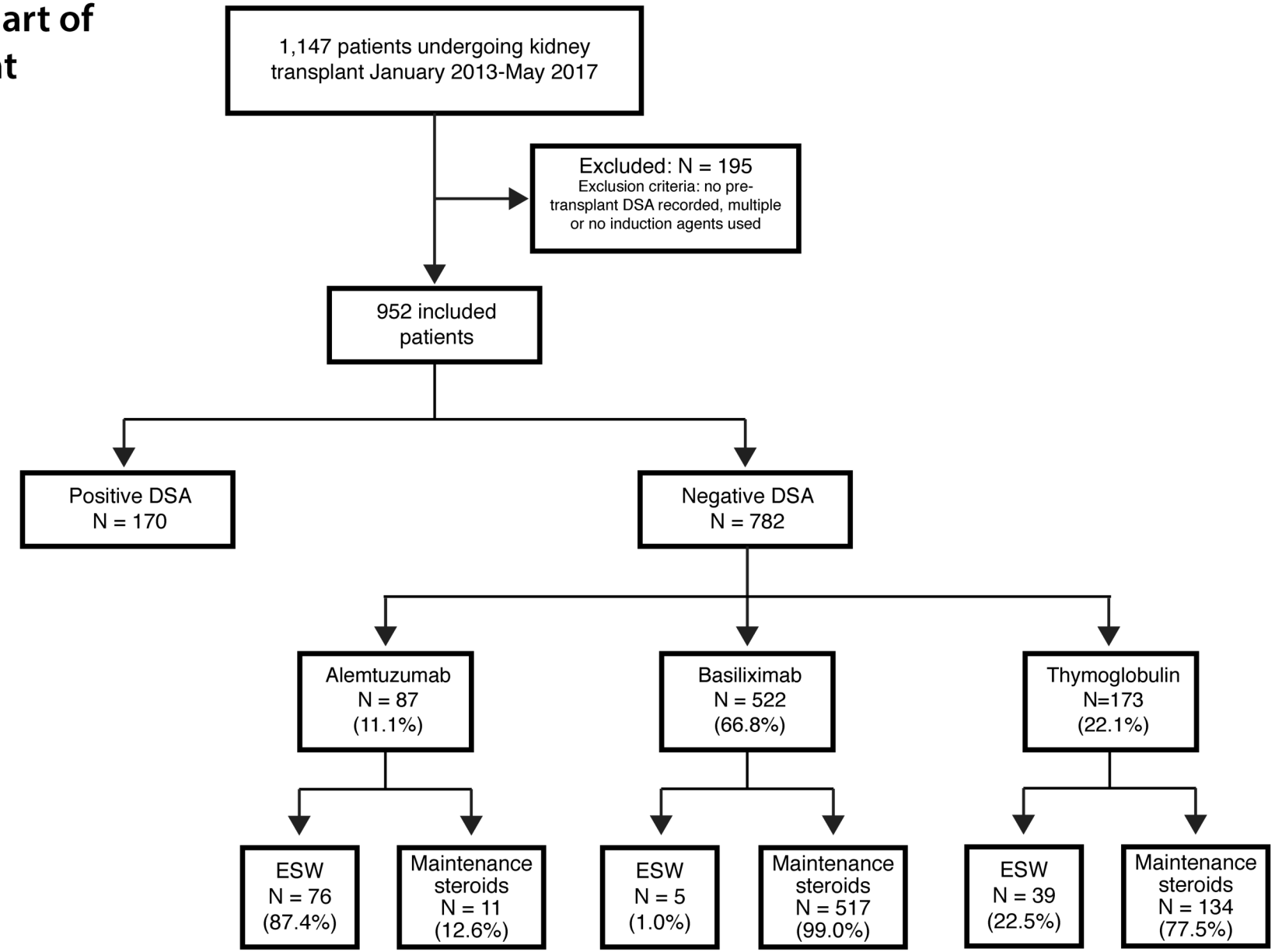
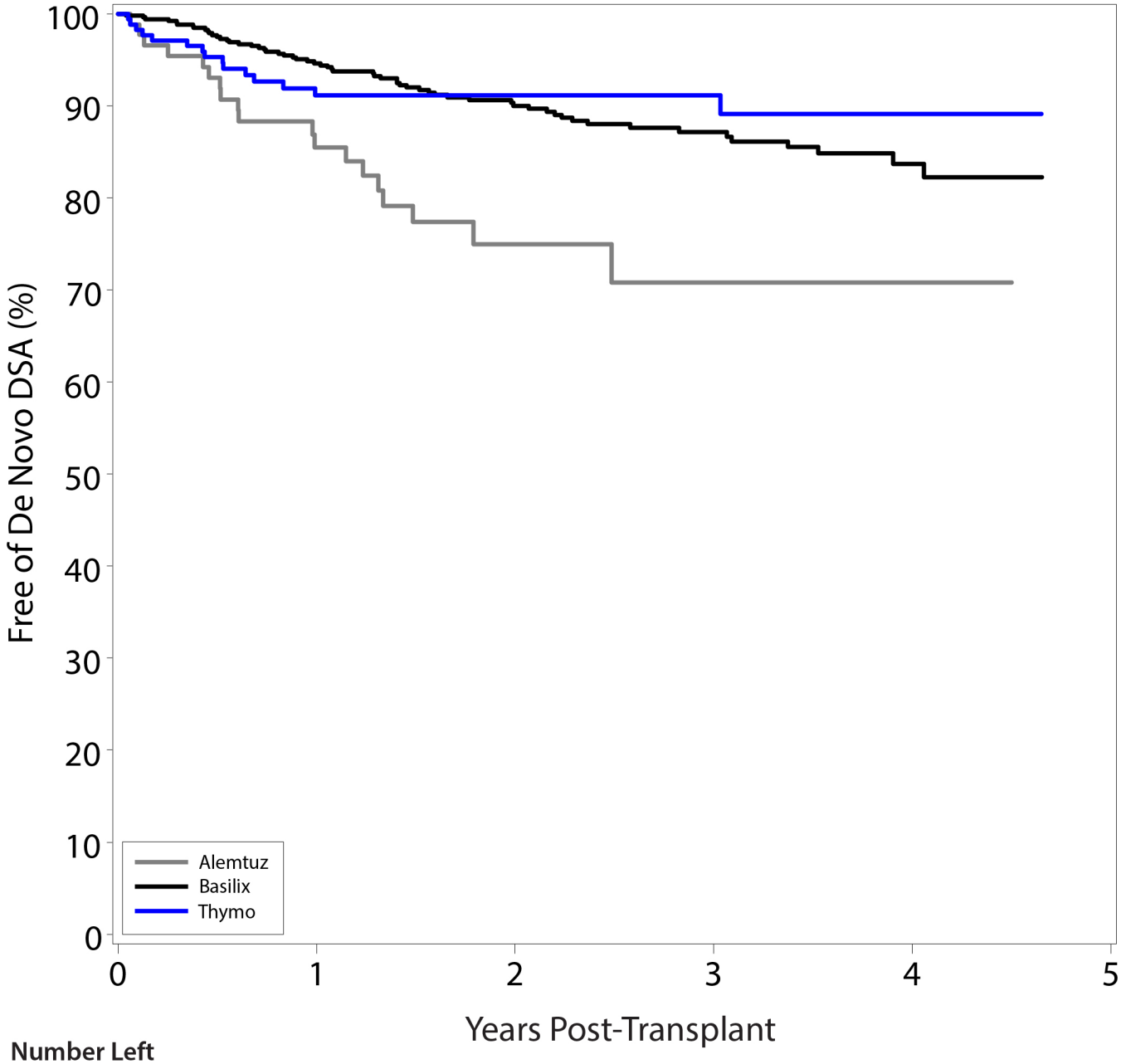


Figure 2. Kaplan-Meier curves for development of de novo DSA by induction group



Number Left

Alemtuz	87	59	26	12	5
Basilix	522	439	290	180	62
Thymo	173	117	82	48	12

Figure 3. Development of dnDSA by HLA Class

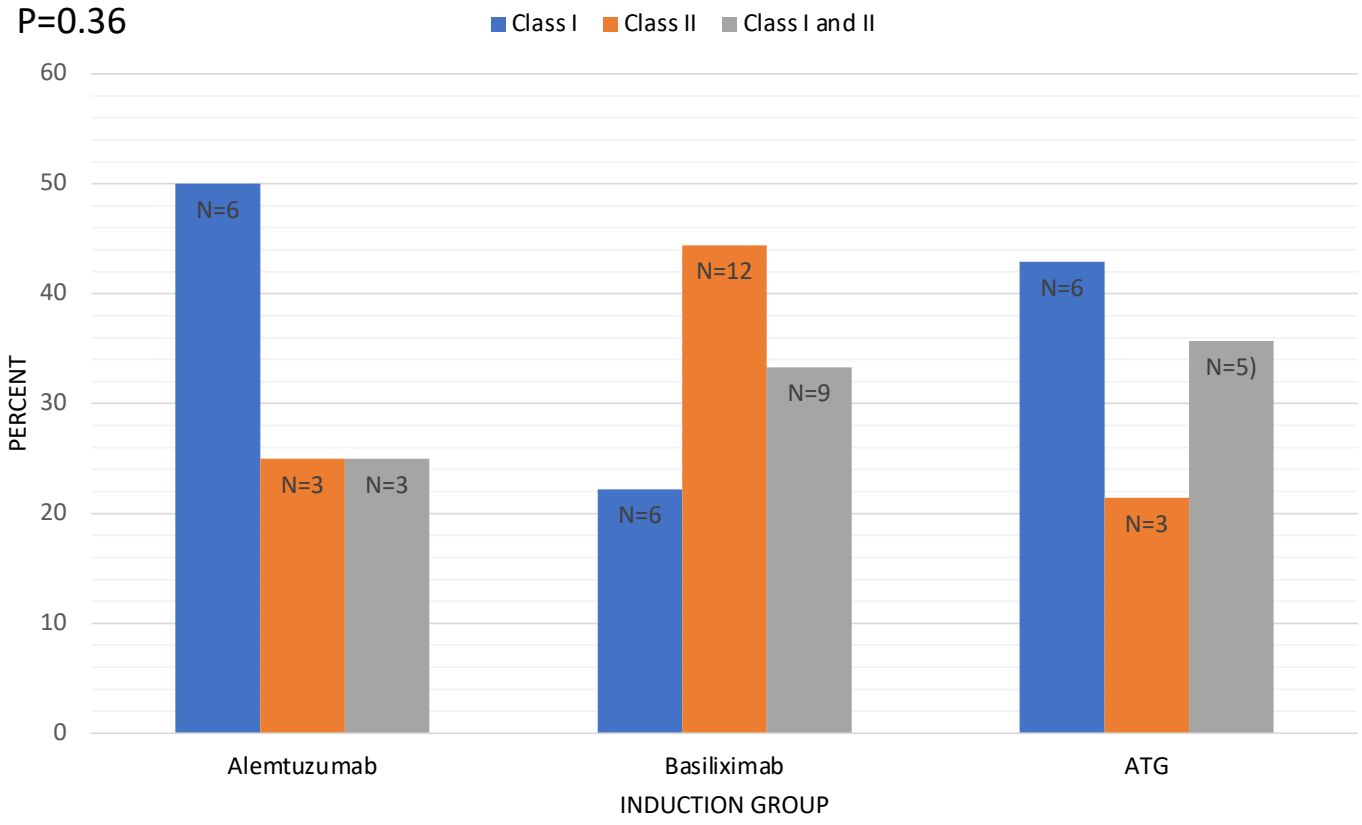
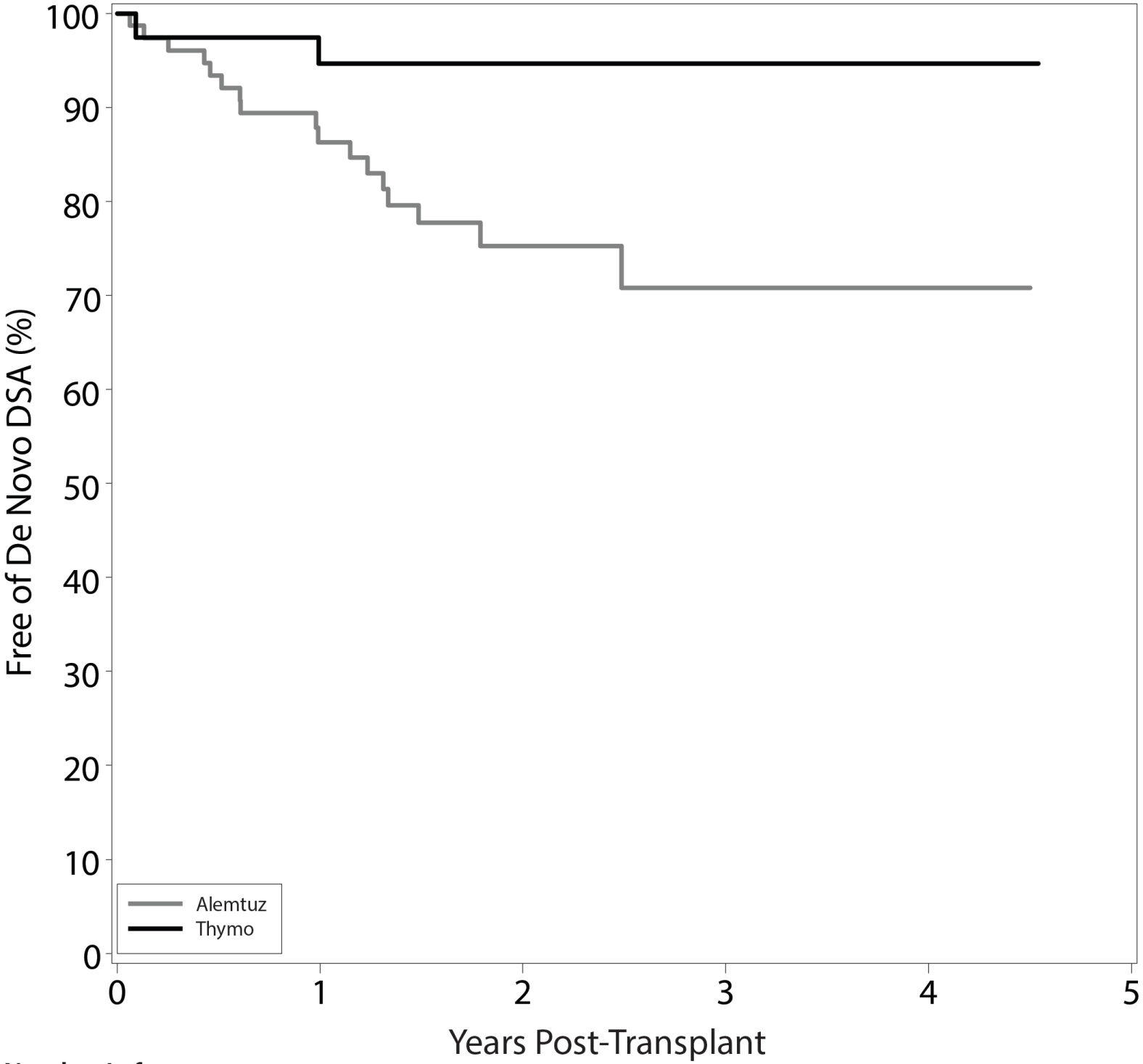


Figure 4. Kaplan-Meier curves for the development of dnDSA in patients with early steroid withdrawal



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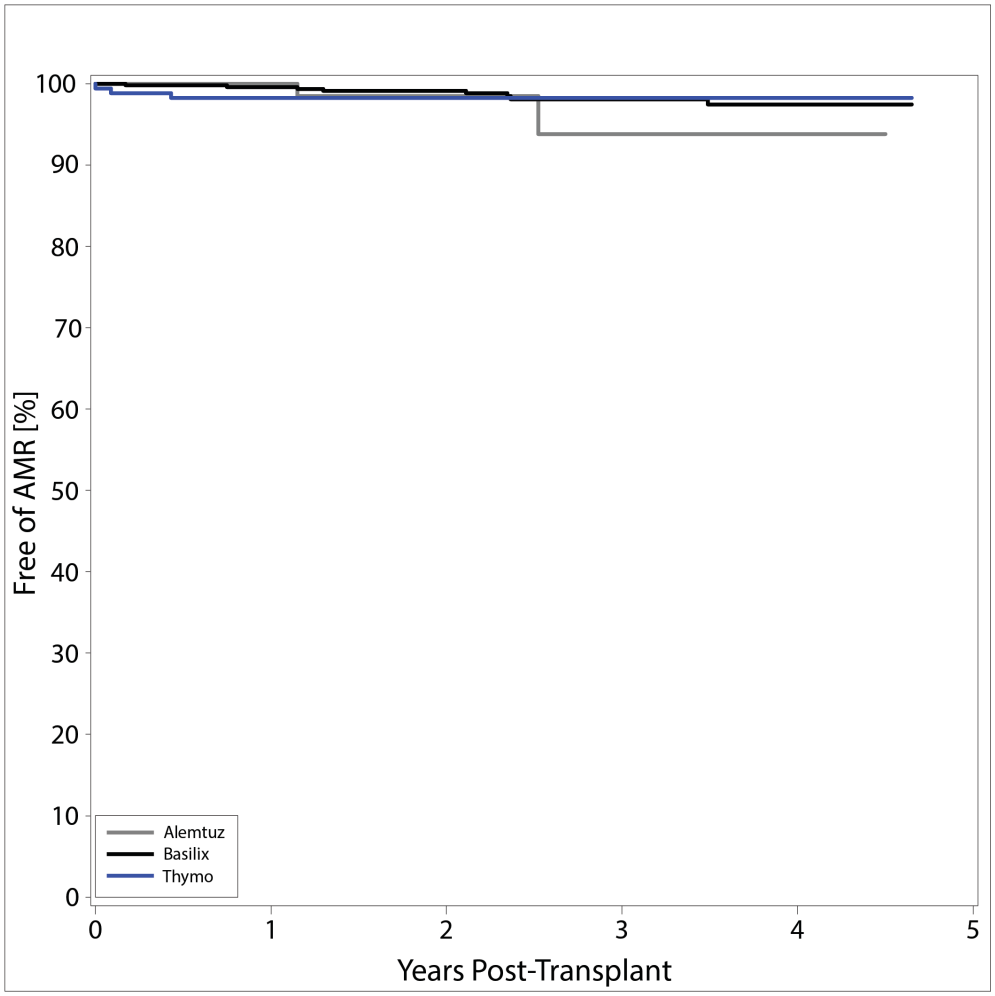
Alemtuz	76	55	25	12	5
Thymo	39	34	26	15	4

Figure 5. Kaplan-Meier curves for (A) AMR and (B) ACR by induction group

A



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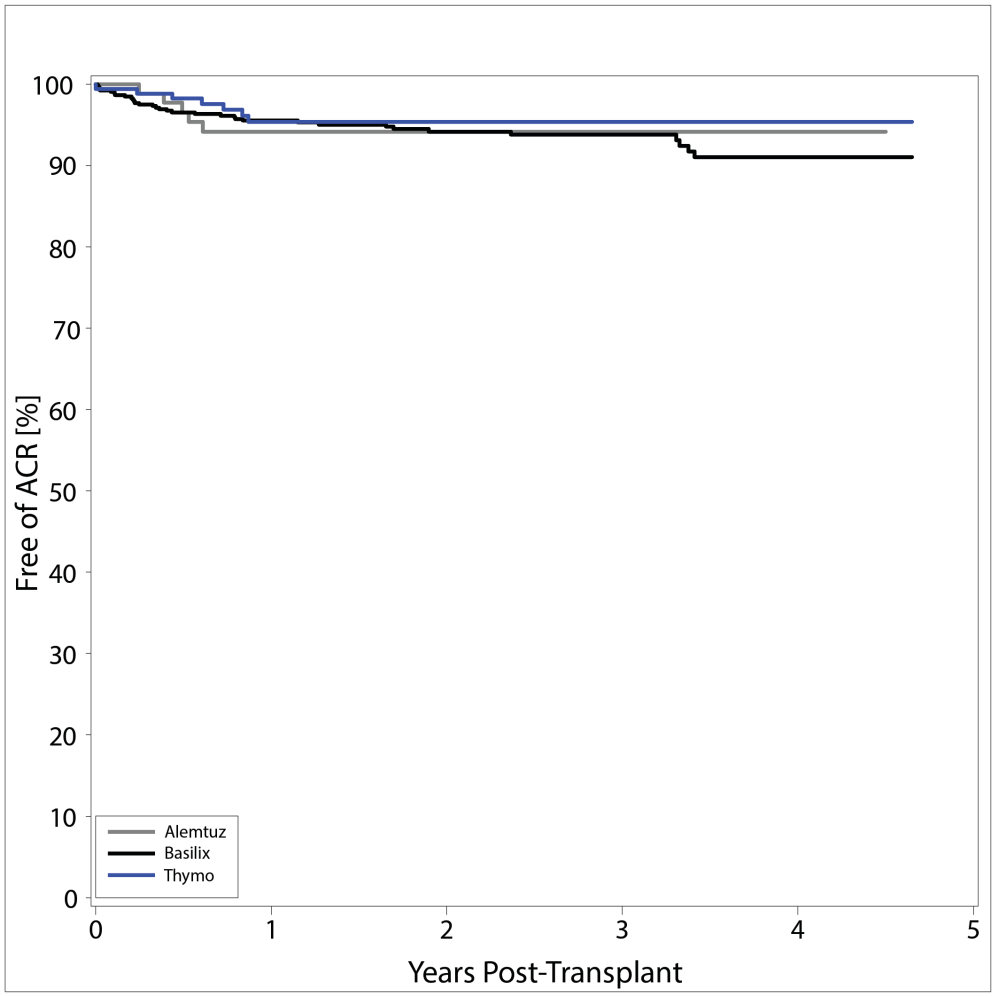
Alemtuz	87	67	32	13	5
Basilix	522	461	320	193	66
Thymo	173	124	87	49	11

B

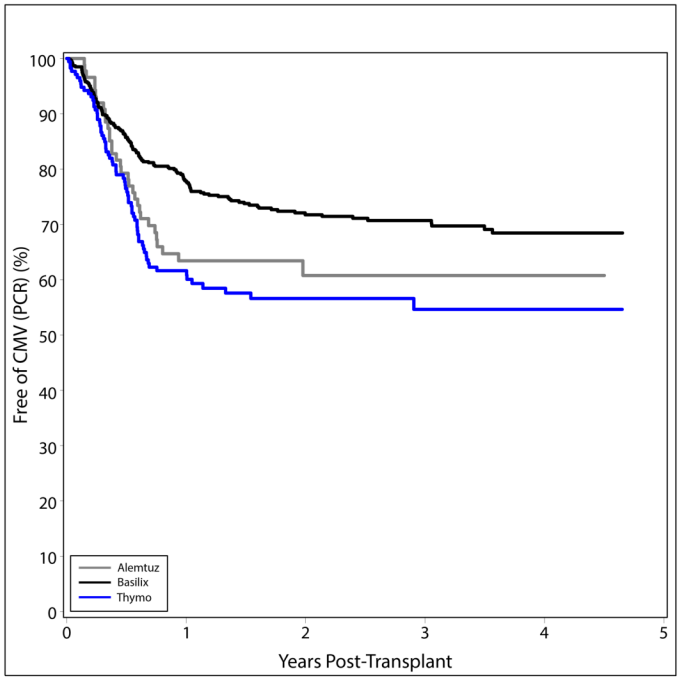


Number Left

Alemtuz	87	62	28	11	5
Basilix	522	440	296	174	59
Thymo	173	120	83	45	9

Figure 6. Kaplan-Meier curves for (A) CMV, (B) BK >1000, (C) BK >10000

A



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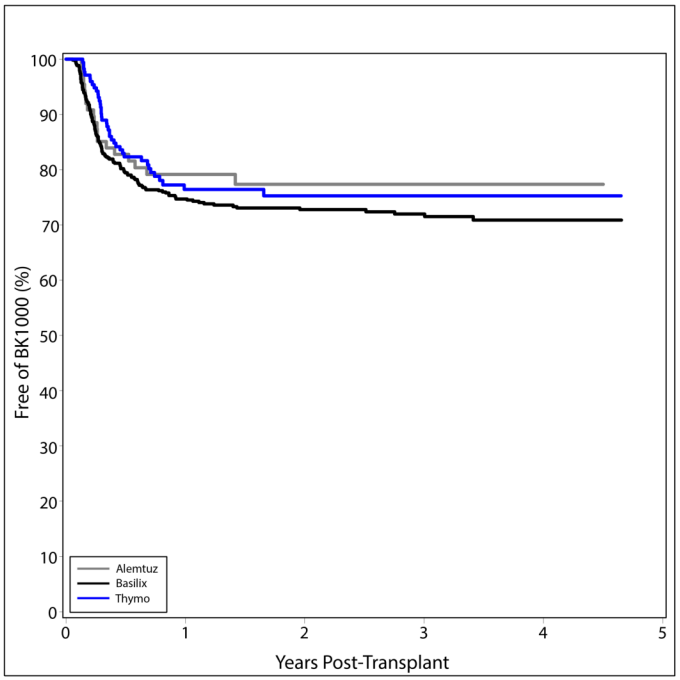
Alemtuz	87	46	23	10	5
Basilix	522	358	233	150	54
Thymo	173	81	53	25	7

B



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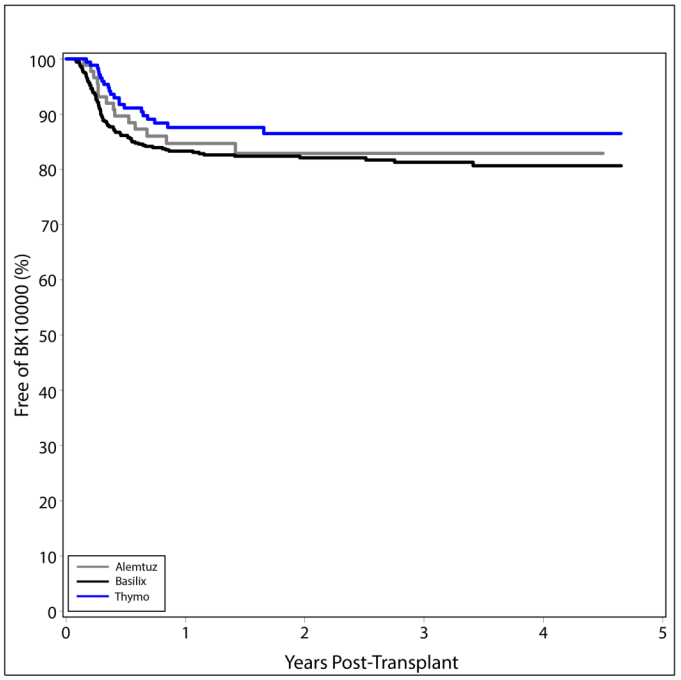
Alemtuz	87	51	24	12	6
Basilix	522	346	239	146	50
Thymo	173	93	59	31	7

C



Number Left

Alemtuz	87	56	27	14	6
Basilix	522	386	265	165	54
Thymo	173	109	72	43	9

Figure 7. MFI of immunodominant DSA in patients who developed rejection
(A) Alemtuzumab, (B) Basiliximab, (C) ATG