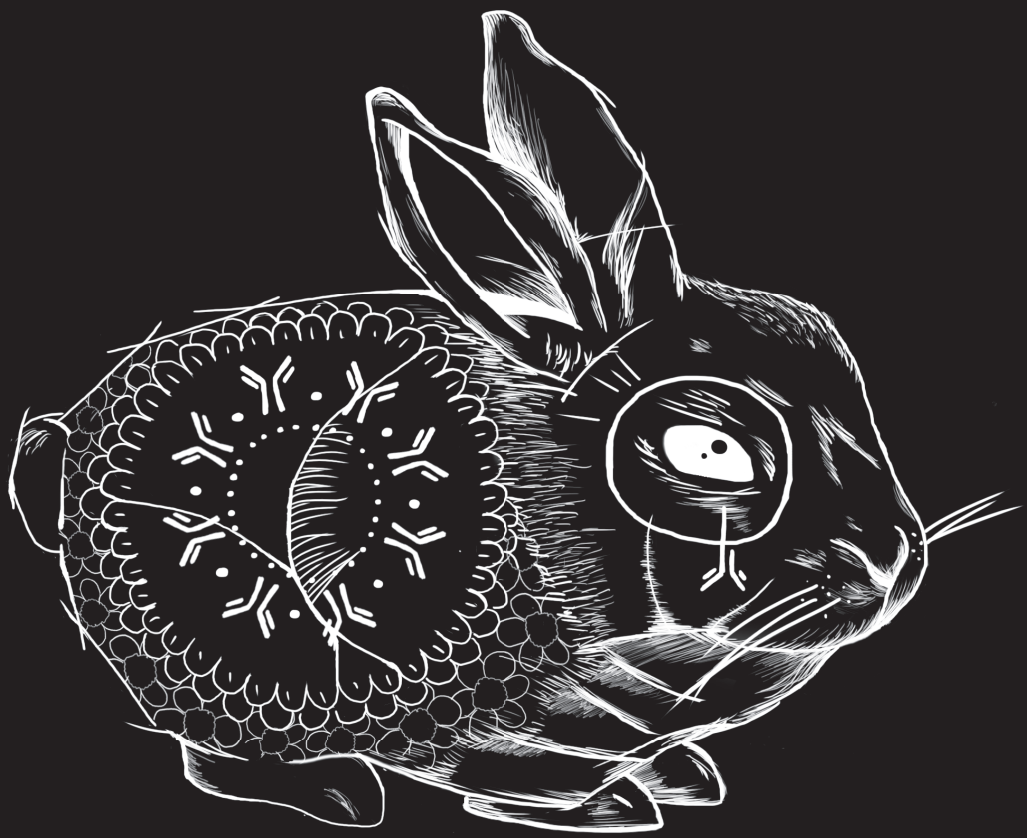
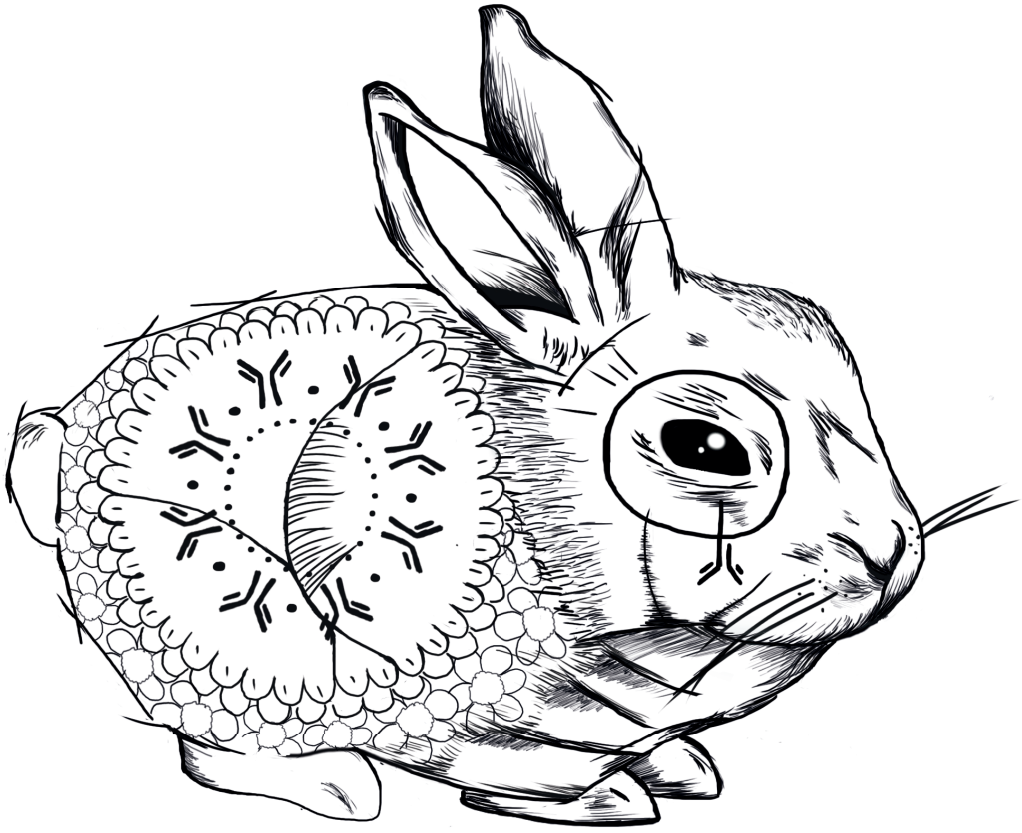


PART III

Exposure-Response Relationships of Serotherapy



Chapter 5

Association between Anti-Thymocyte Globulin Exposure and CD4+ Immune Reconstitution in Paediatric Haematopoietic Cell Transplantation: a Retrospective Pharmacodynamic Cohort Analysis

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SUMMARY

Background

Anti-thymocyte globulin (ATG) was introduced into the conditioning regimen in haemopoietic cell transplantation (HCT) to prevent graft-versus-host-disease (GvHD) and graft failure. However, ATG can also cause delayed immune reconstitution of donor T cells. We studied the relation between exposure to active ATG and clinical outcomes in children.

Methods

In this retrospective analysis, all patients (age 0·2–23 years) receiving their first HCT between April 1, 2004, and April 1, 2012, who received ATG (thymoglobulin) in two Dutch paediatric HCT programmes were included. The cumulative dose of ATG was chosen according to local protocols and was given intravenously over 4 days consecutively. ATG exposure measures (maximum concentration, concentration at time of HCT, clearance, days to reach a concentration below the lympholytic concentration of one arbitrary unit [AU] per mL, total area under the curve [AUC], AUC before HCT, and AUC after HCT) were calculated using a validated population pharmacokinetic model. The main outcome of interest was immune reconstitution (defined as CD4+ T cells >0·05 × 10^9 cells per L in two consecutive measurements within 100 days). Other outcomes of interest were survival, acute and chronic GvHD, and graft failure. We used Cox proportional hazard models, logistic regression models, and Fine-Gray competing risk regressions for analyses.

Findings

251 patients were included. The chance of successful immune reconstitution decreased as the ATG AUC after HCT increased (odds ratio 0·991, 95% CI 0·987–0·996; p<0·0001). Within the cord blood group, we noted decreased immune reconstitution above the lowest AUC quartile (≥20 AU × day/mL; p=0·0024), whereas in the bone marrow or peripheral blood stem cell group, decreased immune reconstitution was noted only in the highest quartile (≥100 AU × day/mL; p=0·0024). Successful immune reconstitution by day 100 was associated with increased overall survival (hazard ratio [HR] 0·49, 95% CI 0·29–0·81; p=0·0047) caused by reduced non-relapse mortality (0·40, 0·21–0·77; p=0·0062), and relapse-related mortality in myeloid leukaemia (0·25, 0·08–0·76; p=0·015). An AUC before transplantation of at least 40 AU × day/mL resulted in a lower incidence of acute GvHD (grade 2–4 HR 0·979, 95% CI 0·963–0·994; p=0·0081; and grade 3–4 0·975, 0·952–0·998; p=0·033), chronic GvHD (0·983, 0·968–0·998; p=0·029), and graft failure (0·981, 0·965–0·997; p=0·020) compared with an AUC of less than 40 AU × day/mL.

Interpretation

These results stress the importance of improving the efficacy and safety of ATG in HCT by amending dosage and timing. Individualised dosing and timing of ATG to aim for optimum exposure before and after HCT could result in improved outcomes after paediatric HCT.

Funding

Dutch Organization for Scientific Research.

INTRODUCTION

Haemopoietic cell transplantation (HCT) is a curative treatment for various underlying malignancies and benign disorders in children. To reduce the risk of graft failure and graft-versus-host disease (GvHD), in-vivo lymphodepletion through serotherapy, such as by anti- thymocyte globulin (ATG), was introduced into conditioning regimens.[1] Although serotherapy has led to a decreased incidence of GvHD and graft failure, side- effects such as viral reactivation and a loss of graft- versus-leukaemia effect have emerged, caused by delayed or absent early T-cell reconstitution after HCT.[2–6]

Although ATG is a commonly used serotherapy drug, its optimum therapeutic window and the timing of treatment have not been defined. Nevertheless, the dosage of ATG has been suggested to affect outcome in terms of GvHD, graft failure, and immune reconstitution.[3,7–9] Since ATG has a half-life of 5–14 days, which means patients are exposed to ATG both before and after HCT,[8–11] the timing of ATG relative to the HCT (i.e., starting day –9 or day –5) also has an effect on outcome.[2] The variable and unpredictable exposure to ATG after comparable doses of ATG further complicates this treatment.[9,12,13] Moreover, T-cell reconstitution[7,14] and the risk of GvHD[2,3,15] differs between cord blood, bone marrow, or peripheral blood stem cell sources, suggesting that optimum exposure might vary between graft types.

Furthermore, different products of ATG are not biosimilar; in this study, we focus on thymoglobulin (Genzyme, Cambridge, MA, USA) because it is the most frequently used type of ATG in HCT. When treating children, developmental pharmacokinetics also needs to be taken into account. The commonly used dosing regimen of thymoglobulin in HCT is a dose of 2·5 mg/kg for 4 days consecutively starting on day –5. This results in markedly different exposure to ATG between age groups, with older children having a disproportionately higher exposure because clearance per kg is lower in older than in younger children.[16] Also, a low lymphocyte count at the time of ATG infusion, which is the target for ATG and therefore its elimination pathway, leads to high ATG exposure.[16]

We aimed to assess the relation between ATG exposure, calculated using a recently developed pharmacokinetic model,[16] and clinical outcome. To achieve this, we did a retrospective analysis to relate different exposure measures of the pharmacologically active fraction of ATG (hereafter referred to as ATG) to various outcome parameters of HCT, such as immune reconstitution, GvHD, graft failure, and survival.

METHODS

Study design and patients

In this analysis, we included all patients (age 0·2–23 years) who received allogeneic HCT with ATG (thymoglobulin only) as part of the conditioning regimen who were enrolled at two paediatric HCT centres in the Netherlands (University Medical Center Utrecht [UMCU], Utrecht, and Leiden University Medical Center [LUMC], Leiden) from April 1, 2004, to April 1, 2012. Only patients undergoing their first HCT were included; there was no restriction on the indication, cell source used, or dose of ATG used. Consecutive patients were included. We excluded patients who were in receipt of serotherapy other than thymoglobulin within 3 months before HCT and those who developed neutralising IgG anti-ATG antibodies within 1 month after HCT. Clinical data were collected prospectively and registered to the clinical database. Additionally, blood samples were prospectively collected for measurement of ATG concentrations.

Minimum follow-up for surviving patients was 6 months. Patients were included and data collected after written informed consent was obtained in accordance with the Declaration of Helsinki. Institutional ethical committee approval for sample and data collection was obtained through trial numbers 05/143 and 11/063-k (UMCU) and P01.028 (LUMC).

Procedures

According to national and international protocols, patients typically received a cumulative dose of 10 mg/kg ATG (thymoglobulin); the infusion of the first dose was started a median of 5 days (range 1–19) before transplantation. The daily dose was administered as a continuous 4 h infusion at constant rate (UMCU) or as an infusion with an increasing rate over 4–5 h (LUMC). According to the local protocol, patients weighing over 40 kg who were treated from 2010 onwards in UMCU received a lower dose of 7·5 mg/kg ATG. Patients treated from 2010 onwards with a cord blood transplant in UMCU received ATG at day –9 instead of day –5. According to local protocol, patients with haemophagocytic lymphohistiocytosis received higher doses of ATG.

Conditioning regimens were given according to national and international protocols. For busulfan-containing regimens, which were given intravenously, therapeutic drug monitoring was used to aim for an area under the curve (AUC) of 75–95 mg × h/day in a myeloablative setting.[17,18] Reduced-intensity conditioning with ATG was reserved for patients with severe aplastic anaemia and Fanconi's anaemia. Patients who were receiving a reduced intensity conditioning for other indications received alemtuzumab as serotherapy and were therefore not included in this study. Patients received gut decontamination, infection prophylaxis, and GvHD prophylaxis according to local protocols, as described previously.[12,18] GvHD prophylaxis mostly consisted of cyclosporin A, with a target trough concentration of 150–250 μg/L controlled by therapeutic drug monitoring, combined with either prednisolone 1 mg/kg per day for patients receiving a cord blood transplant; or mycophenolate mofetil 15 mg/kg per day or methotrexate 10 mg/m² (on days 1, 3, and 6 after transplantation) for patients receiving an unrelated donor transplant. GvHD prophylaxis was given intravenously and cyclosporin A, mycophenolate mofetil, and prednisolone were switched to oral at discharge. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Patients received clemastine and prednisolone (2 mg/kg) intravenously before and during ATG infusion.

After reaching a leucocyte count of at least $0{\cdot}3 \times 10^9$ cells per L, lymphocyte subsets, including CD3+, CD4+, and CD8+ T cells, B cells, and natural killer cells, were measured by flow cytometry at least every other week up to 12 weeks after HCT and monthly thereafter up to 6 months after HCT.

Serum ATG concentrations were measured in blood samples collected before and after each infusion in LUMC only and every other week thereafter in both centres.[16] These data were used to develop and validate a population pharmacokinetic model for ATG.16 Using these findings and this model, concentration–time profiles including all ATG exposure measurements of interest could be accurately calculated in all patients. ATG exposure measures of interest were maximum concentration, concentration at time of HCT, clearance, days to reach a concentration below the lympholytic concentration of one arbitrary unit (AU) per mL,[11] total AUC, AUC before HCT, and AUC after HCT. All ATG exposure measures were calculated using NONMEM 7.2.0.

Outcomes

The main outcome of interest was immune reconstitution, defined as repopulation of CD4+ T lymphocytes. This definition was based on their central role in adaptive immunity and in activating phagocytic cells, as well as their relation with survival.[7] A CD4+ T-lymphocyte count of at least $0{\cdot}05 \times 10^9$ cells per L in two consecutive measurements within 100 days after HCT was deemed successful immune reconstitution. This count was chosen because

counts under this limit are associated with a higher probability of viral reactivations.[2,19] Patients who died before 100 days of follow-up were assessed until the date of death.

We were also interested in the association between ATG exposure and overall survival, event-free survival, non-relapse and relapse mortality, acute and chronic GvHD, graft failure, and effect of graft type. We were also interested in the effect of immune reconstitution on the outcomes of interest. Overall survival was defined as the time from transplantation to last follow-up or death. Event-free survival was defined as survival from HCT to last contact whereby graft failure, relapse of disease, or death were regarded as events. All surviving patients were censored at date of last contact. Non-relapse mortality was defined as death due to causes other than relapse of a malignancy; relapse-related mortality was defined as death due to relapse of a malignancy. Acute GvHD (grade 2–4 and grade 3–4) was classified according to the Glucksberg[20] criteria and chronic GvHD (extensive vs no or limited) was classified according to the Shulman[21] criteria. Graft failure was defined as non- engraftment (i.e., autologous reconstitution) or graft rejection (i.e., secondary loss of donor chimerism). In case of non-engraftment, the time of non-engraftment was set at 60 days after HCT. Additionally, we analysed the association between CD3+, CD8+, and natural killer reconstitution and clinical endpoints.

Statistical analysis

Duration of follow-up was the time to the last assessment for patients who were alive at the end of the study, or death. We assessed the association between outcome and patient-related variables (age at transplant, sex, and cytomegalovirus status); disease (malignancy, primary immune deficiency, bone marrow failure, or benign non-primary immune deficiency); donor factors (HLA disparity and cytomegalovirus status); conditioning regimen (myeloablative or reduced intensity conditioning); and ATG exposure measures (maximum concentration, concentration at time of HCT, days to reach a concentration below the lympholytic concentration of 1 AU/mL, total AUC, AUC before HCT, and AUC after HCT). Clinical outcomes were analysed in subgroups in terms of the different ATG exposure or patient characteristics, including stem cell sources.

Variables associated with a p value less than 0·05 by univariate analysis were selected for testing in a multivariate analysis. Probabilities of event-free survival and overall survival were calculated using the Kaplan- Meier estimate; we used the two-sided log-rank test for univariate comparisons. Time-dependent outcomes were analysed using Cox proportional hazard models. For the endpoints non-relapse mortality, relapse-related mortality, acute GvHD, chronic GvHD, and graft failure we used Fine-Gray competing risk regressions.[22] For dichotomous variables, univariate and multivariate logistic regression analyses were done. Statistical analyses were done using R version 3.0.1.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RA and JJB had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

251 patients were included: 142 at LUMC and 109 at UMCU (table 1). Bone marrow (118 [47%] of 251) and cord blood (91 [36%]) were the main stem cell sources, and 116 (46%) patients had a malignant disease as the indication for HCT. Six (2%) patients (three from each institute) were excluded from the analysis because of neutralising IgG anti-ATG antibodies. Median follow-up was 111 weeks (IQR 32–209). A median of 11 (range 1–32) ATG serum samples were available per patient.

The AUC of ATG after transplantation was predictive of successful immune reconstitution of CD4+ T cells. With increasing exposure after HCT (ranging from 0 to 480 AU × day/mL), the chance of successful immune reconstitution before day 100 decreased for every one-point increase in the AUC (odds ratio 0·991, 95% CI 0·987–0·996; p<0·0001; figure 1A). In multivariate analyses, low AUC after HCT, a matched donor, and bone marrow or peripheral blood source of stem cells were associated with successful immune reconstitution. Table 2 lists a summary of the results of multivariate analyses of the effect of ATG exposure on clinical outcome parameters; the AUC after HCT affected immune reconstitution, whereas the AUC before HCT affected acute and chronic GvHD and graft failure.

We also examined the effect of graft type on immune reconstitution and survival. Figure 2 shows the effect of AUC after transplantation using ATG on CD4+ T-cell reconstitution and overall survival in all patients, those who received cord blood transplants, and those who received bone marrow or peripheral blood stem cell transplants. Few patients who had peripheral blood stem cell transplants were included in this study; therefore, bone marrow and peripheral blood stem cells were analysed as one group since outcomes, including immune reconstitution, were similar (data not shown). We divided the cohort into four groups according to exposure after HCT; cut-off values were chosen according to the quartiles of exposure after HCT (20, 50, and 100 AU × day/mL, respectively). Reconstitution of CD4+ T cells was markedly different between stem cell sources. Within the cord blood group, we noted decreased immune reconstitution above the lowest AUC quartile (<20 AU × day/mL vs ≥20 AU × day/mL; p=0·0024; figure 2B), whereas in the bone marrow or peripheral blood stem cell group, decreased immune reconstitution was noted only in the highest quartile (<100 AU × day/ mL vs ≥100 AU × day/mL; p=0·0024 figure 2C). The amount of CD4+

	Leiden	Utrecht	Total
Number of patients (n)	142	109	251
Male sex [n (%)]	96 (68)	61 (56)	157 (63)
Age (years)	6.2 (0.4-19)	5.9 (0.2-23)	6.2 (0.2-23)
Starting day ATG (days before transplantation)	5 (3-9)	5 (1-19)	5 (1-19)
Cumulative dose [n (%)]			
<9 mg/kg	4 (3)	5 (5)	9 (4)
9-11 mg/kg	136 (96)	97 (89)	233 (92)
>11 mg/kg	2 (1)	7 (6)	9 (4)
Number of concentration samples (mean per patient)	15	6	11
Diagnosis [n (%)]			
Malignancy	69 (49)	47 (43)	116 (46)
Immune deficiency	23 (16)	28 (26)	51 (20)
Bone marrow failure	6 (4)	9 (8)	15 (6)
Benign disorders	44 (31)	25 (23)	69 (28)
Stem cell source [n (%)]			
Bone marrow	89 (63)	29 (27)	118 (47)
Peripheral blood stem cells	30 (21)	12 (11)	42 (17)
Cordblood	23 (16)	68 (62)	91 (37)
Conditioning regimen* [n (%)]			
Reduced intensity	0 (0)	6 (5)	6 (2)
Chemotherapy-based myelo-ablative	103 (73)	88 (81)	191 (76)
TBI-based myelo-ablative	39 (27)	15 (14)	54 (22)
Positive CMV status of recipient [n (%)]	70 (49)	56 (51)	126 (50)
Positive CMV status of donor [n (%)]	57 (40)	19 (17)	76 (30)
Follow up (weeks)	126 (3-427)	84 (1-382)	111 (1-427)

Shown as median (range) unless otherwise specified

Table 1: Patient characteristics. Myelo-ablative chemo is defined as busulfan-based regimens (Targeting to a myelo-ablative exposure: > 70mg(-100)*h/L) or TBI > 7Gy unfractioned or > 10Gy fractioned, as per the international guidelines. RIC (Reduced Intensity Conditioning) contained Cyclophosphomide and Fludarabine. Values are shown as median (range) unless otherwise specified. TBI: total body irradiation, CMV: cytomegalovirus.

T-cell reconstitution over time in the lowest exposure group in cord blood (<20 AU × day/mL) was similar to that noted with bone marrow or peripheral blood stem cells (p=0·54).

Successful immune reconstitution at day 100 was associated with increased overall survival (hazard ratio [HR] 0·49, 95% CI 0·29–0·81; p=0·0047; figure 1B, table 2). In multivariate analyses, diagnosis group and mismatched donor were associated with worse survival. In all patients, overall survival was significantly different in the four post-transplant ATG

Variable	Univariate	Multivariate		
	p	HR	95% CI	p-value
Post-HCT AUC				
CD4+ Immune reconstitution	<0.00001	0.995	(0.991-0.998)	0.0049
Overall survival	<0.00001	1.001	(0.998-1.004)	0.50
Acute GvHD grade 2-4	<0.00001	0.992	(0.984-1.001)	0.078
Acute GvHD grade 3-4	<0.00001	0.995	(0.985-1.006)	0.40
Chronic extensive GvHD	<0.00001	0.998	(0.990-1.005)	0.57
Pre-HCT AUC				
Acute GvHD grade 2-4	<0.00001	0.979	(0.963-0.994)	0.0081
Acute GvHD grade 3-4	<0.00001	0.975	(0.952-0.998)	0.033
Chronic extensive GvHD	<0.00001	0.983	(0.968-0.998)	0.029
Graft failure	<0.00001	0.981	(0.965-0.997)	0.020
Immune reconstitution				
Overall Survival	0.0002	0.489	(0.295-0.809)	0.0047
Non-relapse mortality	0.0002	0.403	(0.213-0.774)	0.0062
Relapse mortality in myeloid leukaemia	0.024	0.248	(0.082-0.761)	0.015
Relapse mortality in lymphoid leukaemia	0.74	4.833	(0.931-25.091)	0.061

Table 2. Multivariate analysis. Overview of multivariate analyses using a Cox proportional hazard model.

exposure groups; there was weak evidence of improved survival in the group with lowest AUC after HCT in the cord blood group compared with other AUC groups (p=0·079, figure 2E). In the bone marrow and peripheral blood stem cell group, patients with the highest exposure showed worse survival compared with the other exposure groups (p=0·00021, figure 2F). Successful immune reconstitution by day 100 was also associated with increased event-free survival (HR 0·45, 95% CI 0·29–0·69; p<0·0001; figure 1B). Non-relapse mortality, relapse, and relapse-related mortality (all patients with relapse died) were affected by CD4+ reconstitution within the first 100 days (figure 3). The incidence of non-relapse mortality was lower in patients with successful than in those with unsuccessful immune reconstitution by day 100 (HR 0·40, 95% CI 0·21–0·77; p=0·0062; figure 3A, table 2). Part of the non-relapse mortality was caused by infectious deaths, which was also affected by immune reconstitution (p=0·010). Having a mismatched donor was associated with decreased non-relapse mortality.

Successful immune reconstitution by day 100 was associated with decreased relapse-related mortality in patients with myeloid malignancy (HR 0·25, 95% CI 0·08–0·76; p=0·015; figure 3B, table 2), but not in lymphoid malignancy (4·83, 0·93–25·09; p=0·061, figure 3C). Since no patients with relapse after HCT survived, data on relapse incidence were similar to those

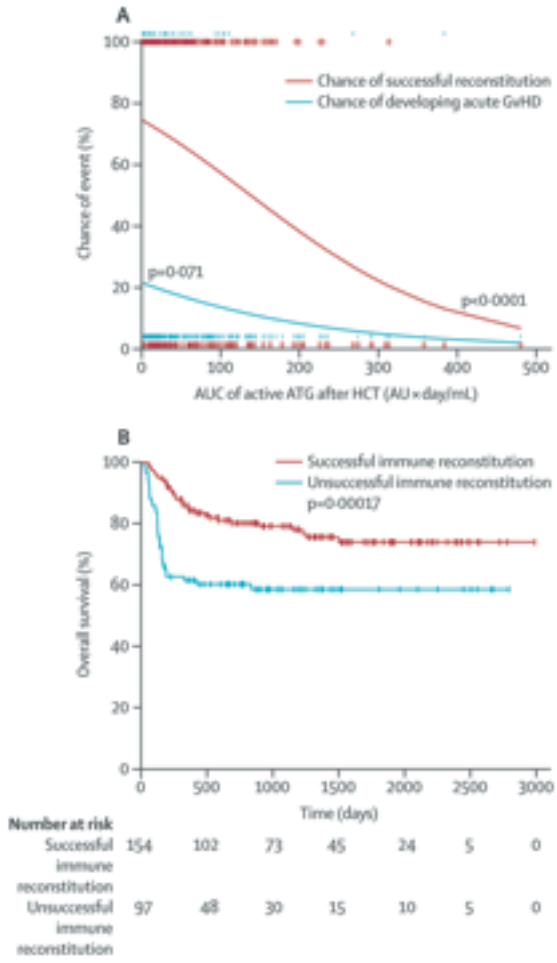

Figure 1. Chance of successful reconstitution, incidence of acute graft-versus-host disease, and overall survival (A) Successful CD4+ T-cell reconstitution before day 100 (red 0's) and grade 2–4 acute GvHD (blue I's) versus AUC of active ATG after HCT. The logistic regression lines show the chance of successful reconstitution versus the AUC after HCT (red line) and the chance of developing acute GvHD of at least grade 2 versus the AUC after HCT (blue line). Every I or O represents a patient with their respective AUC after HCT (x axis) and whether they had an event (y axis, either yes [1; top] or no [0; bottom]). Therefore, the patient with an AUC after HCT of 480 AU × day/mL had no immune reconstitution and no GvHD. (B) Kaplan-Meier survival curve of overall survival according to successful CD4+ T-cell immune reconstitution. ATG=antithymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.

of relapse-related mortality. Similar analyses were done for CD3+ and CD8+ T-cell, B-cell, and natural killer cell reconstitution; these subsets were less predictive for overall survival than CD4+ reconstitution (data not shown).

To identify the optimum therapeutic window for targeted and individualised dosing, we did subgroup analyses within the two stem cell source groups. We noted a direct association between the ATG AUC after transplant and overall survival in a subgroup analysis of 53 patients who received a cord blood transplantation for a benign disorder and 105 who received HLA-matched bone marrow and peripheral blood stem cells. In patients who received a cord blood transplant and had benign underlying disease, the AUC after HCT above or below the median (20 AU × day/mL) affected overall survival (HR 5·1, 95% CI 1·2–23·1, p=0·035; figure 4A). We noted no other associations with overall survival

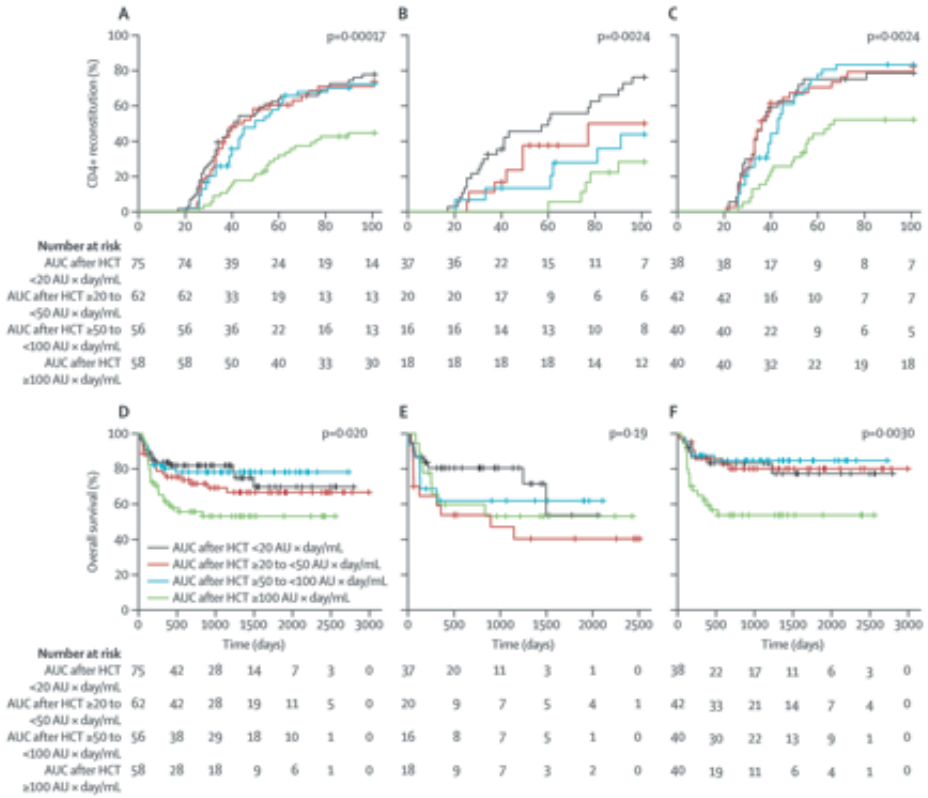

Figure 2. CD4+ T-cell reconstitution and overall survival according to area under the curve after haemopoietic stem cell transplantation by stem cell source The effect of AUC of ATG after HCT on immune reconstitution in all patients (A), those who received cord blood transplants (B), and those who received bone marrow and peripheral blood stem cell transplants (C). The effect of AUC of ATG after HCT on overall survival in all patients (D), those who received cord blood transplants (E), and those who received bone marrow and peripheral blood stem cell transplants (F). P-values are for the comparison between all four groups (log-rank test). ATG=anti-thymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.

in subgroup analyses (data not shown). In patients who received cord blood transplants for a malignant indication, exposure after HCT did not affect overall survival. However, the number of patients with an AUC less than 20 AU × day/mL after HCT in this group was low (n=9), probably because of the low lymphocyte count before conditioning, which leads to low clearance and thus high exposure.[16] In patients who received a matched bone marrow transplant or peripheral blood stem cells, a cut-off of 50 AU × day/mL (median AUC 45 AU × day/mL) gave the best predictive value of overall survival (HR 4·19, 95% CI 1·24–14·18; p=0·021; figure 4B). We identified no multivariate predictors for this analysis. In non-matched bone marrow and peripheral blood stem cells, survival at 5 years was 52%

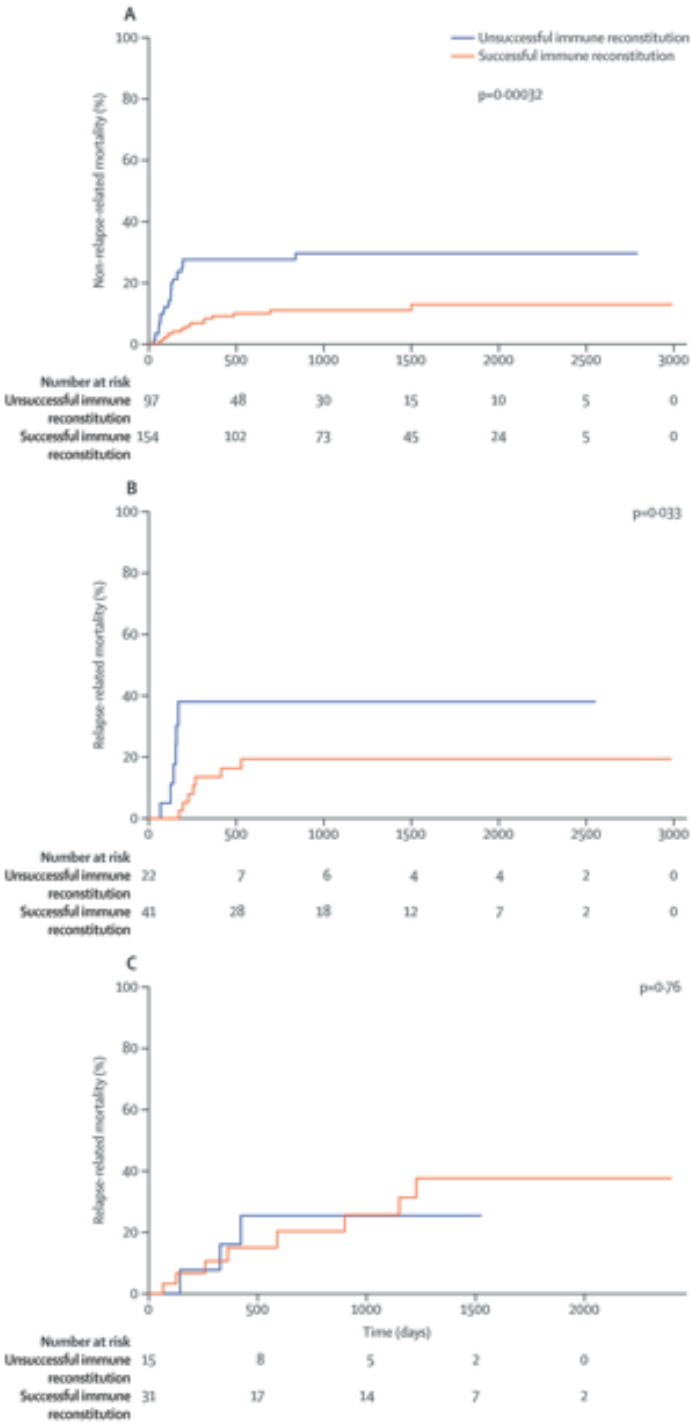

Figure 3. Cumulative incidence of non- relapse-related mortality and relapse- related mortality Cumulative incidence curve of non-relapse-related mortality (A), relapse-related mortality in myeloid leukemia (B), and relapse-related mortality in lymphoid leukemia (C) according to successful CD4+ T-cell immune reconstitution.

(95% CI 37–72) when compared with the matched bone marrow and peripheral blood stem cells, irrespective of AUC after HCT.

The estimated probability of acute GvHD grade 2–4 was not affected by AUC after HCT in a logistic regression analysis (odds ratio 0·999, 95% CI 0·996–1·003; p=0·78, figure 1A). ATG exposure after transplantation, divided in quartiles of exposure (<20, ≥20 to <50, ≥50 to <100, and ≥100 AU × day/mL; appendix p8) did not affect acute GvHD grade 2–4

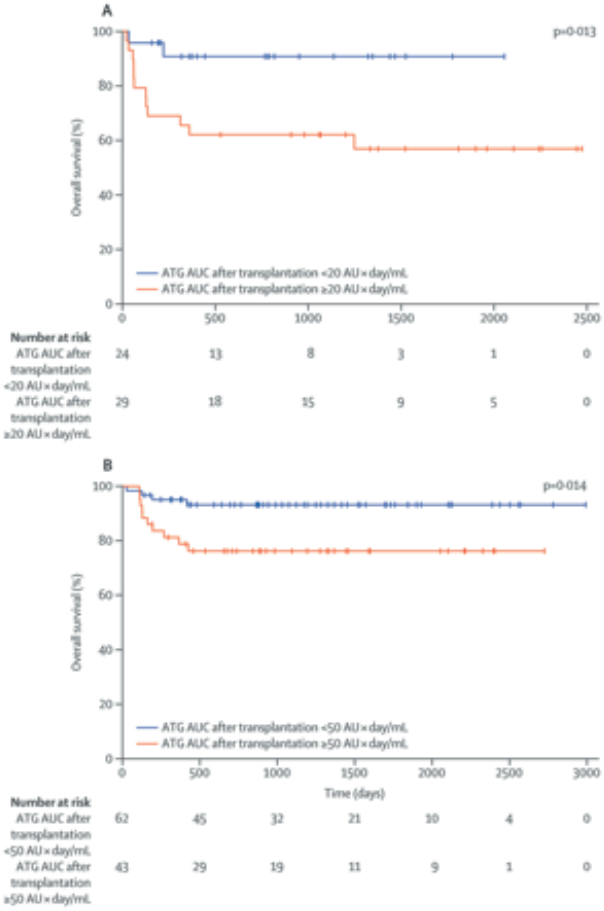

Figure 4. Survival curves according to area under the curve after transplantation in subgroups (A) Overall survival in patients with a benign underlying disease who had cord blood transplants and had an AUC after HCT of 20 AU × day/mL or higher (red line) or below 20 AU × day/mL (blue line). (B) Overall survival in patients who received bone marrow and peripheral blood stem cell transplants from a fully matched donor and who had an AUC after HCT of 50 AU × day/mL or higher (red line) or below 50 AU × day/mL (blue line). ATG=anti-thymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease. HCT=haemopoietic cell transplantation.

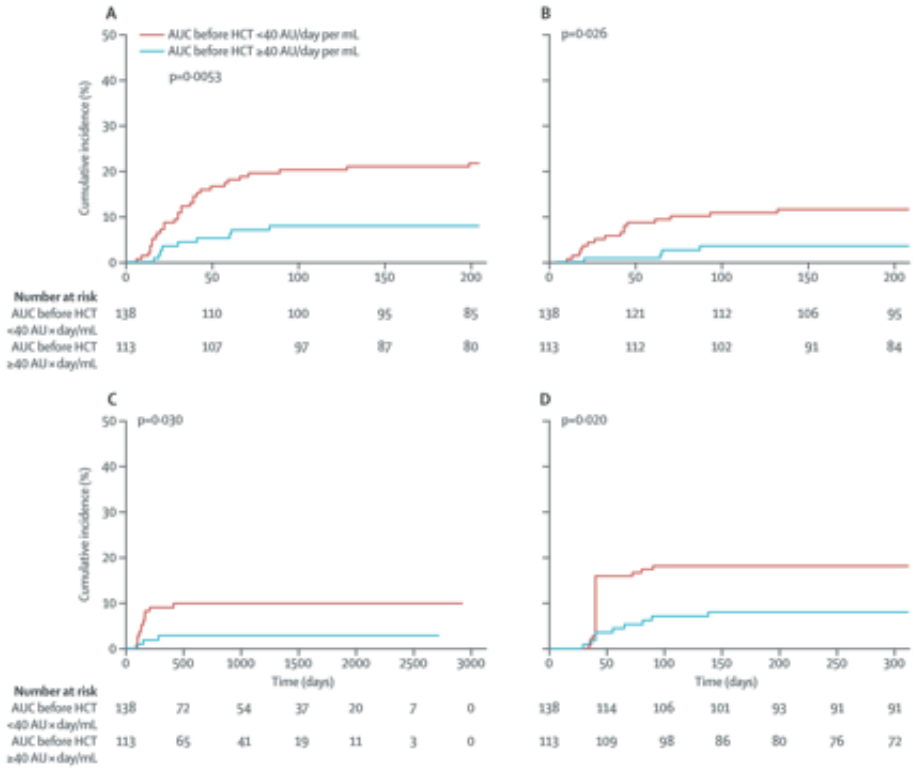

Figure 5. Cumulative incidence curves for acute and extensive chronic graft-versus-host disease and graft failure according to area under the curve before transplantation Cumulative incidence curves of acute GvHD grade 2–4 (A), acute GvHD grade 3–4 (B), extensive chronic GvHD (C), and graft failure (D) according to active ATG AUC before HCT below 40 AU × day/mL (red line) or 40 AU × day/mL or higher (blue line). ATG=anti-thymocyte globulin. AU=arbitrary units. AUC=area under the curve. GvHD=graft-versus-host disease.

(p=0·85), acute GvHD grade 3–4 (p=0·84), or chronic GvHD (p=0·23) in bone marrow and peripheral blood stem cell recipients, whereas in cord blood recipients, exposure after transplantation affected acute GvHD grade 2–4 (p=0·0050), but not acute GvHD grade 3–4 (p=0·15) or chronic GvHD (p=0·60).

However, the association between AUC before HCT and acute and chronic GvHD and graft failure was the strongest association among ATG exposure measures (figure 5). Pre-transplant exposure above 40 AU × day/mL, which was about the median AUC before HCT (37 AU × day/mL) was associated with a significantly lower incidence of grade 2–4 (HR 0·979, 95% CI 0·963–0·994; p=0·0081; figure 5A) and grade 3–4 acute GvHD (0·975, 0·952–0·998; p=0·033; figure 5B). Besides the AUC before HCT, malignant disease and male sex were associated with acute GvHD. A higher AUC before HCT also led to a lower incidence of extensive chronic GvHD (HR 0·983, 95% CI 0·968–0·998; p=0·029; figure 5C)

and graft failure (0·981, 0·965–0·997; p=0·020; figure 5D). The incidence of graft failure in the multivariate analysis was affected by malignant disease. Similar results were found when stratified by cell source.

DISCUSSION

To our knowledge, this is the first large pharmacokinetic and pharmacodynamic study of ATG in children to investigate the relation between exposure and clinical outcome (panel); this study was done with the aim of identifying the optimum therapeutic window of ATG to optimise ATG dosing and thereby to improve outcome, including survival chances, of paediatric HCT. With the limitations of a retrospective cohort study taken into account, our data suggest that active ATG exposure has an effect on the occurrence of successful immune reconstitution and thereby overall survival, and on the prevention of graft failure and GvHD. Low exposure after HCT was most important for ensuring early CD4+ T-cell reconstitution, especially in cord blood transplants, whereas immune reconstitution improved overall survival by reducing both relapse and non- relapse mortality. However, the AUC after HCT had less effect on the prevention of grade 2–4 or grade 3–4 acute and extensive chronic GvHD compared with the AUC before HTC, which were more affected by exposure to ATG before HCT. High exposure before HCT also led to significantly reduced graft failure. The described optimum range of exposure should be used to study future individualised dosing of ATG.

Most published studies on ATG pharmacokinetics reported the concentration at single time-points rather than the more informative exposure of ATG before and after HCT. Because AUC contains information on the whole concentration–time curve rather than the concentration at discrete time-points, it usually gives a better prediction of drug effects.[24] As expected, immune reconstitution was mostly affected by ATG exposure to the graft—i.e., the AUC after HCT. In the first 6–9 months after HCT, patients' T-cell counts are dependent on thymus-independent peripheral expansion of T lymphocytes infused with the graft.[3,25] Adequate immune reconstitution in this period led to fewer viral reactivations and relapses and subsequently improved survival.[2,6,7] We showed that low exposure after HCT was associated with better immune reconstitution, probably because in-vivo lymphodepletion was reduced, as had been suggested on the basis of active ATG concentrations shortly after transplantation.[13] Additionally, this effect was more profound in cord blood transplants than in bone marrow and peripheral blood stem cells, possibly because of the lower number of infused T lymphocytes in the graft combined with improved binding and lysing of the predominant subpopulation of naive cells in cord blood.[7] Although reconstitution worsened with increasing AUC after HCT in cord blood, patients with bone marrow and peripheral

blood stem cells in the three lower exposure groups had equally good reconstitution. Immune reconstitution in cord blood with a low ATG exposure after HCT was comparable to that in bone marrow and peripheral blood stem cells, although a mean of 1 log fewer T cells are infused in cord blood,[3] which is in line with the higher proliferative potential of cord blood cells.[26]

The price for lower exposure to ATG after transplantation might be an increased incidence of GvHD. However, in most studies, no association was found between post- transplantation ATG concentrations and acute or chronic GvHD was found,[8–10,27] whereas omission of serotherapy did result in a higher incidence of both acute and chronic GvHD.[2,4,5,28–31] Our results are mainly in line with these findings. However, we showed that both acute and chronic GvHD were affected by exposure before HCT, and suggest that this was possibly through depletion of antigen-presenting cells, which are pivotal to the induction of acute GvHD after HCT, and the reduction of inflammatory processes before transplantation, which might also be a trigger for induction of acute GvHD.[2] This theory is in line with the absence of association between successful immune reconstitution and acute GvHD. So far, no studies have investigated the role of ATG concentrations before HCT on outcome.

Furthermore, total body irradiation has been suggested to give protection against GvHD;[32] however, we could not find an additional effect of total body irradiation with a high AUC of ATG before HCT in our study. The data in this study suggest the optimum AUC after transplantation in cord blood transplants should be very low—less than 20 AU × day/mL—whereas in bone marrow and peripheral blood stem cell transplants the target AUC after HCT should be less than 50 AU × day/mL.

Exposure before transplantation should be above 40 AU × day/mL, irrespective of the stem cell source. To achieve these target exposures, not only does the dose of ATG need to be revised, the timing is also of importance. Because of the long half-life of active ATG, a low AUC after HCT in cord blood can only be achieved by giving ATG earlier before HCT and possibly at a lower dose depending on weight and lymphocyte count before ATG dosing. More predictable ATG concentrations and predictable immune reconstitution are also important when considering adjuvant cellular treatments, such as donor lymphocyte infusions, or engineered cell treatments, such as cell-based vaccinations and T cells with chimeric antigen receptors. Controlled ATG exposure by dose individualisation is probably also of importance in reduced intensity conditioning in both adults and children. Optimisation of exposure before and after HCT would probably help to reduce the risk of rejection and improve the ability to have fast immune reconstitution, which is of importance for improving the graft-versus-leukaemia effect in a reduced intensity conditioning setting. Studies investigating ATG pharmacokinetics and pharmacodynamics in adults (i.e., myelo-ablative

and reduced intensity conditioning) are being done by our group. To ensure early and robust immune reconstitution in patients transplanted with cord blood for haematological malignancies, some centres have abandoned ATG, because the risk of rejection is low in these patients. In view of our results, this approach seems feasible; early immune reconstitution reduces both non-relapse and relapse mortality, but the latter only in myeloid leukaemia. Still, event-free survival is comparable in this patient group when ATG is compared with no ATG.[2] Future studies are necessary to study the effects of individualised ATG dosing versus no ATG in reducing morbidity (e.g. GvHD) and mortality.

In conclusion, by using an ATG dose that aims to reach the target exposure before and after transplantation, we expect that optimum immune reconstitution and a low incidence of GvHD and graft failure can be achieved, which would probably lead to improved survival.

Panel: Research in Context

Systematic review

We searched Medline on Aug 15, 2014, using the search term "bone marrow transplantation AND antilymphocyte serum". We selected studies that compared antithymocyte globulin (ATG) versus no ATG, diff erent doses of ATG, and diff erent starting days of ATG before transplantation, and studies that related outcome to concentrations of active ATG. No language restrictions were applied. We identified three systematic reviews, seven randomised controlled trials, 12 clinical controlled trials, and two case series. Although in most studies no effects of ATG on overall survival were found, immune reconstitution had a dose-related effect with ATG, whereby a lower dose led to a better immune reconstitution, which translated into less viral reactivations. The incidence of acute and chronic graft-versus-host disease (GvHD) was reduced when ATG was introduced to the conditioning regimen. Also, there seemed to be a concentration- dependent effect, whereby a higher plasma concentration of ATG leads to a lower incidence of acute GvHD, but this was not as evident in chronic GvHD. No studies have reported on effects of ATG on relapse and rejection. Although this review of the published work included randomised controlled trials, clinical controlled trials, and case series, these results are in line with the available systematic reviews, which only included randomised controlled trials that compared ATG versus no serotherapy.

Interpretation

No previous studies have investigated the relation between several exposure measures of ATG and clinical outcome in children. In our study, low ATG exposure after haemopoietic cell transplantation (HCT) led to improved early CD4+ T-cell immune reconstitution, which was associated with increased survival through improved transplant-related mortality and relapse mortality. The incidence of GvHD and graft failure could be reduced by ensuring a sufficiently high ATG exposure before HCT. These exposures before and after HCT determine the therapeutic window of ATG, providing a target for individualised dosing that needs to be confirmed in a prospective study.

REFERENCES

1. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia* 2007; 21: 1387–94.
2. 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: 126–32.
3. Szabolcs P, Niedzwiecki D. Immune reconstitution after unrelated cord blood transplantation. *Cytotherapy* 2007; 9: 111–22.
4. Theurich S, Fischmann H, Chemnitz J, Holtick U, Scheid C, Skoetz N. Polyclonal anti-thymocyte globulins for the prophylaxis of graft-versus-host disease after allogeneic stem cell or bone marrow transplantation in adults. *Cochrane Database Syst Rev* 2012; 9: CD009159.
5. Mohty M, Labopin M, Balère ML, et al. Antithymocyte globulins and chronic graft-vs-host disease after myeloablative allogeneic stem cell transplantation from HLA-matched unrelated donors: a report from the Sociéte Française de Greffe de Moelle et de Thérapie Cellulaire. *Leukemia* 2010; 24: 1867–74.
6. Hiwarkar P, Gaspar HB, Gilmour K, et al. Impact of viral reactivations in the era of pre-emptive antiviral drug therapy following allogeneic haematopoietic SCT in paediatric recipients. *Bone Marrow Transplant* 2013; 48: 803–08.
7. Bartelink IH, Belitser S V, Knibbe CA, et al. Immune reconstitution kinetics as an early predictor for mortality using various hematopoietic stem cell sources in children. *Biol Blood Marrow Transplant* 2013; 19: 305–13.
8. Seidel MG, Fritsch G, Matthes-Martin S, et al. Antithymocyte globulin pharmacokinetics in pediatric patients after hematopoietic stem cell transplantation. *J Pediatr Hematol Oncol* 2005; 27: 532–36.
9. Remberger M, Persson M, Mattsson J, Gustafsson B, Uhlin M. Effects of different serum-levels of ATG after unrelated donor umbilical cord blood transplantation. *Transpl Immunol* 2012; 27: 59–62.
10. Call SK, Kasow KA, Barfi eld R, et al. Total and active rabbit antithymocyte globulin (rATG; thymoglobulin) pharmacokinetics in pediatric patients undergoing unrelated donor bone marrow transplantation. *Biol Blood Marrow Transplant* 2009; 15: 274–78.
11. 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: 460–71.
12. Jol-van der Zijde C, Bredius R, Jansen-Hoogendijk A, et al. IgG antibodies to ATG early after pediatric hematopoietic SCT increase the risk of acute GvHD. *Bone Marrow Transplant* 2012; 47: 360–68.
13. Bosch M, Dhadda M, Hoegh-Petersen M, et al. Immune reconstitution after anti-thymocyte globulin-conditioned hematopoietic cell transplantation. *Cytotherapy* 2012; 14: 1258–75.
14. Servais S, Lengline E, Porcher R, et al. Long-term immune reconstitution and infection burden after mismatched hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2014; 20: 507–17.
15. Jacobson CA, Turki A, McDonough S, et al. Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2012; 18: 565–74.
16. Admiraal R, van Kesteren C, Jol-van der Zijde C, et al. Population pharmacokinetic modeling of thymoglobulin® in children receiving allogeneic-hematopoietic cell transplantation (HCT): towards improved survival through individualized dosing. *Clin Pharmacokinet* 2015; 54: 435–46.

17. Bartelink IH, Boelens JJ, Bredius RGM, et al. Body weight- dependent pharmacokinetics of busulfan in paediatric haematopoietic stem cell transplantation patients: towards individualized dosing. *Clin Pharmacokinet* 2012; 51: 331–45.
18. Bartelink IH, Bredius RGM, Belitser SV, et al. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematologic stem cell transplantation. *Biol Blood Marrow Transplant* 2009; 15: 231–41.
19. Booth C, Veys P. T cell depletion in paediatric stem cell transplantation. *Clin Exp Immunol* 2013; 172: 139–47.
20. 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.
21. 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: 204–17.
22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; 94: 496–509.
23. Apperley J, Carreras E, Gluckman E, Masszi T. EBMT-ESH handbook on haemopoietic stem cell transplantation, 6th edn. Genoa, Italy: Forum Service Editore, 2012.
24. Gabrielsson J, Weiner D. Pharmacokinetic and pharmacodynamic data analysis: concepts and applications, 4th edn. Stockholm: Swedish Pharmaceutical Press, 2015.
25. Chiesa R, Gilmour K, Qasim W, et al. Omission of in vivo T-cell depletion promotes rapid expansion of naive CD4+ cord blood lymphocytes and restores adaptive immunity within 2 months after unrelated cord blood transplant. *Br J Haematol* 2012; 156: 656–66.
26. Cohen Y, Nagler A. Umbilical cord blood transplantation—how, when and for whom? *Blood Rev* 2004; 18: 167–79.
27. Bashir Q, Munsell MF, Giralt S, et al. Randomized phase II trial comparing two dose levels of thymoglobulin in patients undergoing unrelated donor hematopoietic cell transplant. *Leuk Lymphoma* 2012; 53: 915–19.
28. 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: 855–64.
29. Na I-K, Wittenbecher F, Dziubianau M, et al. Rabbit antithymocyte globulin (Thymoglobulin) 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.
30. 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: 2942–47.
31. Champlin RE, Perez WS, Passweg JR, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. *Blood* 2007; 109: 4582–85.
32. Benjamin J, Chhabra S, Kohrt HE, et al. Total lymphoid irradiation-antithymocyte globulin conditioning and allogeneic transplantation for patients with myelodysplastic syndromes and myeloproliferative neoplasms. *Biol Blood Marrow Transplant* 2014; 20: 837–43.