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Impact of Serotherapy on Immune Reconstitution and Survival Outcomes After Stem Cell Transplantations in Children: Thymoglobulin Versus Alemtuzumab



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ABSTRACT

The outcome of allogeneic hematopoietic stem cell transplantation (HSCT) is strongly affected by the kinetics of reconstitution of the immune system. This study compared the effects of antithymocyte globulin (ATG) and alemtuzumab on various outcome parameters after HSCT. The study cohort consisted of 148 children, with a median age of 9.6 years (range, .4 to 19.0), who underwent HSCT for malignant and benign hematological disorders in a single HSCT unit. Conditioning included ATG ($n = 110$) or alemtuzumab ($n = 38$). Cox proportional hazard regression analysis showed that alemtuzumab significantly delayed the recovery of CD3⁺ T cells and CD4⁺ as well as CD8⁺ T cell subsets ($P \leq .001$) and natural killer (NK) cells ($P = .008$) compared with ATG. In both ATG- and alemtuzumab-treated patients, shorter drug exposure lead to significantly faster recovery of T cells. Alemtuzumab was associated with lower donor chimerism 3 and 6 months after transplantation and a higher risk of disease relapse ($P = .001$). The overall survival and event-free survival risks were significantly lower for alemtuzumab-treated patients ($P = .020$ and $P < .001$, respectively). Patients who received alemtuzumab showed a trend to lower risk of acute graft-versus-host disease, more human adenovirus, and less Epstein-Barr virus reactivations compared with patients who received ATG. These data indicate that children treated with alemtuzumab as part of the conditioning regimen have a slower T cell and NK cell reconstitution compared with those treated with ATG, which compromises the overall and event-free survival. Prolonged length of lympholytic drug exposure delayed the T cell recovery in both ATG- and alemtuzumab-treated patients. Therefore, we recommend detailed pharmacokinetic/pharmacodynamic (PK/PD) analyses in a larger cohort of patients to develop an algorithm aiming at optimization of the serotherapy containing conditioning regimen.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy in children with a variety of malignant diseases and nonmalignant diseases [1,2]. Still, transplantation-related morbidity, particularly graft-versus-

host disease (GVHD), and recurrence of initial disease remain the major causes of an unsuccessful outcome [3–5].

Before HSCT, patients are conditioned with chemotherapy, irradiation, and/or serotherapy to eliminate residual malignant cells, prevent acute and chronic GVHD, and facilitate engraftment. Serotherapy usually consists of antithymocyte globulin (ATG), a polyclonal antibody, or the monoclonal antibody alemtuzumab. ATG is a polyclonal immunoglobulin preparation obtained by immunization of rabbits or horses with human thymocytes or T cell lines. Alemtuzumab is a humanized monoclonal antibody specific

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for CD52. In the HSCT setting, both types of serotherapy are used to eliminate T cells, but they also target other cell types, eg, B cells and natural killer (NK) cells [6–10]. ATG and alemtuzumab are considered instrumental in reducing the risk of rejection by suppressing the reaction of host T cells against the graft. However, because of their long half-lives, the antibodies will usually remain present after transplantation and eliminate the donor T cells infused with the graft as well. In this way, these antibodies not only reduce the occurrence of GVHD [5,7,11–13], but they may also have a negative impact on the occurrence of a graft-versus-leukemia effect [1,13]. Furthermore, the negative impact of ATG and alemtuzumab on the recovery of lymphocytes after HSCT is associated with an increased risk of viral infections/reactivations [5,6,14–16]. Alemtuzumab has a longer half-life (15 to 21 days) than ATG (4 to 14 days), leading to a more prolonged effect on lymphocyte recovery [14,17–19].

Although ATG and alemtuzumab are frequently used in pediatric stem cell transplantations, there are only a few studies comparing the effects of ATG and alemtuzumab exposure on the outcomes after transplantation in children. In a large multi-center study in a cohort of children with acute lymphoblastic leukemia after unrelated donor transplantation, Veys et al. [5] showed that alemtuzumab was more effective than ATG in lowering the risk of severe acute GVHD, whereas serotherapy as such did not compromise leukemia-free survival. Myers et al. [20] evaluated the incidence of adenovirus infection in 111 pediatric recipients of bone marrow transplants with either ATG or alemtuzumab in their conditioning regimen. Besides an increased risk of adenovirus infection in alemtuzumab-treated patients, they found no significant differences in complications, eg, GVHD, and overall survival between both serotherapy groups. Shah et al. [13] compared 14 alemtuzumab-treated patients with 13 ATG-treated patients after pediatric HSCT and concluded that alemtuzumab is more effective than ATG in decreasing the incidence of GVHD without increasing the risk of relapse or infectious complications. They also evaluated T cell recovery after transplantation and reported a significantly slower T cell recovery after alemtuzumab compared with ATG. To our knowledge, other single-center studies comparing the recovery of the different lymphocyte subsets (T, B, and NK cells) in children after applying these 2 types of serotherapy are lacking.

The aim of this study is to compare the effects of ATG with alemtuzumab as part of the conditioning regimen and to evaluate the impact of the length of lympholytic exposure to these drugs after HSCT on various outcome parameters after allogeneic stem cell transplantation, ie, immune recovery, GVHD, infections and survival, in children receiving HSCT as part of the treatment of different benign and malignant hematological diseases.

METHODS

Patients

Between January 2003 and May 2012, 235 pediatric patients with malignant and benign hematological diseases received their first HSCT at the pediatric HSCT unit of Leiden University Medical Center (LUMC). All patients who underwent transplantation with a bone marrow or peripheral blood stem cell graft from an unrelated or matched family donor and who received serotherapy as part of their conditioning regimen and GVHD prophylaxis after HSCT were eligible for this study. By applying these inclusion criteria, patients receiving no serotherapy ($n = 48$), a cord blood transplant ($n = 19$), a graft from a haploidentical donor ($n = 19$), or those not receiving GVHD prophylaxis ($n = 1$) were excluded. Consequently, the final study cohort consisted of 148 patients. Analysis was performed using July 1, 2014 as the cut-off date for follow-up.

All data used for this study were obtained from the databases of the pediatric HSCT unit at the LUMC and the European Bone Marrow Transplant Group. The medical ethical committee of the LUMC approved this study (P01.028). Informed consent was obtained from all patients included in the study and/or their parents.

Serotherapy

To determine the impact of the different types of serotherapy on various outcome parameters, patients were divided into 2 groups: (1) those receiving ATG (Thymoglobulin; Genzyme, Naarden, the Netherlands) at an intended cumulative dose of 10 mg/kg body weight (BW) divided over 3 to 5 days, mostly starting at day -5 ; and (2) those receiving alemtuzumab (Campath; Genzyme) at an intended cumulative dose of 1 mg/kg BW, divided over 3 to 5 days, mostly starting at day -5 . Steroids (prednisone 2 mg/kg, in 4 doses) were given throughout the course of serotherapy, starting the evening before first dose of serotherapy, throughout the entire course of serotherapy. Over the years, we changed the dose (from 1 to 2 mg/kg) and starting time of steroids (from just before infusion to the day before infusion), which led to less ATG infusion-related side effects. Clemastine has always been given just before the start of each infusion. Upon a systemic inflammatory reaction (ie, fever, tachycardia, hypotension), additional steroids (200 mg/m² hydrocortisone) and clemastine were given.

In total, 15 patients showed a severe systemic reaction upon the first administration of ATG necessitating a switch to alemtuzumab. These patients, receiving a median dose of 2.5 mg ATG/kg BW and of .6 mg alemtuzumab/kg BW, were classified in the alemtuzumab group. Importantly, active ATG serum concentrations were already below the level of detection, ie, $<.1$ arbitrary units (AU)/mL, at the time of HSCT in these patients. Separate analysis of these “switchers” indicated that the outcomes, including immune recovery, were comparable to those of the 23 children only receiving alemtuzumab (see [Supplementary Text S1](#)).

Active ATG, the fraction of the product capable of binding to cells, and alemtuzumab levels were measured using quantitative flow cytometry assays, both in modifications of the method described [12,18]. In short, HUT-78 T cells were incubated with 4-fold dilutions of patients' serum, starting with a dilution of 1:8, followed by washing and incubation with conjugated secondary antibodies; for active ATG, Alexa Fluor 647–labeled goat anti-rabbit IgG (Life Technologies, Carlsbad, CA) and for alemtuzumab, Alexa Fluor 647–labeled goat antihuman IgG (Life Technologies). To construct a reference curve, HUT cells were incubated with known amounts of ATG or alemtuzumab. Finally, cells were washed and analyzed by flow cytometry on a FACS Scan (Becton Dickinson Biosciences, Franklin Lakes, NJ). Mean fluorescence intensities obtained at the different standard dilutions were plotted against the active ATG or alemtuzumab concentrations. Active ATG is measured in AU. Five mg/mL ATG was arbitrarily set at containing an active ATG concentration of 5000 AU/mL. The lower limit of detection for active ATG was .1 AU/mL [12] and for alemtuzumab, .01 μ g/mL.

Standard active ATG measuring was done from April 2004. Active ATG levels were available for 102 of the 111 ATG-treated patients and alemtuzumab levels were available for all 38 alemtuzumab-treated patients.

Conditioning Regimen

Depending on their diagnosis and condition, patients received chemotherapy or total body irradiation–based regimens. Transplantation procedures and conditioning regimens were generally according to the European Group for Blood and Marrow Transplantation recommendations for the various underlying diseases. To investigate the impact of irradiation and chemotherapy, conditioning regimens were divided into 2 groups: (1) myeloablative conditioning (MA) and (2) nonmyeloablative conditioning (NMA). Any regimen containing high-targeted busulfan (above 65 to 100 mg/hour/L), total body irradiation, or treosulfan combined with thiotepa were considered MA. Low-targeted busulfan (45 to 65 mg/hour/L), cyclophosphamide with or without fludarabine, or treosulfan with fludarabine (without thiotepa) were considered NMA or reduced intensity [21]. All patients who received reduced-intensity conditioning regimens were classified as NMA.

Supportive Care

Standard care consisted of strict protective isolation and oral administration of antimicrobial drugs for gut decontamination. No cytomegalovirus (CMV), Epstein-Barr virus (EBV), or human adenovirus (HAdV) prophylaxis were used. Viral load monitoring by PCR was performed at least once weekly during the first 8 weeks after transplantation and, thereafter, at each visit to the outpatient clinic until the number of peripheral blood T cells exceeded 300 cells/ μ L. GVHD prophylaxis consisted of calcineurin inhibitor (CI; cyclosporine [CsA] or tacrolimus in case of CsA intolerance) or a combination of CI and methotrexate in almost all patients. Three patients received

additional mycophenolate mofetil (CellCept) or corticosteroids and 1 patient received no GVHD prophylaxis.

Donors

Stem cell sources used in this study consisted of bone marrow grafts and peripheral blood stem cells from related or unrelated donors. All patients and donors were typed using PCR high resolution typing for HLA class I and II antigens (10 antigens: A, B, C, DRB, and DQB). HLA-matched donors were defined as 10 out of 10 matched.

Definitions

Cell reconstitution

The day of engraftment was defined as the first day of 2 consecutive measurements at which an absolute neutrophil count of at least $.5 \times 10^9/L$ was achieved in the absence of granulocyte infusion. Recovery of monocytes and $CD3^+$ T cells was defined as the first day of 2 consecutive measurements with an absolute cell count of at least $100/\mu L$. Recovery of $CD3/CD4^+$ T cells, $CD3/CD8^+$ T cells, $(CD3^- CD56^+ CD16^{+/-})$ NK cells, and $(CD19/CD20^+)$ B cells was defined as the first day of 2 consecutive measurements with an absolute cell count of at least $50/\mu L$ for the respective cell (sub)population. Neutrophil and monocyte counts were obtained from hematological leukocyte counting and differentiation, which was performed every 1 to 3 days during the first 2 months after HSCT. T cell, T cell subsets, NK cell, and B cell analysis in peripheral blood mononuclear cells (PBMC) was performed every week, when sufficient lymphocytes were present (usually from 2 to 3 weeks after HSCT onward) by immunostaining and flow cytometry (FACS Calibur II, Becton Dickinson Biosciences). Data were analyzed using BD Cellquest software. The progress of lymphocyte counts (T, B, and NK cells) was evaluated over time (1, 2, 3, 6, and 12 months after HSCT). All results on test days closest to day 30, day 60, day 90, day 180, and day 365 were included in these analyses of cell recovery. The effect of ATG and alemtuzumab on naïve and memory/effector T cells was evaluated using absolute naïve ($CD45RA^+CCR7^+$) and memory/effector ($CD45RA^+$ and/or $CCR7^-$) $CD3/CD4^+$, and $CD3/CD8^+$ T cell counts.

Length of lympholytic drug exposure

To investigate the impact of drug exposure on immune reconstitution, the length of lympholytic drug exposure after transplantation was determined. For this purpose, the day after HSCT that the drug level fell below 1 AU/mL and .2 $\mu g/mL$ was calculated for ATG and alemtuzumab, respectively. Above this level, in none of the patients T cells recovered to ≥ 100 cells/ μL (Text S2 and Figure S1, supplementary data).

GVHD

Incidences of acute and chronic GVHD were classified using the Glucksberg and Shulman criteria, respectively [22,23]. Acute and chronic GVHD referred to all grades (I to IV and limited/extended, respectively), whereas severe acute GVHD was defined as grade II to IV acute GVHD.

Viral infection/reactivation

CMV, EBV, and HAdV infections/reactivations were defined as 2 consecutive viral DNA loads of at least 1000 copies/mL in serum or plasma samples separated by at least 3 days, determined with real time quantitative (RQ) PCR in the first 100 days after transplantation [24–27]. In these cases, preemptive treatment with ganciclovir (CMV), rituximab (EBV), or cidofovir (HAdV) was initiated. Pretransplantation CMV and EBV serostatus of patient and donor were determined for all HSCT couples and only CMV and EBV-seropositive patients and seronegative patients with a seropositive donor (at risk patients) were included in CMV and EBV infection/reactivation analyses, respectively.

Chimerism

Donor chimerism, analyzed in PBMC using the Powerplex 16 assay (Promega, Leiden, the Netherlands [28]), was arbitrarily categorized as: (1) donor chimerism of at least 95% and (2) less than 95%.

Survival

Overall survival (OS) was defined as time to death, regardless of the cause, or last follow-up (censoring). Event-free survival (EFS) referred to the time to disease recurrence, retransplantation, death, or last follow-up (censoring). Overall mortality and treatment failure were used as the inverse of OS and EFS, respectively, in multivariate analysis. Nonrelapse mortality (NRM) was defined as death not related to recurrence or progression of the original disease. Relapse referred to the incidence of recurrence of malignant diseases. Only patients with malignant diseases were included in relapse analysis.

Statistical Analysis

All analyzed variables and outcomes were compared between the ATG group and the alemtuzumab group. Differences in patient characteristics among the treatment groups (Table 1) were compared using Mann-Whitney rank tests for continuous data, chi-squared test for categorical data, and Fisher's exact tests for binomial data. The cumulative OS and EFS and the cumulative incidence of NRM, relapse, GVHD, viral reactivations, and the recovery of immune cells (neutrophils, monocytes, $CD3$, $CD4$, $CD8$, NK, and B cells) were calculated using the Kaplan-Meier method. All were descriptive values. The probabilities of OS, EFS NRM, relapse, GVHD, viral reactivations, and recovery of immune cells were compared with log-rank tests.

Table 1

Patient Characteristics (n = 148)

Characteristic	ATG Group	Alemtuzumab Group	P Value
Patients, n	110	38	
Patient age, median (range), yr	7.8 (.4–18.6)	13.3 (3.9–19.0)	<.001
Patient sex			.545
Male	74 (67)	28 (74)	
Female	36 (33)	10 (26)	
Diagnosis			.180
Benign hematological disease	46 (42)	11 (29)	
Malignant disease	64 (58)	27 (71)	
Donor			.430
Identical related donor	17 (15)	7 (18)	
Other related donor	3 (3)	3 (8)	
Unrelated donor	90 (82)	28 (74)	
Donor-recipient HLA match			1.000
10 of 10 HLA match	72 (66)	25 (66)	
1 or more HLA mismatch(es)	38 (34)	13 (34)	
Graft type			.455
Bone marrow	93 (85)	30 (79)	
Peripheral blood stem cells	17 (15)	8 (21)	
Graft manipulation			<.001
No depletion	107 (97)	29 (76)	
T cell–depleted graft [†]	3 (3)	9 (24)	
Conditioning regimen [‡]			<.001
MA	102 (92)	24 (63)	
NMA	8 (8)	14 (37)	
Nucleated cells infused, $\times 10^8/kg$, (average \pm SEM)			
Bone marrow	2.8 \pm .2	2.1 \pm .7	.17
Peripheral blood stem cells	14.6 \pm 3.0	12.5 \pm 4.6	.72
GVHD prophylaxis			.001
CI	3 (3)	8 (21)	
CI + MTX [‡]	107 (97)	30 (79)	
CMV status			.848
Recipient and/or donor seropositive	67 (61)	22 (58)	
Recipient and donor both seronegative	43 (39)	16 (42)	
EBV status			.339
Recipient and/or donor seropositive	104 (95)	38 (100)	
Recipient and donor both seronegative	6 (5)	0 (0)	
Dosage serotherapy			
Cumulative dose ATG, median (range), mg/kg	10 (5–11)		
Cumulative dose alemtuzumab, median (range), mg/kg		.8 (.2–1.4)	
Follow-up, median (range), mo			
All patients	57 (2–135)	57 (0–138)	
Survivors	66 (27–135)	112 (27–138)	

CI includes cyclosporine or tacrolimus; MTX, methotrexate.

Data presented are n (%) unless otherwise indicated.

* Depletion either by negative selection ($CD3$ and $CD19$ depletion) or positive selection ($CD34$ selection).

[†] Conditioning regimens that contained either high-dose total body irradiation or high-dose busulfan or treosulfan were classified as MA conditioning. All other regimens were classified as NMA conditioning (cyclophosphamide alone or fludarabine in combination with cyclophosphamide, thiopeta or melfalan) [21].

[‡] Three patients received additional mycophenolate mofetil (CellCept) or corticosteroids.

Distribution of chimerism percentages between the 2 groups was calculated using crosstabs and compared with chi-squared tests.

The effects of serotherapy and other covariates on OS, treatment failure, NRM, relapse, GVHD, viral reactivations, and recovery of immune cells were analyzed by Cox proportional hazard regression models. The effects on chimerism were assessed using binary regression models. The effects on immune cell counts (CD3, CD4, CD8, NK, and B cells) were analyzed using linear mixed models (fixed main effects), in which multiple comparisons at different time points were taken into account.

The primary objective of this study was to compare ATG and alemtuzumab. Therefore, serotherapy was included in all multivariate analyses and ATG was set as reference category. Besides serotherapy, patient age, patient sex (female versus male), diagnosis (malignant disease versus benign hematological disease), conditioning regimen (MA versus NMA), graft manipulation (T cell depletion versus no T cell depletion), graft type (bone marrow versus peripheral blood stem cells), and HLA match (compete match versus incomplete match) were included in all multivariate analyses using the “enter” method. Other variables considered were relationship to donor, ie, unrelated versus identical related or nonhaploidentical other related, age of the donor, sex match between patient and donor (matched versus mismatched), CMV and EBV serostatus patient (seropositive versus seronegative), CMV and EBV serostatus donor (seropositive versus seronegative), GVHD prophylaxis (CI and methotrexate versus CI alone or no prophylaxis), and year of transplantation (2003 to 2005, 2006 to 2008, or 2009 to 2012).

For all multivariate and univariate analyses, *P* values < .05 were considered statistical significant. All *P* values were 2-sided. For the evaluation of viral reactivations and relapse, patients were censored at time of retransplantation, death from any cause, or last follow-up. Additional censoring was performed at time of relapse or donor lymphocyte infusion for evaluation of cell reconstitution, chimerism, and GVHD. Patients who died or underwent retransplantation within 100 days were excluded for chronic GVHD analyses. Patients receiving rituximab (MabThera, Roche, Basel, Switzerland), were excluded from B cell analyses from the first time point they received rituximab. In NRM analyses, patients were censored at time of retransplantation, relapse, or last follow-up. Analyses were done with SPSS20 software (IBM SPSS Inc., Chicago, IL). Graphs were made in Prism Graphpad 6.02 (Graphpad Software, La Jolla, CA).

RESULTS

Patient Characteristics

A total of 110 patients received ATG and 38 patients received alemtuzumab as part of their conditioning regimen. As shown in Table 1, there are some significant differences in patient characteristics between the 2 serotherapy groups. Patients treated with alemtuzumab were older, more often received a T cell–depleted graft and CsA only as GVHD prophylaxis, and were less likely to have a MA conditioning regimen than those treated with ATG. These differences between the 2 serotherapy groups did not bias the results of the analyses of T cell recovery and overall mortality risk (see section “Differences in Patient Characteristics between Serotherapy Groups”).

The median total dose of ATG was 10 mg/kg (range, 5 to 11 mg/kg); 97% of the ATG recipients actually received a total dose of 10 mg/kg. The median total dose of alemtuzumab was .8 mg/kg (range, .2 to 1.4 mg/kg). The pretransplantation clinical condition of the 38 alemtuzumab-treated patients was not significantly different from that of a subcohort of ATG-treated patients matched with respect to original disease, donor type, and year of HSCT (see Supplementary Text S3). Regarding the various outcome parameters, the 15 ATG to alemtuzumab switchers did not differ significantly from the 23 children receiving alemtuzumab only (see Supplementary Text S1).

Engraftment and Monocyte Recovery

In total, 145 patients (98%) engrafted. The median time to engraftment and to appearance of monocytes was shorter in the ATG group compared with the alemtuzumab group (Table 2). Multivariate analysis showed a trend to slower

Table 2

Median Day, Univariate, and Multivariate Analysis of Appearance of Various Cell Subsets

	Appearance of Cells, Median Day (95% CI)*		Log Rank	Multivariate Analysis†	
	ATG	Alemtuzumab	<i>P</i> Value	HR (95% CI)	<i>P</i> Value
Neutrophils	22 (20–24)	28 (26–30)	.030	.67 (.40–1.14)	.139
Monocytes	21 (20–22)	28 (22–34)	.001	.53 (.31–.91)	.022
CD3 T cells	29 (25–33)	98 (79–117)	<.001	.35 (.20–.61)	<.001
CD4 T cells	36 (32–40)	104 (93–115)	<.001	.33 (.18–.60)	<.001
CD8 T cells	34 (28–40)	114 (98–130)	<.001	.35 (.19–.65)	.001
NK cells	22 (21–23)	34 (30–38)	<.001	.47 (.27–.82)	.008
B cells	54 (50–58)	54 (49–59)	.722	1.07 (.58–1.98)	.819

95% CI indicates 95% confidence interval.

* Median first day of constitutive cell counts above $.5 \times 10^9/L$ for neutrophils, above 100/ μL for CD3 T cells and monocytes, and above 50/ μL for CD4 T cells, CD8 T cells, CD3⁺ CD56⁺ CD16⁺ NK cells and CD19/CD20⁺ B cells.

† Cox proportional hazard regression models, corrected for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, and HLA match.

engraftment and a significant slower recovery of monocytes after alemtuzumab (Table 2).

Immune Reconstitution

The median day of appearance of T cells in the ATG group was 29 days for total CD3 T cells, 36 days for CD4, and 34 days for CD8 T cell subsets, whereas the recovery of T cells was delayed in the alemtuzumab group (98 days for CD3, *P* < .001; 104 days for CD4, *P* < .001; and 114 days for CD8, *P* < .001) (Table 2). Multivariate analysis showed that alemtuzumab was associated with a slower recovery of CD3 T cells (hazard ratio [HR], .35; *P* < .001) (Table 2), CD4 T cells (HR, .33; *P* < .001), and CD8 T cells (HR, .35; *P* = .001).

The recovery of NK cells was slower in alemtuzumab-treated patients compared with in ATG-treated patients (34 and 22 days, respectively; *P* < .001). This association was also observed in multivariate analysis (HR, .47; *P* = .008) (Table 2). There was no difference in the median time to appearance of B cells (54 days for both groups, Table 2) between the ATG and alemtuzumab-treated patients.

To further evaluate the effect of serotherapy, lymphocyte subset counts at 1, 2, 3, 6, and 12 months after HSCT were compared between the ATG and alemtuzumab groups. Significant differences in T cells counts between the treatment groups were observed during the first 3 months after HSCT (Figure 1). In the alemtuzumab group, CD3, CD4, and CD8 T cell counts were lower in the first 3 months after HSCT compared with those of the ATG group (*P* = .025 for CD4 T cell counts at 3 months and *P* ≤ .001 for all others). NK cell counts were significantly lower in alemtuzumab-treated patients 1 month after HSCT (*P* < .001) (Figure 1). No differences in any of the T cell populations, NK cell, or B cell counts were observed 6 and 12 months after HSCT.

Naïve and memory/effector CD4 and CD8 T cell counts after HSCT were compared between both serotherapy groups. Memory/effector CD4 and CD8 T cell were significantly higher after ATG (*P* < .001 for both) (Figure 2). No difference was observed in naïve CD4 and CD8 T cells counts.

Impact of the Length of Lympholytic Drug Exposure on Immune Reconstitution

Based on the length of lympholytic exposure to active ATG after HSCT, ATG-treated patients were divided in 3

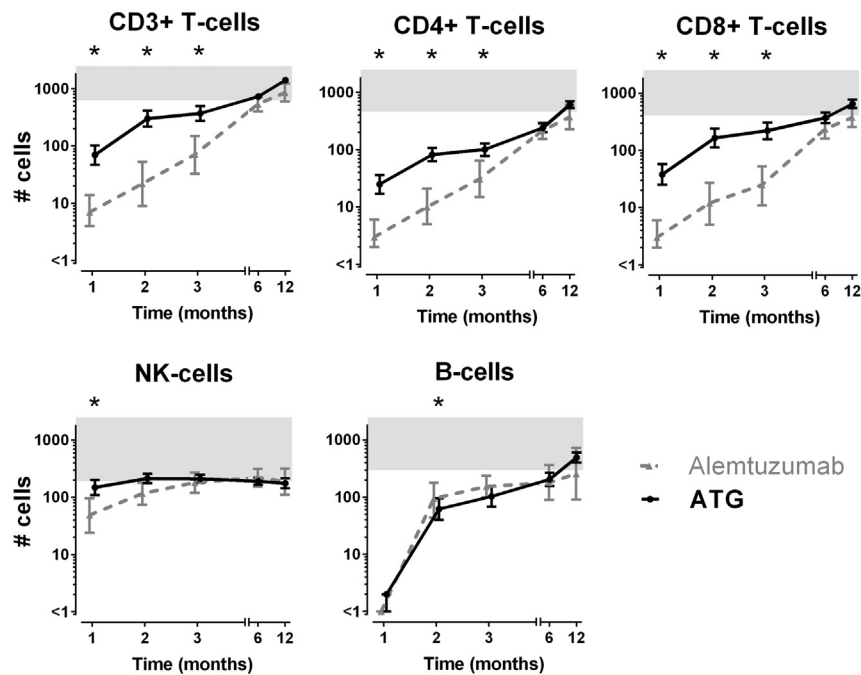


Figure 1. Recovery of lymphocyte subsets after transplantation. Geometric mean of total T cell (CD3), CD4 T cell subset, CD8 T cell subset, NK, and B cell counts at 1, 2, 3, 6, and 12 months after transplantation of patients who received ATG (black lines) and alemtuzumab (grey dashed lines). The grey shaded areas represent the range of healthy children. Error bars indicate the lower and upper 95% CI of the geometric mean. Months after transplantation are shown on all x-axes. On all y-axes, values represent cells/ μ L. Subset counts were compared using multivariate linear regression analyses with correction for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, HLA match, CMV serostatus of the patient, and CMV serostatus of the donor. An asterisk indicates a significant difference between ATG and alemtuzumab-treated patients ($P < .05$). All patients receiving rituximab (MabThera), usually for EBV infections, were excluded from B cell analyses from the moment they received Rituximab.

equally sized groups, ie, “short exposure” group (length of lympholytic exposure was <10 days), “median exposure” group (10 to 18 days), and “long exposure” group (≥ 18 days). Alemtuzumab-treated patients were accordingly divided in 3 groups, with limits being ≤ 34 days, 35 to 48 days, and ≥ 48 days.

Figure 3 shows the recovery of neutrophils, monocytes, and lymphocyte subsets for the different exposure groups among the ATG-treated (Figure 3A) and the alemtuzumab-treated (Figure 3B) patients. Multivariate analysis showed that CD3, CD4, and CD8 T cell recovery was delayed in patients with long exposure compared with patients with short or median exposure in the ATG-treated group ($P = .001$, $P = .005$,

and $P = .003$, respectively). In contrast, NK cell recovery tended to be slower in patients with short exposure compared with those with long or median exposure ($P = .054$). No differences were observed in B cell, neutrophil, and monocyte recovery ($P = .619$, $P = .683$, and $P = .675$, respectively).

In alemtuzumab-treated patients, similar effects of drug exposure on T cell recovery were seen (Figure 3B). CD8 T cell recovery was significantly faster ($P < .001$) and CD3 T cell recovery tended to be faster ($P = .125$) in patients with short alemtuzumab exposure compared with those with long or median exposure. NK cell recovery was also faster in patients with short alemtuzumab exposure ($P = .027$). No differences were observed in B cell, neutrophil, and monocyte recovery.

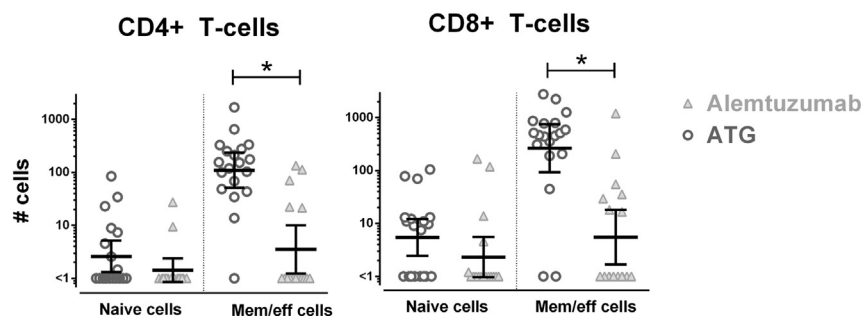


Figure 2. Naïve and memory/effector T cell counts. Absolute counts of naïve and memory/effector CD4 and CD8 T cell counts 2 months after HSCT of patients who received ATG (dark circles) and alemtuzumab (light grey triangles). Within the CD3/CD4 $^{+}$ and CD3/CD8 $^{+}$ T cell subsets naïve cells were phenotypically defined as CD45RA $^{+}$ CCR7 $^{+}$ and memory/effector cells as CD45RA $^{-}$ and/or CCR7 $^{-}$. The horizontal black lines and error bars indicate geometric mean and its lower and upper 95% CI. Values on y-axes represent cells/ μ L. Cell counts were compared using multivariate linear regression analyses with correction for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, HLA match, CMV serostatus of the patient, and CMV serostatus of the donor. An asterisk indicates a significant difference between ATG and alemtuzumab-treated patients ($P < .05$).

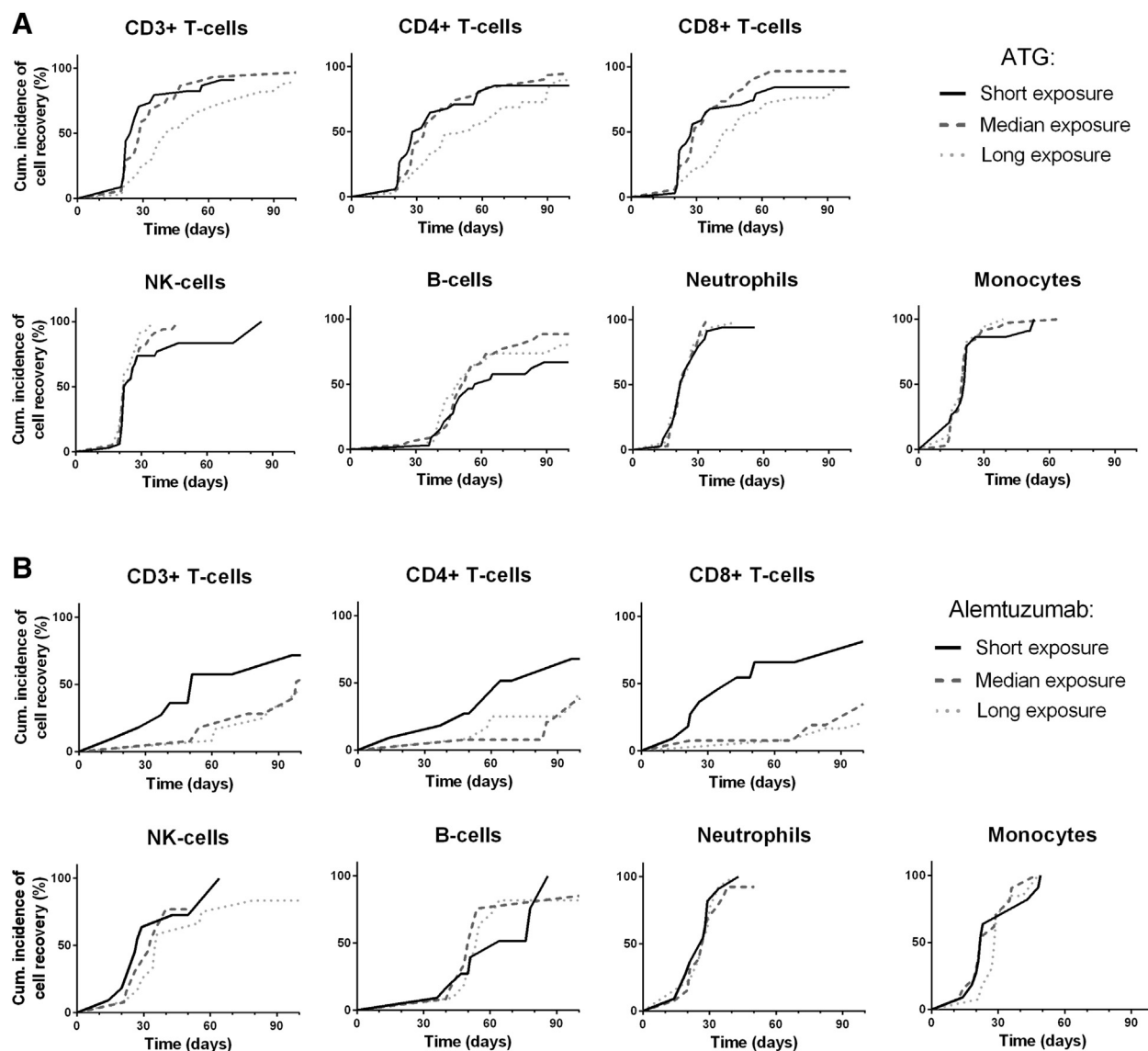


Figure 3. Impact of drug exposure on immune recovery. Cumulative incidence of recovery of cell (sub)populations related to length of exposure to (A) ATG and (B) alemtuzumab after HSCT. Recovery was defined as reaching absolute cell counts above $.5 \times 10^9/L$ for neutrophils, above $100/\mu L$ for CD3 T cells and monocytes, and above $50/\mu L$ for CD4 T cells, CD8 T cells, NK cells, and B cells. Patients were divided in 3 groups based on the length of lympholytic drug exposure: short exposure (black lines), median exposure (grey dashed lines), and long exposure (grey dotted lines). Days after transplantation are shown on all x-axes.

GVHD

The incidence of any grade of acute GVHD (aGVHD) was higher in the ATG group ($P = .030$). In multivariate analysis, the risks of aGVHD and severe aGVHD were lower in the alemtuzumab group compared with the ATG group, although not significantly (HR, .34; $P = .175$ and HR, .20; $P = .130$, respectively) (Table 3).

No differences were observed in the incidence and risk of chronic GVHD (cGVHD) between the ATG group and the alemtuzumab group ($P = .703$ and $P = .758$, respectively).

Viral Reactivations

No differences in CMV reactivations were observed in univariate or multivariate analysis (Table 3). The incidence of EBV reactivations was higher in ATG-treated patients (30%) compared with alemtuzumab-treated patients (8%, $P = .011$). Multivariate analysis also showed a lower risk of EBV reactivations in alemtuzumab-treated patients (HR, .23; $P = .039$)

(Table 3). Whereas patients who received alemtuzumab were less likely to have an EBV reactivation, their risk of HAdV reactivation tended to be higher compared with ATG-treated patients (HR, 3.56; $P = .103$) (Table 3).

Chimerism

The proportion of patients with $\geq 95\%$ donor chimerism in PBMC was similar for the ATG group and the alemtuzumab group early after transplantation (Table 4). In multivariate analysis, which, among others, included correction for diagnosis and conditioning regimen, no differences in donor chimerism were observed 1 and 2 months after transplantation between the 2 serotherapy groups (Table 4). Alemtuzumab was associated with a significant decrease of the proportion of patients with $\geq 95\%$ donor chimerism 3 and 6 months after transplantation (HR, .17; $P = .042$ and HR, .06; $P = .037$, respectively) (Table 4).

Table 3
Incidence, Univariate, and Multivariate Analysis of GVHD and Viral Reactivations

	Numbers/Total (%) [*]		Log Rank	Multivariate Analysis [†]	
	ATG	Alemtuzumab	P Value	HR (95% CI)	P Value
GVHD					
aGVHD I-IV	28/110 (25)	3/38 (8)	.030	.34 (.07–1.62)	.175
aGVHD II-IV	17/110 (15)	2/38 (5)	.119	.20 (.02–1.62)	.130
cGVHD	10/97 (10)	3/26 (12)	.703	.76 (.13–4.34)	.758
Viral reactivations					
CMV [‡]	30/67 (45)	8/21 (38)	.522	.78 (.30–2.04)	.611
EBV [§]	31/104 (30)	3/38 (8)	.011	.23 (.06–.93)	.039
HAdV	9/110 (8)	5/38 (13)	.367	3.56 (.78–16.3)	.103

* Descriptive values: number of patients with the described condition/total of evaluated patients (%).

† Cox proportional hazard regression models, corrected for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, and HLA match (and GVHD prophylaxis in case of GVHD analyses).

‡ Only CMV-seropositive patients and seronegative patients with a seropositive donor were included in this analysis.

§ Only EBV-seropositive patients and seronegative patients with a seropositive donor were included in this analysis.

Survival and Relapse

Overall mortality was 42% in the alemtuzumab group versus 21% in the ATG group ($P = .003$) (Figure 4A, Table 5). Multivariate analysis also showed that alemtuzumab was associated with a higher overall mortality risk than ATG (HR, 2.59; $P = .020$) (Table 5).

Compared with the ATG group, EFS was lower in the alemtuzumab group ($P < .001$) (Figure 4B). The risk of treatment failure (relapse, death, or retransplantation; inverse of EFS) was higher for patients who received alemtuzumab (HR, 3.14; $P < .001$) (Table 5). The probability of NRM was higher for patients treated with alemtuzumab in univariate analysis ($P = .018$) (Figure 4C) but not in multivariate analysis (HR, 2.89; $P = .138$) (Table 5). Furthermore, the incidence of relapse was higher in the alemtuzumab group ($P = .024$) (Figure 4D). Multivariate analysis also showed that alemtuzumab-treated patients were more at risk to relapse compared with ATG-treated patients (HR, 5.10; $P = .001$) (Table 5).

Table 4
Incidence, Univariate, and Multivariate analysis of Donor Chimerism

	Numbers/Total (%) [*]		Log Rank	Multivariate Analysis [†]	
	ATG	Alemtuzumab	P Value	HR (95% CI)	P Value
1 Month			1.000	.43 (.10-1.77)	.244
<95%	19 (17)	6 (18)			
≥95%	90 (83)	27 (82)			
2 Months			1.000	.34 (.04-2.81)	.319
<95%	12 (12)	4 (13)			
≥95%	90 (88)	27 (87)			
3 Months			.072	.17 (.03-.94)	.042
<95%	15 (16)	8 (36)			
≥95%	77 (84)	14 (64)			
6 Months			.050	.06 (.00-.84)	.037
<95%	15 (18)	5 (45)			
≥95%	69 (82)	6 (55)			
12 Months			.355	.11 (.08-1.53)	.100
<95%	9 (13)	3 (27)			
≥95%	61 (87)	8 (73)			

* For each time point (1, 2, 3, 6, and 12 months) the distribution of patients who had a donor chimerism of at least 95% or less than 95% is shown.

† Binary regression models, corrected for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, and HLA match.

Differences in Patient Characteristics Between Serotherapy Groups

As shown in Table 1, there are some differences in patient characteristics between the 2 serotherapy groups. Patients treated with alemtuzumab significantly more often received a T cell–depleted graft, were less likely to have an MA conditioning regimen, and were significantly older than patients treated with ATG. Therefore, we investigated whether these 3 characteristics introduced a possible bias. Excluding the 22 patients who received an NMA conditioning regimen, alemtuzumab was still associated with a significantly higher overall mortality risk (HR, 2.30; $P = .049$; $n = 126$) in multivariate regression analysis. Similarly, when the 12 patients with a T cell–depleted graft were excluded, the overall mortality risk remained significantly higher in the alemtuzumab group compared with the ATG group (HR, 3.00; $P = .009$; $n = 136$). The age of the patients in the ATG group ranged from .4 to 18.6 years, whereas in the alemtuzumab group, the youngest patient was 3.9 years old (Table 1). The overall mortality risk remained significantly higher in the alemtuzumab group compared with the ATG group (HR, 3.03; $P = .015$; $n = 116$) after excluding all patients younger than 4 years.

The same was observed for T cell recovery. Alemtuzumab was associated with a significantly delayed T cell recovery compared with ATG in the total cohort (Table 2), in the subcohort with only MA conditioning regimens included, in the subcohort with all T cell–depleted grafts excluded, and in the subcohort with all patients younger than 4 years excluded ($P \leq .002$ for all [sub]cohorts).

DISCUSSION

In this study, the effect of ATG as part of the conditioning regimen on clinical and immunological outcome parameters after stem cell transplantation in children was compared with alemtuzumab. Our results show that after HSCT, the recovery of lymphocytes, ie, CD3 T cells, CD4, and CD8 T cell subsets, and to a less extent NK cells, was significantly delayed after alemtuzumab treatment compared with after ATG. In addition, length of lympholytic exposure to active ATG or alemtuzumab after HSCT had an impact on the kinetics of immune recovery. Furthermore, our data show that ATG was associated with a higher OS and EFS, lower relapse risk, more EBV reactivations, and reached $\geq 95\%$ donor chimerism in a significantly higher proportion of patients. Patients treated with alemtuzumab showed a trend to a lower incidence of acute GVHD and more HAdV reactivations.

A similar differential effect of alemtuzumab and ATG on T cell recovery as found in our study has been reported in adults receiving allo-HSCT mainly after reduced-intensity conditioning [14]. Shah et al. [13] studied the impact of serotherapy on the incidence of severe GVHD in a small cohort of children and reported a univariate significantly slower CD3 T cell recovery after alemtuzumab compared with after ATG. To our knowledge, there are no other studies that have compared the effects of ATG and alemtuzumab on the recovery of the different lymphocyte subsets in children.

Alemtuzumab affected both naïve and memory/effector CD4 and CD8 T cells, whereas ATG mostly affected naïve CD4 and CD8 T cells. This differential effect of ATG on T cell differentiation stages was also reported by Bosch et al. [6]. No studies are available for alemtuzumab.

Previously, it has been shown that both a higher dose of ATG [29] and administration of ATG closer to the day of transplantation [30] were associated with a slower T cell recovery after HSCT. In the present study, we used the blood

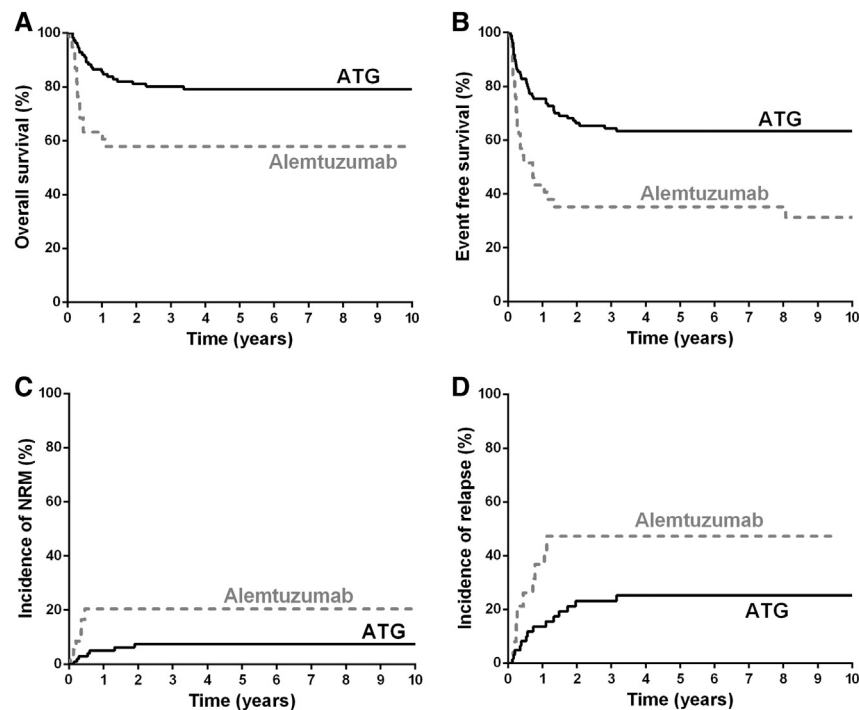


Figure 4. Kaplan-Meier survival curves. Probability of (A) overall survival, (B) EFS, (C) NRM, and (D) relapse (risk of recurrence of malignant disease) after HSCT with ATG (black lines) and alemtuzumab (grey dashed lines) as part of the conditioning regimen.

concentrations of active ATG and alemtuzumab to determine the impact of the length of lympholytic exposure to these antibodies on immune reconstitution. Our results show that this determines the pace of CD3, CD4, and CD8 T cell recovery. This result is concordant with data from Bosch et al. [6], in which higher levels of active ATG 1 week and 1 month after transplantation were associated with decreased CD4 and CD8 T cell counts in adults. In a recent publication on the impact of ATG serum levels on acute and chronic GVHD, Chawla et al. [31] also reported a significant correlation between the decrease in active ATG level and the T cell count

1 month after HSCT. Whereas longer ATG exposure correlated with a slower T cell recovery, NK cell recovery was faster in patients with long active ATG exposure in our study. Bosch et al. reported increased NK cell counts at day 28 after HSCT in patients with higher ATG levels 1 week after transplantation [6]. The reason for the faster NK cell recovery after longer ATG exposure observed in this and our study remains unclear, but might be related to favorable expansion of NK cells in a T lymphopenic setting [32]. A direct effect of ATG on NK cells cannot be excluded [33].

In the alemtuzumab group, longer drug exposure was associated with delayed T cell and NK cell recovery. This is in line with data from Juliusson et al. [17] showing that a higher dose of alemtuzumab, subcutaneously administered to adults receiving allogeneic HSCT after NMA conditioning, correlated with lower CD4 and CD8 counts after HSCT. Gärtner et al. [34] reported that infusion of a higher dose of alemtuzumab was associated with a significantly lower increase of NK cell counts 1 month after transplantation, which is concordant with our findings.

Our data are the first to connect the level of lympholytic drug exposure to immune recovery and outcome after HSCT in children. We were able to show that longer lympholytic drug exposure delayed T cell recovery in both ATG- and alemtuzumab-treated patients. Our data suggest that the length of lympholytic ATG exposure was not associated with OS, EFS, NRM, and relapse (Figure S2, Supplementary Data). Multivariate analysis showed a nonsignificant trend to less severe aGVHD and more EBV reactivations after long ATG exposure (Figure S2). These observations are comparable to those recently published by Chawla et al., which showed in adults an association of high levels of exposure to ATG with a low incidence of acute (grade II to IV) and cGVHD and with a high incidence of EBV post-transplantation lymphoproliferative disorder. They reported no significant associations

Table 5
Incidence, Univariate, and Multivariate Analysis of Overall Mortality, Treatment Failure, NRM, and Relapse

	Numbers/Total (%) ^a		Log Rank P Value	Multivariate Analysis [†]	
	ATG	Alemtuzumab		HR (95% CI)	P Value
Overall mortality [‡]	23/110 (21)	16/38 (42)	.003	2.59 (1.16–5.75)	.020
Treatment failure [§]	39/110 (36)	25/38 (66)	<.001	3.14 (1.66–5.95)	<.001
NRM	7/110 (6)	6/38 (16)	.018	2.89 (.71–11.77)	.138
Relapse	14/64 (22)	10/27 (37)	.024	5.10 (1.95–13.37)	.001

^a Descriptive values: number of patients with described condition/total evaluated patients (%).

[†] Cox proportional hazard regression models, corrected for age, patient sex, conditioning regimen, graft manipulation, diagnosis, graft type, and HLA match.

[‡] Overall mortality (risk of death, regardless of the cause) is the inverse of overall survival.

[§] Treatment failure (risk of relapse, retransplantation, or death) is the inverse of EFS.

^{||} Only patients with malignant diseases were included in relapse (risk of recurrence of malignant disease) analyses.

between ATG levels and relapse, death, or nonrelapse-associated death [31]. Our alemtuzumab group was too small to determine the impact of exposure levels. Comparison of the low exposure alemtuzumab subgroup with the high exposure ATG subgroup showed no significant difference in recovery of the various cell population, but a higher incidence of overall mortality, treatment failure, and relapse of malignant disease was observed in the low exposure alemtuzumab subgroup. (Tables S1 and S2, Supplementary Data).

T cell depletion with ATG or alemtuzumab is a commonly used strategy for prevention of GVHD [5,7,16,35,36]. Our data showed a nonsignificant lower incidence of aGVHD after alemtuzumab. In multivariate analysis, the risks of aGVHD and severe aGVHD were lower in the alemtuzumab group, although not significantly. This is in accordance with data from a large multicenter study by Veys et al. [5] as well other studies [13,37]. It is attractive to speculate that a reduced alloreactivity after alemtuzumab treatment caused by the strong delay of the recovery of naïve T cells as well as memory/effector T cells may also explain the relatively high proportion of patients with donor chimerism <95% in children receiving this serotherapy.

In the present study, we found more EBV reactivations in the ATG group than in the alemtuzumab group. On the other hand, alemtuzumab-treated patients tended to be more at risk for HAdV reactivations. The latter was supported by data on HAdV infection and HAdV-related disease reported by Myers et al. [20]. However, they did not observe a difference in EBV infections between both serotherapy groups. On the other hand, Cohen et al. reported in pediatric patients after HSCT significantly more EBV viremia after ATG compared with after alemtuzumab treatment [38].

Although ATG and alemtuzumab are frequently used in pediatric HSCT, there are only a few studies comparing the effect of ATG and alemtuzumab on survival outcomes in children [5,13,20]. In these studies, no significant differences in survival rates between ATG- and alemtuzumab-treated patients were found. Our study is the first in which significantly lower survival rates became apparent after alemtuzumab compared with after ATG treatment. Also, the risk of treatment failure and relapse were significantly higher in alemtuzumab-treated patients. This is possibly related to the delayed T cell recovery after an alemtuzumab-containing conditioning regimen. Although alemtuzumab was associated with a lower incidence of aGVHD and slower T cell recovery, treatment with alemtuzumab did not translate in an overall higher incidence of viral reactivations. On the other hand, sustained complete donor chimerism was established in a lower proportion of alemtuzumab-treated patients and they more often received a second HSCT procedure, a stem cell boost, or donor lymphocyte infusion to enhance hematopoiesis, to increase donor chimerism, and to support immune reconstitution, respectively (data not shown). This indicates that alemtuzumab-treated patients had more transplantation-related complications.

One of the limitations of the present study is the diversity of the study population. Although we performed multivariate analyses to determine the impact of serotherapy on different outcome parameters using known prognostic factors, there may still be yet undefined factors that influence the prognosis. Furthermore, this is a retrospective study and, therefore, more subjected to bias than a prospective randomized controlled trial.

This is the first single-center study in a large cohort of children to investigate the impact of ATG and alemtuzumab

on immune reconstitution and clinical outcomes using multivariate regression analyses. Our data show that a conditioning regimen containing alemtuzumab delays recovery of CD3, CD4, CD8 T, cells and NK cells compared with a regimen containing ATG. This translated into a lower incidence of aGVHD but also into higher risks of overall mortality, treatment failure, and relapse of the original disease in children with different benign and malignant diseases. Despite these results, the use of alemtuzumab is still recommended in patients who received ATG treatment before because of the risks of sensitization and antibody formation against ATG [12,39]. Our data are also the first to connect the length of lympholytic drug exposure to immune recovery and outcome after HSCT in children: longer drug exposure delayed the T cell recovery in both ATG- and alemtuzumab-treated patients. Detailed pharmacokinetic/pharmacodynamic (PK/PD) analyses in a large cohort of patients are needed to develop an algorithm aiming at optimizing the serotherapy-containing conditioning regimen for individual patients.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.bbmt.2014.11.674>

REFERENCES

- Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006;354:1813–1826.
- Wayne AS, Baird K, Egeler RM. Hematopoietic stem cell transplantation for leukemia. *Pediatr Clin North Am*. 2010;57:1–25.
- Moore AS, Shaw PJ, Hallahan AR, et al. Haemopoietic stem cell transplantation for children in Australia and New Zealand, 1998–2006: a report on behalf of the Australasian Bone Marrow Transplant Recipient Registry and the Australian and New Zealand Children's Haematology Oncology Group. *Med J Aust*. 2009;190:121–125.
- Vossen JM, Donker AE, Heemskerk MB, et al. Unrelated donor marrow transplantation in children: transplant policy and outcome in Leiden Paediatrics SCT-Centre. *Bone Marrow Transplant*. 2010;45:87–95.
- Veys P, Wynn RF, Ahn KW, et al. Impact of immune modulation with in vivo T cell depletion and myeloablative total body irradiation conditioning on outcomes after unrelated donor transplantation for childhood acute lymphoblastic leukemia. *Blood*. 2012;119:6155–6161.
- Bosch M, Dhadda M, Hoegh-Petersen M, et al. Immune reconstitution after anti-thymocyte globulin-conditioned hematopoietic cell transplantation. *Cytotherapy*. 2012;14:1258–1275.
- Hoegh-Petersen M, Amin MA, Liu Y, et al. Anti-thymocyte globulins capable of binding to T and B cells reduce graft-vs-host disease without increasing relapse. *Bone Marrow Transplant*. 2013;48:105–114.
- Mohty M. Mechanisms of action of antithymocyte globulin: T cell depletion and beyond. *Leukemia*. 2007;21:1387–1394.
- Na IK, Wittenbecher F, Dziubianau M, et al. Rabbit antithymocyte globulin (Thymoglobulin(R)) impairs the thymic output of both conventional and regulatory CD4+ T cells after allogeneic hematopoietic stem cell transplantation in adult patients. *Haematologica*. 2013;98:23–30.
- Stauch D, Dernier A, Sarmiento ME, et al. Targeting of natural killer cells by rabbit antithymocyte globulin and campath-1H: similar effects independent of specificity. *PLoS One*. 2009;4:e4709.
- Ayuk F, Diyachenko G, Zabelina T, et al. Comparison of two doses of antithymocyte globulin in patients undergoing matched unrelated

- donor allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2008;14:913–919.
12. Jol-van der Zijde CM, Bredius RG, Jansen-Hoogendijk AM, et al. IgG antibodies to ATG early after pediatric hematopoietic SCT increase the risk of acute GVHD. *Bone Marrow Transplant*. 2012;47:360–368.
 13. Shah AJ, Kapoor N, Crooks GM, et al. The effects of Campath 1H upon graft-versus-host disease, infection, relapse, and immune reconstitution in recipients of pediatric unrelated transplants. *Biol Blood Marrow Transplant*. 2007;13:584–593.
 14. Schmidt-Hieber M, Schwarck S, Stroux A, et al. Immune reconstitution and cytomegalovirus infection after allogeneic stem cell transplantation: the important impact of in vivo T cell depletion. *Int J Hematol*. 2010;91:877–885.
 15. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. *Curr Opin Hematol*. 2012;19:324–335.
 16. Soiffer RJ, Leraedemacher J, Ho V, et al. Impact of immune modulation with anti-T cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood*. 2011;117:6963–6970.
 17. Juliusson G, Theorin N, Karlsson K, et al. Subcutaneous alemtuzumab vs ATG in adjusted conditioning for allogeneic transplantation: influence of Campath dose on lymphoid recovery, mixed chimerism and survival. *Bone Marrow Transplant*. 2006;37:503–510.
 18. Rebello P, Cwynarski K, Varughese M, et al. Pharmacokinetics of CAMPATH-1H in BMT patients. *Cytotherapy*. 2001;3:261–267.
 19. Waller EK, Langston AA, Lonial S, et al. Pharmacokinetics and pharmacodynamics of anti-thymocyte globulin in recipients of partially HLA-matched blood hematopoietic progenitor cell transplantation. *Biol Blood Marrow Transplant*. 2003;9:460–471.
 20. Myers GD, Krance RA, Weiss H, et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant*. 2005;36:1001–1008.
 21. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant*. 2009;15:1628–1633.
 22. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974;18:295–304.
 23. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204–217.
 24. Niesters HG, van Esser J, Fries E, et al. Development of a real-time quantitative assay for detection of Epstein-Barr virus. *J Clin Microbiol*. 2000;38:712–715.
 25. Lugthart G, van Oostaijen-Ten Dam MM, Jol-van der Zijde CM, et al. Early cytomegalovirus reactivation leaves a specific and dynamic imprint on the reconstituting T cell compartment long-term after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20:655–661.
 26. Kalpoe JS, Kroes AC, de Jong MD, et al. Validation of clinical application of cytomegalovirus plasma DNA load measurement and definition of treatment criteria by analysis of correlation to antigen detection. *J Clin Microbiol*. 2004;42:1498–1504.
 27. Claas EC, Schilham MW, de Brouwer CS, et al. Internally controlled real-time PCR monitoring of adenovirus DNA load in serum or plasma of transplant recipients. *J Clin Microbiol*. 2005;43:1738–1744.
 28. Lankester AC, Bierings MB, van Wering ER, et al. Preemptive alloimmune intervention in high-risk pediatric acute lymphoblastic leukemia patients guided by minimal residual disease level before stem cell transplantation. *Leukemia*. 2010;24:1462–1469.
 29. Duval M, Pedron B, Rohrlach P, et al. Immune reconstitution after haematopoietic transplantation with two different doses of pre-graft antithymocyte globulin. *Bone Marrow Transplant*. 2002;30:421–426.
 30. Lindemans CA, Chiesa R, Amrolia PJ, et al. Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood*. 2014;123:126–132.
 31. Chawla S, Dharmani-Khan P, Liu Y, et al. High serum level of antithymocyte globulin immediately before graft infusion is associated with a low likelihood of chronic, but not acute, graft-versus-host disease. *Biol Blood Marrow Transplant*. 2014;20:1156–1162.
 32. Jamieson AM, Isnard P, Dorfman JR, et al. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *J Immunol*. 2004;172:864–870.
 33. Dalle JH, Dardari R, Menezes J, et al. Binding of thymoglobulin to natural killer cells leads to cell activation and interferon-gamma production. *Transplantation*. 2009;87:473–481.
 34. Gartner F, Hieke S, Finke J, Bertz H. Lowering the alemtuzumab dose in reduced intensity conditioning allogeneic hematopoietic cell transplantation is associated with a favorable early intense natural killer cell recovery. *Cytotherapy*. 2013;15:1237–1244.
 35. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98:2942–2947.
 36. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10:855–864.
 37. Norlin AC, Remberger M. A comparison of Campath and Thymoglobulin as part of the conditioning before allogeneic hematopoietic stem cell transplantation. *Eur J Haematol*. 2011;86:57–66.
 38. Cohen J, Gandhi M, Naik P, et al. Increased incidence of EBV-related disease following paediatric stem cell transplantation with reduced-intensity conditioning. *Br J Haematol*. 2005;129:229–239.
 39. Jol-van der Zijde CM, Bredius RG, Jansen-Hoogendijk AM, et al. Antibodies to anti-thymocyte globulin in aplastic anemia patients have a negative impact on hematopoietic SCT. *Bone Marrow Transplant*. 2012;47:1256–1258.