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Comparison of Benefits and Risks Associated with Anti-T-Lymphocyte Globulin (ATLG) Serotherapy in Methotrexate (MTX)- versus Mycophenolate Mofetil (MMF)-Based Hematopoietic Stem Cell Transplantation

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Abstract: Background: Serotherapy with anti-T lymphocyte globulin (ATLG, Grafalon, formerly ATG-Fresenius) is established for the prevention of severe graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (HSCT). The evidence from prospective studies is predominantly derived from a setting where methotrexate (MTX) and a calcineurin inhibitor (CNI) are used as the backbone of GVHD prophylaxis. The efficacy of ATLG in combination with CNI and mycophenolate mofetil (MMF) has not been investigated as much, particularly in terms of a direct comparison with its effects when combined with CNI/MTX. A total of 401 HSCTs from two Austrian transplant centers were retrospectively evaluated. We included peripheral blood transplants from early- or intermediate-stage (excluding advanced/refractory) hematological diseases from matched siblings or 10/10 or 9/10 matched unrelated donors with CNI/MTX or CNI/MMF prophylaxis, either without (n = 219) or with ATLG (n = 182). Overall, ATLG significantly reduced the risk for all-cause mortality by multivariate Cox analysis (HR 0.53; p = 0.002). Stratification by postgrafting prophylaxis type revealed a significant survival advantage for ATLG in the CNI/MMF cohort (HR 0.49; p = 0.001; n = 193), while its effect on survival in the CNI/MTX cohort was not significant (HR 0.87; p = 0.56; n = 208). In unrelated HSCT with CNI/MMF prophylaxis, ATLG exhibited its greatest survival benefit (HR 0.34; p = 0.001; n = 104). In the context of CNI/MMF, ATLG may provide even greater benefits than in the setting of CNI/MTX for post-grafting immunosuppression. Future prospective studies on ATLG should therefore focus on CNI/MMF-based transplants, which are widely performed in elderly or comorbid patients not expected to tolerate a standard course of MTX.

Keywords: ATLG; MTX; MMF; HSCT



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1. Introduction

The rabbit-derived anti-human T-lymphocyte globulin (ATLG, Grafalon, formerly ATG-Fresenius/ATG-F), represents, besides ATG (anti-thymocyte globulin, Thymoglobulin), one of two T cell directed serotherapeutic preparations licensed in Europe for the prevention of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). ATLG's effectiveness and safety have been demonstrated in three prospective, randomized controlled trials, two in HSCT from unrelated donors, at a total dose of 60 mg/kg body weight [1,2], and one in HSCT from HLA-matched sibling donors, using a dose of 30 mg/kg [3]. All studies applied myeloablative pre-transplant conditioning,

and patients received standard post-grafting GVHD prophylaxis with cyclosporin A (CsA) and methotrexate (MTX). Likewise, ATG (Thymoglobulin) was prospectively evaluated exclusively [4,5], and predominantly [6], in the myeloablative HSCT setting with an MTX-based post-grafting GVHD prophylaxis regimen. Data on the use of both ATLG and ATG in reduced-intensity conditioning (RIC) HSCT, and particularly in the MTX-free setting, are primarily limited to retrospective studies [7–12]. In RIC transplants, mycophenolate mofetil (MMF), instead of MTX, is frequently used as an antimetabolite agent combined with the calcineurin inhibitor (CNI). Of note, in several published transplant series, ATG entirely replaced the antimetabolite component (MTX or MMF) of GVHD prophylaxis, i.e., it was used with CsA alone in the case of matched sibling HSCT [11,12]. These retrospective studies have demonstrated the feasibility and effectiveness of anti-T cell serotherapy for the prevention of GVHD after RIC HSCT, and thus may dispel concerns about a higher relapse risk associated with ATG or ATLG in RIC compared to myeloablative (MAC) HSCT, at least if low to intermediate doses of ATG/ATLG are applied [8,10,13]. Neither of the studies have primarily addressed the impact of ATG/ATLG according to conditioning intensity, and even less is known regarding the impact of ATG/ATLG in the intensification of GVHD prophylaxis with CNI/MMF compared to CNI/MTX. A differential impact of ATG/ATLG pretreatment according to the given post-grafting prophylactic regimen may be assumed, since studies have suggested a more potent GVHD-preventive effect for MTX in comparison to MMF, particularly in HSCT from unrelated donors [14–17]. Therefore, with the present large retrospective, bi-centric study, we aimed to investigate as to whether *in vivo* T cell depletion with ATLG has a differential impact on major HSCT outcomes when given in the CNI/MMF setting compared to CNI/MTX, assuming an at least comparable clinical benefit conferred by ATLG in the MMF versus the MTX setting. In contrast to a previous study [8], which included serotherapy with ATG and alemtuzumab in addition to ATLG, the present study was restricted to ATLG (Grafalon) for the purpose of homogeneity.

2. Methods

2.1. Patients and Treatment

This retrospective study was performed to address whether there is a differential impact of ATLG on the major HSCT outcomes, overall mortality, non-relapse mortality (NRM), severe acute GVHD (aGVHD), aGVHD-associated NRM, moderate/severe chronic GVHD (cGVHD), and relapse of the underlying malignancy in HSCTs with CNI/MTX versus CNI/MMF-based post-grafting immunosuppression. Therefore, data from 401 consecutive allogeneic (fully or partially HLA-matched) HSCTs for hematological diseases in adult patients at two Austrian EBMT centers (EBMT-CIC 271-Innsbruck, and EBMT-CIC 594-Linz) were analyzed. Only the first HSCTs for hematological indications in early/intermediate disease phase were included (no active leukemia or other uncontrolled malignancy). In addition, HSCTs using post-grafting immunosuppression other than CNI/MTX or CNI/MMF were excluded (e.g., post-transplant cyclophosphamide- or sirolimus-based immunosuppression). We also excluded haploidentical grafts, *ex vivo* T cell-depleted grafts, and cord blood grafts. Transplants were performed between 1996 and 2018, with the majority of transplants (96%) performed later than 1 January 2000; the median year of HSCT was 2010. The median follow-up of the surviving patients was 5.4 years (range, 0.4–20.7 years). The graft source was G-CSF-mobilized PB in all cases. In accordance with the Declaration of Helsinki, all patients provided written informed consent to the treatment and to data collection and analysis.

This study obtained written consent from all patients in accordance with the Declaration of Helsinki and analyzed major patient, disease, and transplant details outlined in Table 1. Tables S1 and S2 summarize the cohort characteristics by ATLG use for the groups CNI/MMF (Table S1) and CNI/MTX (Table S2). Administration of ATLG typically involved two (up to three) doses, with the last infusion given the day before transplant. To prevent allergic reactions and fever, patients were given high-dose steroids, antihistamines, and 1 g metamizole before each ATLG infusion. The infusion was administered over 12 h

or 6 h per day with the continuous monitoring of circulatory and respiratory functions. Prior to 2016, ATLG was primarily used for unrelated donor transplants, but from 2016 onwards it was also used in the majority of matched, related transplants.

Table 1. Cohort Characteristics.

Patients and Transplant Details	All	CNI/MMF	CNI/MTX	<i>p</i> -Value
Total number of transplants (n)	401	193	208	
Recipient age at Tx years (median/range)	48.6 (17.0–73.0)	53.25 (18.0–73.0)	42.75 (17.0–69.0)	<0.001
Diagnosis (%)				
AML	192 (47.9%)	86 (44.6%)	106 (51.0%)	
MDS, MPN, and MDS-MPN overlap syndromes	82 (20.4%)	41 (21.2%)	41 (19.7%)	
ALL	72 (18.0%)	35 (18.1%)	37 (17.8%)	
Lymphoma, Myeloma, BPDCN	45 (11.2%)	25 (13.0%)	20 (9.6%)	
Non-malignant	10 (2.4%)	6 (3.1%)	4 (1.9%)	0.69
Disease stage (%)				
Early	211 (52.6%)	86 (44.6%)	125 (60.1%)	
Intermediate	190 (47.4%)	107 (55.4%)	83 (39.9%)	0.002
Median year of HSCT (median/range)	2010 (1996–2018)	2011 (1999–2018)	2007 (1996–2018)	
Median follow-up of survivors years (median/range)	5.4 (0.4–20.7)	65.3 (4.5–189.0)	64.2 (4.4–248.5)	0.72
Conditioning (%)				
myeloablative (full intensity MAC or RTC)	248 (61.8%)	70 (36.3%)	178 (85.6%)	
reduced intensity (RIC or NMA)	153 (38.2%)	123 (63.7%)	30 (14.4%)	<0.001
Donor (%)				
matched sibling	228 (56.9%)	89 (46.1%)	139 (66.8%)	
unrelated (9/10 or 10/10 matched)	173 (43.1%)	104 (53.9%)	69 (33.2%)	<0.001
Graft source (%)				
G-CSF mobilized peripheral blood	401 (100%)	193 (100.0%)	208 (100.0%)	
Anti-T cell Serotherapy (%)				
Yes (ATLG)	182 (45.4%)	118 (61.1%)	64 (30.8%)	
No	219 (54.6%)	75 (38.9%)	144 (69.2%)	<0.001
ATLG dose in mg/kg (median/range)	35 (15–60)	30 (15–60)	35 (15–60)	0.08

Table 1. Cont.

Patients and Transplant Details	All	CNI/MMF	CNI/MTX	<i>p</i> -Value
Donor/Recipient sex matching (%)				
female donor to male recipient	94 (23.4%)	43 (22.3%)	51 (24.5%)	
Other	307 (76.6%)	150 (77.7%)	157 (75.5%)	0.64

AML, acute myeloid leukemia; MPN, myeloproliferative neoplasia; MDS, myelodysplastic syndromes; ALL, acute lymphoblastic leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; MAC, myeloablative conditioning; RTC, reduced toxicity conditioning; RIC, reduced-intensity conditioning; NMA, non-myeloablative conditioning; G-CSF, granulocyte-colony-stimulating factor; ATLG, anti-human T-lymphocyte globulin; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate.

Post-grafting immunosuppression consisted of CNI (in the majority of cases CsA, initial trough level 200–300 ng/mL) combined with either MTX (15 mg/m² on day + 1, 10 mg/m² on days +3, +6, +11; day +11 MTX was omitted in the case of relevant toxicities such as active infection or severe mucositis), or with MMF (MMF dose was either 1000 mg 2 times per day, 1000 mg 3 times per day, or 12.5 mg/kg 3 times per day). MMF was routinely discontinued on day + 35 or day + 56 in the absence of GVHD.

2.2. Definitions and Grading

In this study, overall survival (OS) and progression-free survival (PFS) were defined as the time from transplant to the last follow up without death or without prior death or relapse. Non-relapse mortality (NRM) was termed as the last-follow up without prior relapse and was considered for cases both with and without acute GVHD grade II–IV. Acute GVHD is graded according to Glucksberg or modified Glucksberg criteria, [18], while chronic GVHD is graded as limited or extensive according to Seattle criteria, or as mild, moderate, or severe according to the NIH consensus [19]. The distinction between myeloablative and reduced intensity conditioning was used as published before [20]. Disease stage was categorized as early- or low-risk in cases of malignancies in the first complete remission/CR, and as non-malignant disease or low-risk MDS or MPN Intermediate risk was categorized as malignancies in their second remission or MDS/MPN intermediate risk. This study did not include transplant cases for advanced diseases such as active and uncontrolled leukemia, high/very-high-risk MDS or MPN as determined by (IPSS, DIPSS/DIPSS plus), or malignancies that relapsed after a previous allogeneic HSCT.

R statistics and the packages survival, riskRegression, cmprsk, cowplot, survminer, finalfit, and ggplot2 were used for the statistical analyses and plot creation [21–28]. This study used the Kaplan–Meier method to estimate survival probabilities and the log-rank test to compare survival curves for OS and PFS. Cumulative incidences for relapse (or progression), non-relapse mortality (NRM), acute graft-versus-host disease grade III–IV (aGVHD), and chronic graft-versus-host disease (cGVHD) were calculated considering competing risks. Multivariate analyses were performed for OS and PFS using the Cox proportional hazards model, including variables such as age of donor or recipient, serotherapy (ATLG), disease stage, HLA mismatch (matched versus partially matched), related versus unrelated donors, sex mismatch, and the conditioning intensity (MAC versus RIC). Sub-hazard ratios were calculated for endpoints with competing risks using the multivariable Fine and Gray regression. In multivariate models, covariates were stepwise excluded until all variables were *p*-value ≤ 0.2, and variables of interest were left in the model independent of their *p*-values.

3. Results

3.1. Overall Survival by ATLG in the MMF versus MTX Setting

In the entire cohort, the 3 year survival probability after ATLG-based transplants was 66.3% (95% confidence interval [95%CI], 59.7–73.5%; n = 182), while after non-ATLG-based HSCT, it was 59.3% (95%CI, 53.1–66.4%, n = 219; log-rank $p = 0.04$; Figure 1A). By multivariable Cox proportional hazards analysis, ATLG was significantly associated with a reduced risk for death (hazard ratio (HR), 0.53, $p = 0.002$; Table 2).

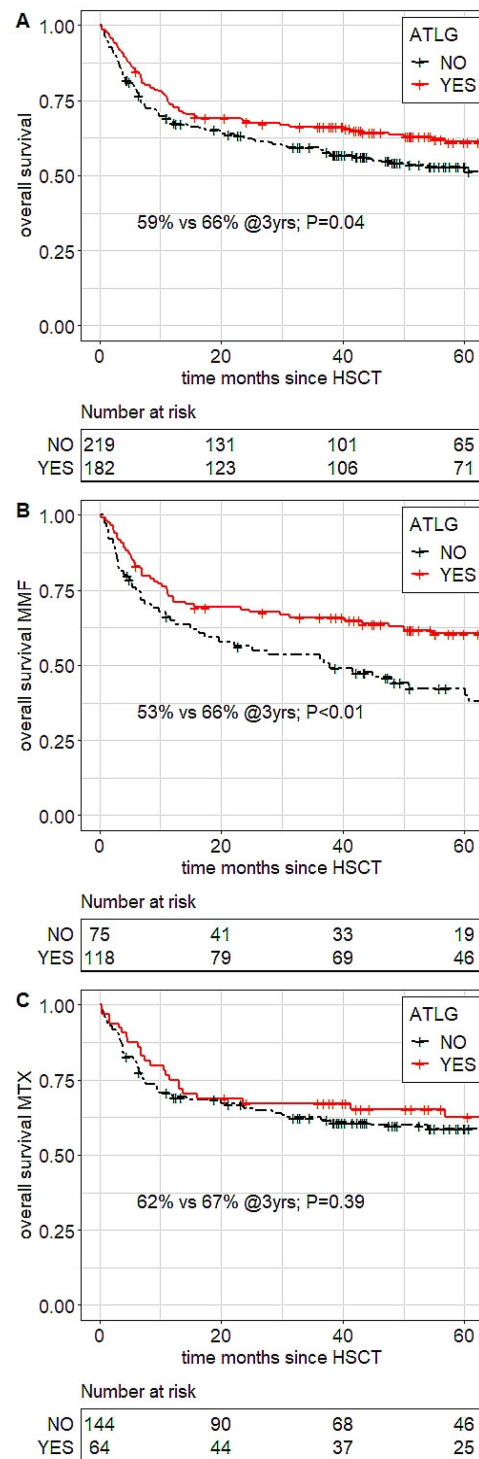


Figure 1. OS by ATLG treatment. Red line, ATLG; black line, no ATLG. (A) All HSCT; (B) MMF-based HSCT. (C) MTX-based HSCT.

Table 2. Multivariable analyses for the adjusted effect of ATLG on various endpoints in the overall cohort (n = 401), as well as in the MMF (n = 193) and the MTX (n = 208) subgroup, respectively. For overall mortality, the Cox proportional hazards model was used, and for the other endpoints, the Fine and Grey regression was used, considering competing risks.

Endpoint/Cohort	Hazard Ratio */Sub-Hazard Ratio	p Value
Overall Mortality *		
Overall	0.53	0.002
MMF	0.49	0.001
MTX	0.87	0.56
Non-Relapse Mortality		
Overall	0.29	<0.001
MMF	0.23	<0.001
MTX	0.29	<0.001
aGVHD 3–4		
Overall	0.51	0.004
MMF	0.38	0.006
MTX	0.58	0.11
aGVHD-associated mortality		
Overall	0.29	<0.001
MMF	0.22	<0.001
MTX	0.3	0.01
cGVHD moderate/severe		
Overall	0.38	<0.001
MMF	0.46	0.02
MTX	0.29	<0.001
Relapse		
Overall	1.87	0.01
MMF	1.78	0.1
MTX	2.4	0.009

MMF, mycophenolate mofetil; MTX, methotrexate; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease. * It marks the endpoint (Overall survival) where hazard ratios are given.

Stratification for the post-grafting immunosuppressive regimen revealed a significantly higher 3 year OS probability in the ATLG versus non-ATLG group within the cohort, with CNI/MMF being used as the post-grafting GVHD prophylaxis (65.8%; 95%CI, 57.8–75.0%; n = 118; versus 53.4%; 95%CI, 43.1–66.3%; n = 75; log-rank $p = 0.005$; Figure 1B). Multivariable Cox analysis confirmed the significant survival benefit for ATLG in the CNI/MMF group (Table 2).

By contrast, following HSCT with CNI/MTX-based GVHD prophylaxis, the 3 year OS after ATLG-based HSCT was not significantly different from that after non-ATLG based HSCT (67.2%; 95%CI, 56.6–79.7%, n = 54; versus 62.5%; 95%CI, 54.9–71.0%; n = 142; log-rank $p = 0.39$; Figure 1C). Furthermore, by multivariable analysis, no significant survival benefit for ATLG was revealed in the CNI/MTX cohort (Table 2).

3.2. Overall Survival—Sibling Transplants

In matched sibling HSCT, following ATLG-based HSCT, the 3 year OS probability was 76.1% (95%CI, 64.2–90.2; n = 42), compared to 60.3% (95%CI, 53.6–67.9; n = 186) following non-ATLG based HSCT (log-rank $p = 0.02$; Figure 2A; multivariable adjusted HR, 0.47;

$p = 0.02$). The respective 3 year OS probabilities after stratification for post-grafting GVHD prophylaxis were as follows in the CNI/MMF cohort: 79.0% (95%CI, 64.1–97.2%; $n = 24$) for ATLG-based transplants, versus 53.9% (95%CI, 42.8–67.8%; $n = 65$) for non-ATLG-based HSCT ($p = 0.01$; Figure 2C; multivariable adjusted HR, 0.40; $p = 0.04$). In the CNI/MTX cohort, 3 year OS in the ATLG group was 72.2% (95%CI, 54.2–96.2%; $n = 18$) compared to 63.8% (95%CI, 55.6–73.1%; $n = 121$) following non-ATLG based HSCT ($p = 0.28$; Figure 2E; multivariable adjusted HR, 0.67; $p = 0.39$).

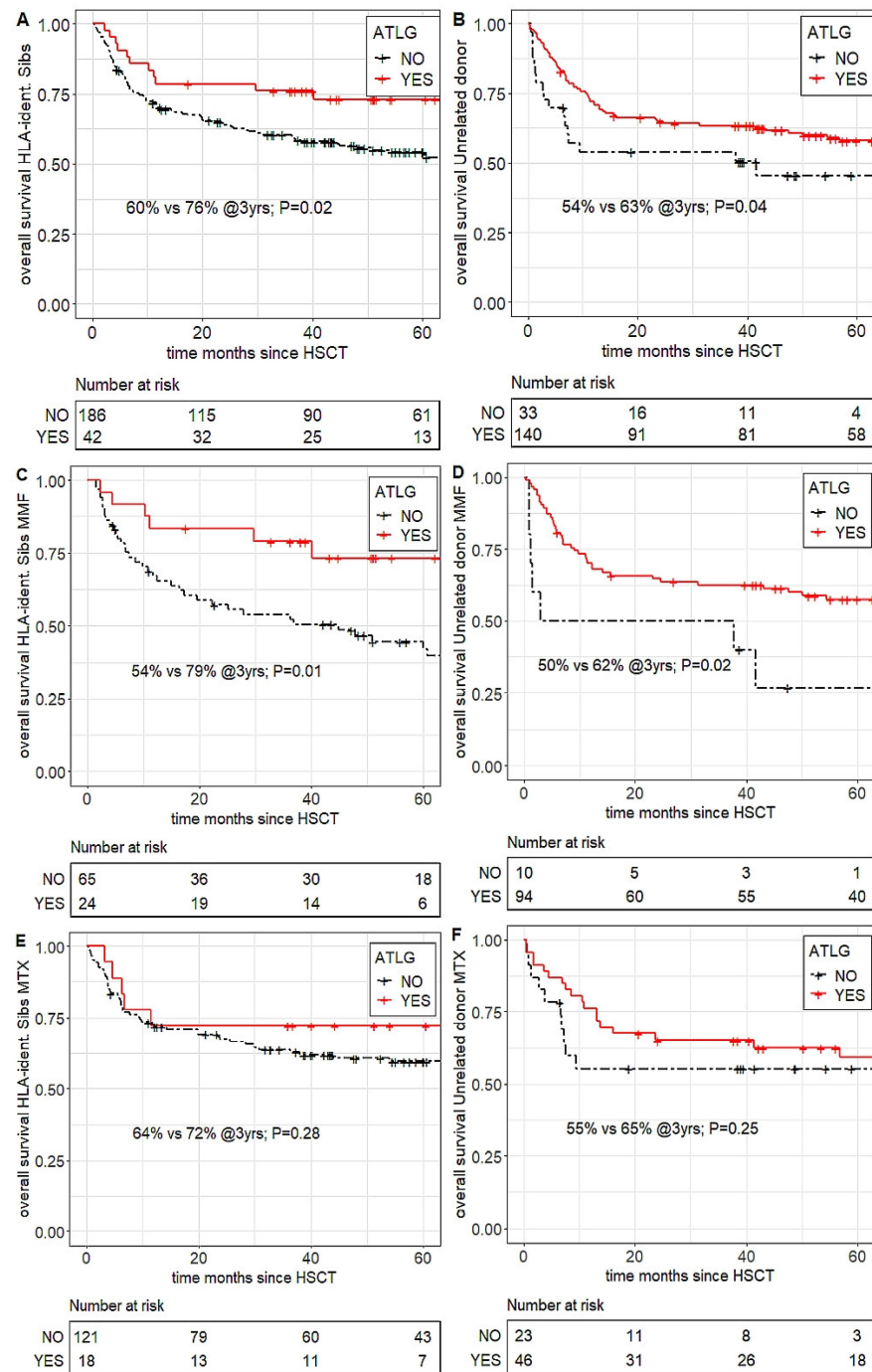


Figure 2. OS by ATLG treatment and donor relation. Red line, ATLG; black line, no ATLG. (A) all matched-related HSCT; (B) all unrelated HSCT; (C) MMF-based matched-related HSCT; (D) MMF-based unrelated HSCT; (E) MTX-based matched-related HSCT; (F) MTX-based unrelated HSCT.

3.3. Overall Survival—Unrelated Transplants

In unrelated HSCT, ATLG-treated recipients experienced a superior 3 year OS probability of 63.3% (95%CI, 55.8–71.9; n = 140), compared to the non-ATLG recipients (53.9%; 95%CI, 39.1–74.1; n = 33; log-rank $p = 0.04$; Figure 2B; multivariable adjusted HR, 0.56; $p = 0.03$). The respective outcomes in the CNI/MMF cohort were 62.5% (95%CI, 53.3–73.1%; n = 94) for ATLG-based transplants, versus 50.0% (95%CI, 26.9–92.9%; n = 10) for non-ATLG-based HSCT ($p = 0.02$; Figure 2D; multivariable adjusted HR, 0.34; $p = 0.001$). In the CNI/MTX cohort, 3 year OS in the ATLG cohort was 65.1 (95%CI, 52.7–80.5%; n = 46), compared to 55.2% (95%CI, 38.0–80.4%; n = 23) following non-ATLG-based HSCTs (log-rank $p = 0.25$; Figure 2F, multivariable adjusted HR, 0.69; $p = 0.34$).

Impact of ATLG on OS in MTX- versus MMF-Based HSCT by Conditioning Intensity

To investigate whether conditioning intensity, rather than GVHD prophylaxis type, may have been causative for the observed ATLG benefits mainly seen in MMF-based HSCT, the MTX and MMF subgroups were separately stratified for RIC vs. MAC transplants and assessed by multivariable analysis. In the MTX cohort, ATLG had no significant impact on OS, neither in the MAC HSCT (HR 1.00, $p = 1.00$, n = 178) nor in RIC HSCT (HR 0.50, $p = 0.25$, n = 30). By contrast, in the MMF cohort, ATLG was associated with superior OS both after MAC HSCT (HR 0.20, $p = 0.002$, n = 70) and after RIC HSCT (HR 0.52, $p = 0.01$, n = 123).

3.4. Impact of ATLG on OS in MTX- versus MMF-Based HSCT by Recipient Age

In the MMF cohort, ATLG was associated with a survival benefit regardless of recipient age (younger than median: HR 0.31, $p = 0.006$, n = 97; older than median: HR 0.39, $p = 0.04$, n = 96). No such association could be seen in the CSA/MTX group, neither for recipients younger than the median (HR 0.69, $p = 0.30$, n = 104), nor for those older than the median (HR 1.06, $p = 0.85$, n = 104).

3.5. ATLG-Associated OS Benefit Following MMF-Based HSCT Is Independent of Disease Risk

Similarly, the OS benefit associated with ATLG in recipients undergoing MMF-based prophylaxis was independent of the disease risk (low disease risk: HR 0.50, $p = 0.04$, n = 86; intermediate disease risk: HR 0.32, $p < 0.001$, n = 107). In contrast, in recipients with MTX-based prophylaxis, ATLG was not associated with a significant OS benefit, irrespective of the disease risk (low disease risk: HR 0.43, $p = 0.06$, n = 125; intermediate disease risk: HR 0.86, $p = 0.70$, n = 83).

3.6. ATLG-Associated OS Benefit Following MMF-Based HSCT Has Emerged in the More Recent Transplant Era

In the CSA/MMF group, ATLG was associated with a survival benefit in the group transplanted after the median transplantation date, but not in the group transplanted before the median transplantation date (after median: HR 0.42, $p = 0.02$, n = 97; before median: HR 0.82, $p = 0.83$, n = 96). No such association could be seen in the CSA/MTX group, neither for recipients transplanted before nor after the median transplantation date (after median: HR 1.27, $p = 0.49$, n = 103; before median: HR 0.61, $p = 0.51$, n = 105).

3.7. Non-Relapse Mortality (NRM) by ATLG in MMF- versus MTX-Based HSCT

In the entire cohort, the 3 year cumulative incidence of NRM was significantly lower following ATLG-based HSCT (17.9%, 95%CI, 12.6–23.8%; n = 182) than after non-ATLG based HSCT (30.8%; 95%CI, 24.7–37.0%; n = 219; $p < 0.001$; Figure 3A and Table 2). Following CNI/MMF-based HSCT, the 3 year cumulative incidence of NRM was 21.5% in the ATLG cohort (95%CI, 14.5–29.4%; n = 118), which was significantly lower than that in the non-ATLG cohort (34.8%; 95%CI, 24.2–45.7%; n = 75; $p = 0.005$; Figure 3B and Table 2). Similarly, following CNI/MTX-based HSCT, the 3 year NRM was significantly lower in the ATLG

cohort (11.1%; 95%CI, 4.8–20.2%; $n = 64$) than in the non-ATLG cohort (28.6%; 95%CI, 21.5–36.2%; $n = 144$; $p = 0.008$; Figure 3C and Table 2).

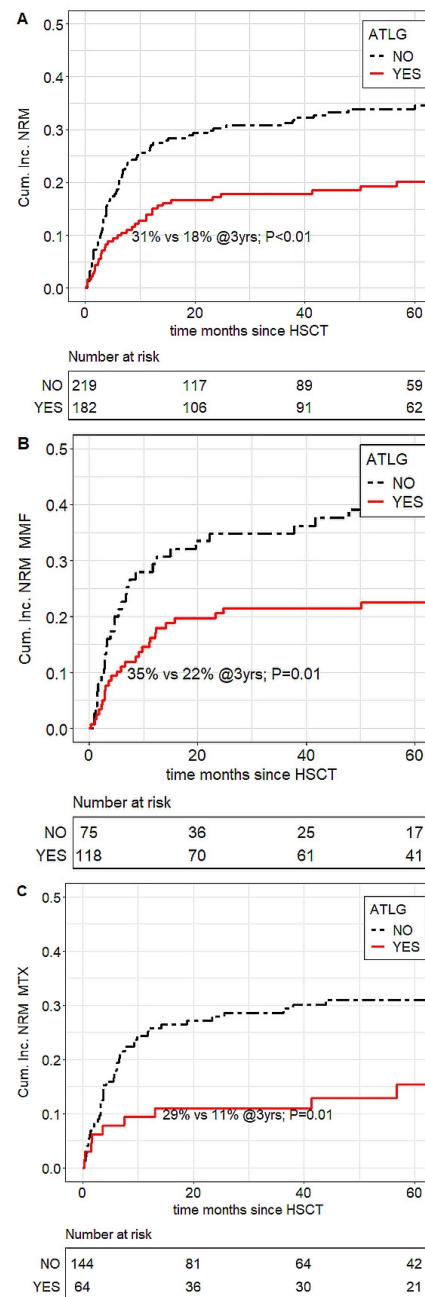


Figure 3. NRM by ATLG. Red line, ATLG; black line, no ATLG. (A) Entire cohort. (B) MMF-based HSCT. (C) MTX-based HSCT.

3.8. Acute GVHD Grade III–IV by ATLG in MMF- versus MTX-Based HSCT

In the entire cohort, the 1 year cumulative incidence of aGVHD grade III–IV was significantly lower following ATLG-based HSCT (19.1%; 95%CI, 13.7–25.2%; $n = 182$) compared to that after non-ATLG-based HSCT (27.4%; 95%CI, 21.7–33.4%; $n = 219$; $p = 0.04$; Table 2). In the subgroup of CNI/MMF-based HSCT, the respective incidences were 20.7% for the ATLG cohort (95%CI, 13.8–28.5%; $n = 116$), compared to 32.0% for the non-ATLG cohort (95%CI, 21.7–42.7%; $n = 75$; $p = 0.07$; Table 2). In contrast, in the MTX subgroup, no significantly different aGVHD grade III–IV incidence was observed after ATLG-based HSCT (16.1%; 95%CI, 8.2–26.3%; $n = 54$) versus non-ATLG-based transplants (25.0%; 95%CI, 18.2–32.3%; $n = 142$; $p = 0.12$; Table 2).

3.9. aGVHD-Associated Mortality

The 3 year cumulative incidence of aGVHD-associated mortality, defined as any NRM occurring during or after an episode of aGVHD grade II–IV and attributable to either GVHD itself or to infection, was significantly lower after ATLG-based transplants, reaching 13.4% (95%CI, 8.9–18.9%; n = 182), compared to after non-ATLG based transplants, which reached 23.0% (95%CI, 17.6–28.8%; n = 219; $p = 0.003$; Figure 4A and Table 2). This difference was significant also in the CNI/MMF subgroup, with an incidence of 17.2% (95%CI, 11.0–24.6%; n = 118) for ATLG-based transplants versus 24.0% after non-ATLG transplants (95%CI, 15.0–34.2%; n = 75; $p = 0.04$; Figure 4B and Table 2). In the CNI/MTX subgroup, the use of ATLG resulted in a particularly low incidence of aGVHD-associated mortality at 3 years, only 6.3% (95%CI, 2.0–14.2; n = 64), which was lower than that after transplants without ATLG (22.4%; 95%CI, 16.0–29.6%; n = 144), which had a high significance ($p = 0.006$; Figure 4C and Table 2).

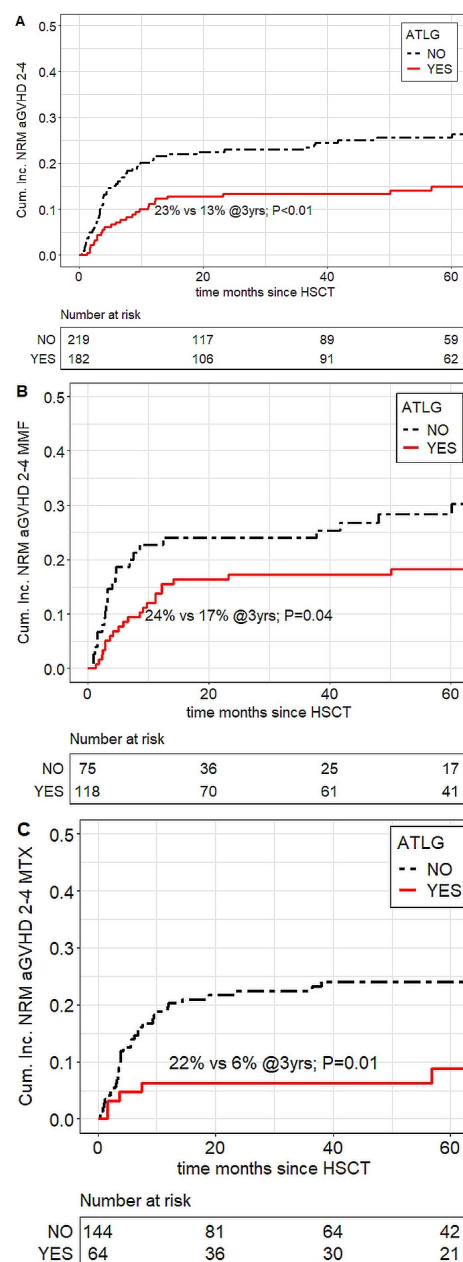


Figure 4. aGVHD-associated mortality by ATLG. Red line, ATLG; black line, no ATLG. (A) Entire cohort. (B) MMF subgroup. (C) MTX subgroup.

3.10. Chronic GVHD, Moderate/Severe

The use of ATLG was significantly associated with a reduced incidence of moderate to severe chronic GVHD, both in the overall cohort and in the MMF and the MTX subgroups (Table 2).

3.11. Relapse

The 3 year cumulative incidence of relapse following ATLG-based transplants was 25.4% (95%CI, 16.4–27.4; n = 182), while following HSCT without ATLG, it was 21.7% (95%CI, 16.4–27.4; n = 219; $p = 0.24$; Figure 5A and Table 2). While in the MMF subgroup the relapse incidence was not increased in the ATLG cohort (20.3%; 95%CI, 13.6–28.0; n = 118) compared to the non-ATLG cohort (25.6%; 95%CI, 16.2–36.0; n = 75; Figure 5B and Table 2), the relapse incidence following MTX-based transplants was significantly higher in the ATLG cohort (34.9%; 95%CI, 23.4–46.7, n = 64) than in the non-ATLG cohort (19.6%; 95%CI, 13.5–26.5; n = 144; $p = 0.04$; Figure 5C and Table 2).

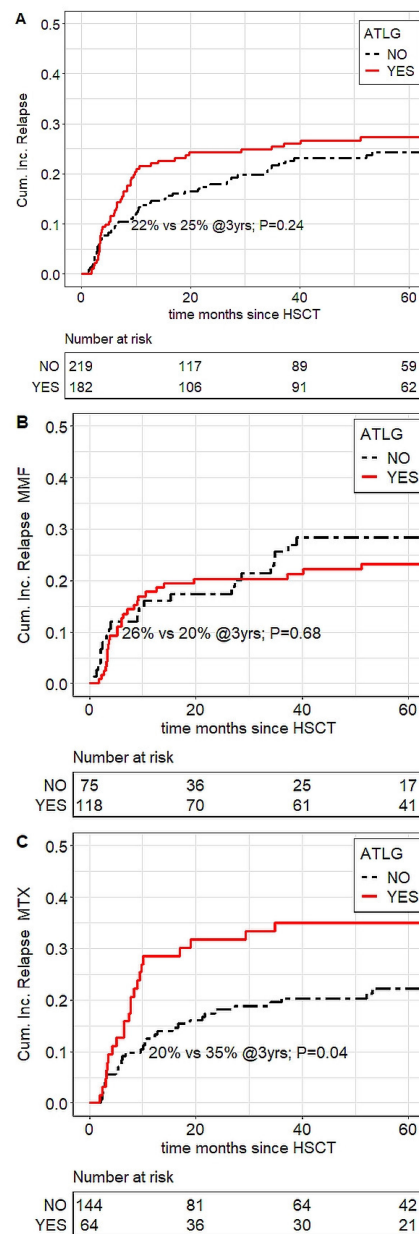


Figure 5. Cumulative relapse incidence. Red line, ATLG; black line, no ATLG. (A) Entire cohort. (B) MMF subgroup. (C) MTX subgroup.

4. Discussion

Evidence of the benefits of anti-T cell serotherapy for the intensification of GVHD prophylaxis is largely derived from the setting of myeloablative HSCT, with CNI and MTX serving as the backbone in the GVHD prophylactic regimen. This particularly applies to the prospective studies of ATLG [1–3], which solely included MTX-based, MAC HSCT. Likewise, the prospective studies of ATG [4–6] were conducted in the context of MTX-based immunosuppression, and two of them [4,5] were restricted to myeloablative HSCT, while one study [6] included both MAC and RIC/non-myeloablative (NMA) HSCT. While there is evidence from retrospective studies for a beneficial effect of ATG in the setting of RIC HSCT, provided the dose is restricted to a maximum of 5–6 mg/kg [10,13], ATLG has not yet been studied systematically in the RIC setting. In addition, the effects of the two preparations have not been addressed with a primary, comparative focus on the role of the backbone GVHD prophylaxis, i.e., MTX-based versus MMF-based regimens.

Since MTX-based GVHD prophylaxis is often linked to MAC HSCT, while MMF is frequently used in the RIC setting, it has remained unsolved as to whether a putative interaction of anti-T cell serotherapy exists with either conditioning intensity, with GVHD prophylaxis type, or with both. Our present findings indicate that GVHD prophylaxis type, rather than conditioning intensity, may interact with the benefits and risks of an intensification of GVHD prophylaxis with ATLG.

We show here, in a large, bi-centric, retrospective analysis of a cohort that is homogeneous with regard to disease phase (early/intermediate) and the stem cell source (peripheral blood), that ATLG was associated with a significant reduction of the risk for death in the overall cohort, and in the subgroup of patients receiving CNI/MMF as post-grafting immunosuppression. In contrast, in patients with CNI/MTX-based immunosuppression, there was no significant overall survival benefit associated with the use of ATLG. Indeed, ATLG was significantly associated with a reduced risk for NRM in the overall cohort, as well as in both subgroups, and also with reduced cGVHD and reduced aGVHD-associated mortality. However, an association of ATLG with a reduced risk for severe aGVHD (grade III–IV) was observed exclusively in the CNI/MMF subgroup. In the context of relapse, on the other hand, a significantly increased risk associated with the use of ATLG was observed only in CNI/MTX-treated recipients, but not in the CNI/MMF cohort.

Our findings indicate that adding ATLG to a standard GVHD prophylactic regimen is a particularly suitable option when the basic intervention (CNI/antimetabolite) is only moderately effective at preventing GVHD. This is consistent with previous research which has shown the CNI/MMF regimen [14–16,29] to be less effective at preventing GVHD compared to the CNI/MTX regimen. Accordingly, two other recent studies have demonstrated a limited benefit of MMF for GVHD prophylaxis in unrelated donor HSCT, (i) by showing that the addition of sirolimus to CSA/MMF reduced the incidence aGVHD grade II–IV and NRM, resulting in improved survival [30], and (ii) by the demonstration of a merely equivalent prophylactic potential of anti-T cell serotherapy plus CNI/MMF, compared to anti-T cell serotherapy plus CNI alone [31]. Differential MMF dosing, generally in the range between 30 mg/kg (or 2×1 g flat dose) and 45 mg/kg per day, likely plays a role in this context, however, in the above study, the CSA/MMF control group even received the higher MMF dose of 45 mg/kg per day [30].

According to our present findings, the addition of ATLG to CNI/MMF, as opposed to CNI/MTX, on the one hand seemed to provide a greater benefit in terms of the reduction of severe aGVHD and NRM, and, on the other hand, was more feasible in terms of a potentially increased relapse risk. Since the issue of an increased relapse risk associated with anti T cell serotherapy has been shown to be dose-dependent, both in case of ATG [10,13] and ATLG [8], it might be reasonable to use ATLG or ATG at a lower dose in combination with CNI/MTX, as compared to their use together with CNI/MMF. Such a differential dosing would take into account both the GVHD-protective properties, and the potential impairment of graft-versus-leukemia effects by the respective backbone GVHD prophylaxis.

The concept of anti T cell serotherapy has been purposefully studied in the myeloablative setting, based on the conclusion from a large retrospective study that a possible abrogation of the GVL effect might be more harmful in the reduced intensity than in the myeloablative conditioning setting [32]. In that study however, which also included a large proportion of patients prepared with alemtuzumab in addition to ATG, the majority of patients received CNI/MTX as basic immunosuppression. Based on the present findings, it might be worth studying ATLG in the RIC (or reduced toxicity conditioning) setting in a prospective manner, particularly if CNI/MMF is chosen as the baseline GVHD prophylaxis. Based on findings from the Marseille group [11,12], MMF might even be omitted in the case of matched sibling transplants if anti-T cell serotherapy is applied.

One might speculate that even in matched-unrelated transplantation, it is important to avoid the overdosing of ATG or ATLG. Among the factors that have been identified to increase the risk for relapse through an excess immunosuppression with ATG/ATLG are (i) a low recipient lymphocyte count at the time of ATG initiation, as shown for both ATG [33,34], and ATLG [2], and (ii) an HLA-C2 homozygous killer cell immunoglobulin receptor (KIR) ligand status [8]. The present findings suggest that the type of post-grafting immunosuppression, i.e., MTX-based or not, should be added to the list of factors that need to be considered to interact with the effects of an additional anti-T cell serotherapy.

The leading limitation in our study is the heterogeneity between the groups and the long observation period. We have addressed this issue by various substratified analyses, in addition to standard multivariable analyses, to rule out any bias through the most divergent group characteristics. Interestingly, these analyses have revealed that the most significant benefit of ATLG was revealed in the more recent era of our cohort. This may be a result of a more differentiated, risk-adapted ATLG dosing (i.e., considering donor–recipient relationships), which was implemented during the more recent transplant era. This study examined adult patients who received PBSC as a graft source. It is worth noting that the findings may not be applicable to allogeneic HSCT in children or to cases where bone marrow is used as the graft source.

In summary, in the context of CNI/MMF, ATLG may provide even greater benefits than in the setting of CNI/MTX for post-grafting immunosuppression. Future prospective studies on ATLG should, therefore, focus on, or at least include, CNI/MMF-based transplants, which are widely performed in the elderly, or comorbid patients not expected to tolerate a standard course of MTX.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/transplantology4010005/s1>. Table S1: Cohort Characteristics CNI/MMF by ATLG use. Table S2: Cohort Characteristics CNI/MTX by ATLG use.

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Abbreviations

aGVHD	acute GVHD
ALL	acute lymphocytic leukemia
AML	acute myeloid leukemia
ATG	Antithymocyte globulin
ATLG	anti-human T-lymphocyte globulin
BPDCN	blastic plasmacytoid dendritic cell neoplasm
cGVHD	chronic GVHD
CNI	calcineurin inhibitor
CsA	cyclosporin A
G-CSF	granulocyte-colony stimulating factor
GVHD	graft-versus-host disease
HR	hazard ratio
HSCT	allogeneic hematopoietic stem cell transplantation
MAC	myeloablative
MDS	myelodysplastic syndromes
MMF	mycophenolate mofetil
MPD	myeloproliferative disorder
MPN	myeloproliferative neoplasia
MPS	myeloproliferative syndromes
MTX	methotrexate
NMA	non-myeloablative
NRM	non-relapse mortality
OS	overall survival

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