Individualized dosing of serotherapy in allogeneic hematopoietic cell transplantation - a delicate balance

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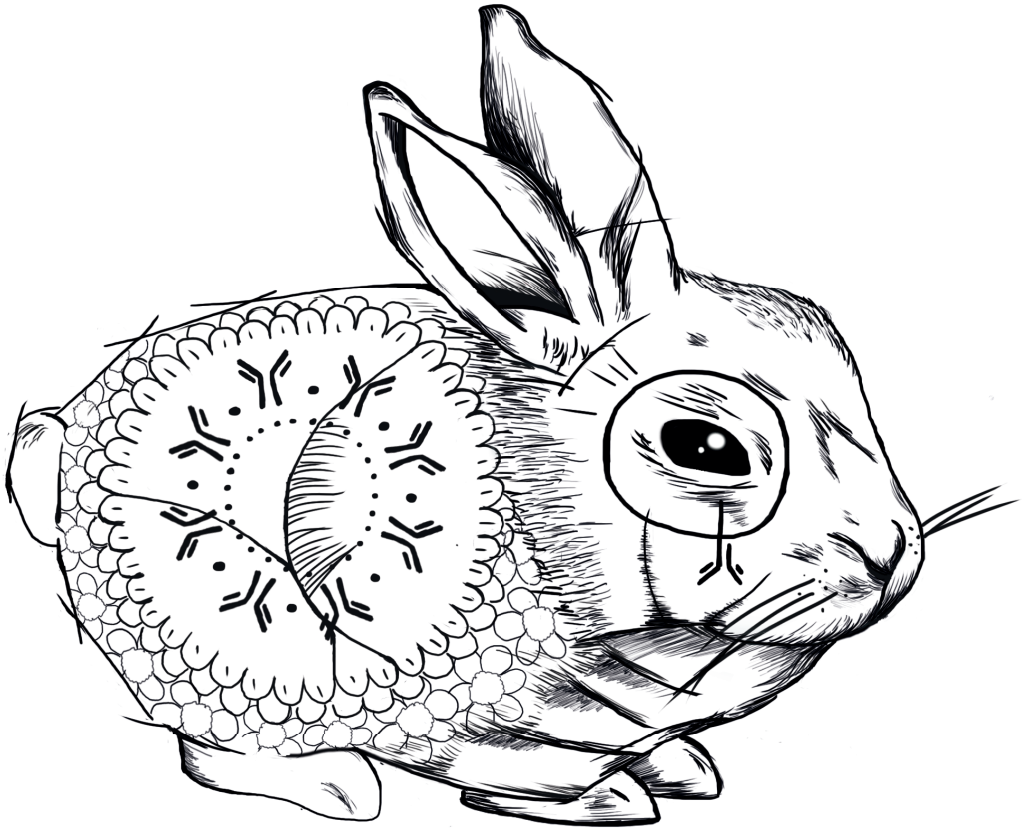

Chapter 6

Excellent T-cell Reconstitution and Survival Depend on Low ATG exposure after Pediatric Cord Blood Transplantation

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ABSTRACT

Successful immune reconstitution (IR) is associated with improved outcomes following pediatric cord blood transplantation (CBT). Usage and timing of anti-thymocyte globulin (ATG), introduced to the conditioning to prevent graft-versus-host-disease and graft failure, negatively influences T-cell IR. We studied the relation between ATG exposure, IR and clinical outcomes.

All pediatric patients receiving a first CBT between 2004-2015 at the University Medical Center Utrecht were included. ATG-exposure measures were determined with a validated PK-model. Main outcome of interest was early CD4+ IR, defined as CD4+ T-cell counts over $50x10^6$/L twice within 100 days after CBT. Other outcomes of interest included event free survival (EFS). Cox proportional-hazard and Fine-Gray competing-risk models were used.

A total of 137 patients, median age of 7.4 years (range 0.2-22.7), were included, of whom 82% received ATG. Area under the curve (AUC) of ATG after infusion of the CB transplant predicted successful CD4+ IR. Adjusted probability on CD4+ IR was reduced with 26% for every 10 points increase in AUC after CBT (hazard ratio (HR) 0.974, $p<0.0001$). Chances on EFS were higher in patients with successful CD4+ IR (HR 0.25, $p<0.0001$) and lower ATG exposure after CBT (HR 1.005, $p=0.0071$).

This study stresses the importance of early CD4+ IR after CBT, which can be achieved by reducing the exposure to ATG after CBT. Individualized dosing of ATG to reach optimal exposure, or in selected patients omission of ATG, may contribute to improved outcomes in pediatric CBT.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has been introduced as a curative option for diseases including malignancies, immune deficiencies, bone marrow failure and selected metabolic disease. Since the late eighties, umbilical cord blood has become available as an alternative donor source in pediatric HCT[1]. Compared to bone marrow (BM) or peripheral blood stem cells (PBSC), umbilical cord blood transplantation (CBT) has the advantage of less stringent human leukocyte antigen (HLA) matching requirements, increasing the chances of finding a suitable donor and is rapidly available[2]. Furthermore, CBT shows at least comparable survival compared to BM and PBSC[3–6], lower rates of chronic GvHD and is suggested to have a more potent anti-leukemic effect compared to the traditional stem cell sources[7,8].

When focusing on early T-cell immune reconstitution (IR) (i.e. within 3 months after HCT), CBT is suggested to have a slower IR compared to non-T-cell depleted BM or PBSC[9–11]. Anti-thymocyte globulin (ATG), introduced to the conditioning regimen to prevent graft-versus-host disease (GvHD) and graft failure, is suggested to influence IR after CBT more profound compared to BM and PB [6,12–14]. This more profound in vivo depletion may be due to the fact that ATG is produced in rabbits using infant human thymus, which may result in more antibodies against naïve receptors, which are more present on CB T-cells[15,16].

Successful immune reconstitution following HCT is associated with improved survival chances following HCT[9,11,15,17,18], making efforts to improve IR imperative. Strategies to enhance IR may include optimizing the dosing and thereby the exposure to ATG. Using the same linear dosing regimen in mg/kg for individuals of all sizes, exposure to ATG has been highly variable between patients[19–23], especially in pediatric populations. This is mainly due to large variability in clearance, which depends on both body weight and recipient lymphocyte counts[20,23]. Individualized dosing of ATG targeted at a predefined optimal exposure, thereby reducing variability in exposure for all patients, may subsequently lead to better predictable and improved IR. However, for individualized dosing of ATG, the optimal target exposure (i.e. the therapeutic window) needs to be further investigated in cord blood recipients.

Studies investigating the relationship between ATG exposure and either IR or clinical outcome are scarce, especially for CBT, where it may be most relevant[15,20]. We recently described the relationship between ATG exposure and outcome, however the CB patients in this analysis were heterogeneous in terms of conditioning regimens, GvHD-prophylaxis and supportive care. In the current study, these limiting aspects will be overcome by including a large cohort with uniformly treated children receiving a CBT in an experienced CBT center

to study the relationship between ATG exposure, IR and clinical outcomes. An identified therapeutic window may be used as the target for individualized dosing.

METHODS

Study design and Patients

In this analysis, we included all patients receiving a single-unit CBT treated in the pediatric Blood and Marrow Transplantation Program of the University Medical Center Utrecht, the Netherlands between December 2004 and October 2015. Cord blood was selected in patients where an identical sibling donor was not available, or based on indication (high risk acute myeloid leukemia [AML], inborn errors of metabolism, primary immune deficiencies). Only first allogeneic HCT's were included. There was no restriction on the underlying disease or dose of ATG used. Consecutively treated patients were included. Patients developing IgG anti-ATG antibodies were excluded[24]. Data were collected prospectively in a local clinical database. Blood samples were collected weekly; ATG concentrations were measured in batches. Patient inclusion and data collection started after informed consent was obtained in accordance to the declaration of Helsinki. For data and sample collection, institutional ethical committee approval was obtained through trial numbers 05/143 and 11/063-K.

Procedures

UCB units were obtained from (inter)national cord blood banks. Human Leukocyte Antigen (HLA)-matching was performed on HLA-A, HLA-B and HLADRB1, and had to be at least 4/6 matched to the patient. The minimum total nucleated cell dose required for the transplant was 2.5, 3, or $5x10^7$ nucleated cells per kilogram in case of a 6/6, 5/6, or 4/6 HLA-matched UCB-unit, respectively. In rare cases, a 3/6 matched UCB unit was chosen with a minimum of $5x10^7$ nucleated cells per kilogram. Grafts were thawed and diluted 1:1 with NaCl 0.9% before infusion, no washing was performed. Conditioning regimens were selected according to national and international study protocols open in the program, most frequently busulfan-based myeloablative regimens. Busulfan was given intravenously with therapeutic drug monitoring aiming for a target cumulative area under the curve (AUC) of 85-95 mg*h/L in a myeloablative setting[25,26]. ATG (Thymoglobulin®, Genzyme, MA, USA) was administered at a dose of 10 mg/kg in 4 consecutive days according to (inter)national protocols. From 2010 onwards, patients with a body weight over 40 kg received a reduced dose of 7.5 mg/kg. Additionally, the first infusion of ATG relative to the CBT was moved from day -5 (before 2010) to day -9 (2010 onwards). Starting from 2013, patients treated for acute myeloid leukemia (AML) or high-risk acute lymphoid leukemia (ALL) did not receive ATG as part of the conditioning regimen to ensure early T cell reconstitution[27].

Patients not receiving ATG were included in the analysis to investigate the full spectrum of ATG exposures, including no exposure. GvHD-prophylaxis consisted of cyclosporin A with a target trough concentration of 200-250 µg/L combined with prednisolone 1 mg/kg. Prednisolone was tapered in 2 weeks starting 4 weeks post-HCT in benign disorders, in malignant disorders in one week after engraftment. Cyclosporin A was continued until 3 months (malignant disease) or 6 months (benign disorders) after HCT. Patients were prophylactically treated with aciclovir; treatment for viral reactivations of adenovirus, cytomegalovirus and Epstein–Barr virus was started after reaching 1000 copies/mL. All patients received gut decontamination and pneumocystis jiroveci prophylaxis according to local protocol as previously described[15]. Patients were treated in high-efficiency, positive pressure, particle-free isolation rooms.

ATG concentrations

Active ATG concentrations were measured in serum every week following the first infusion of ATG[23]. Using a validated pediatric population pharmacokinetic model[23], full concentration-time curves could be estimated, and pharmacokinetic exposures measures were calculated. These included the total AUC, the AUC before (defined as the AUC from start of first infusion up to the infusion of the graft) and after (defined as the AUC from start of infusion to infinite time, i.e. total elimination of all ATG) CBT, maximum concentration and concentration at the time of infusion of the graft. ATG exposure measures were calculated using NONMEM 7.3.0 (Icon, Ireland).

Lymphocyte subsets

Lymphocyte subsets, consisting of CD3+, CD4+ and CD8+ T-cells, B-cells (CD19+) and NK-cells (CD56+CD3-), were measured after reaching a leucocyte count of > 0.4 x 10^9 cells/L. Cell counts were followed every other week up to 12 weeks after CBT, thereafter monthly.

Outcomes

Main outcome of interest was CD4+ T-cell immune reconstitution, defined as having > 50 x 10^6 CD4+ T-cells in 2 consecutive measurements within 100 days. The definition was based on the previously demonstrated association with survival as well as the central role of T-helper cells in adaptive immunity[6,9,12,15]. Patients who deceased within 100 days were evaluated until date of death. Other outcomes of interest were overall survival (OS), defined as days to death of any cause or last follow up, event free survival (EFS), defined as days to first event (death due to any cause, relapse, or graft failure) or last follow up. Non-relapse mortality (NRM) and relapse related mortality (RRM) were defined as death due to any cause other than relapse and death due to relapse, respectively, or last follow up. Acute and chronic GvHD were classified according to the Glucksberg[28] and Shulman[29] criteria.

Graft failure was defined as non-engraftment or secondary rejection. Viral reactivations of cytomegalovirus (CMV), adenovirus (AdV) and Epstein-Barr virus (EBV) were defined as >1000 copies/ml in blood. Both AUC of ATG before and after CBT, and CD4+ immune reconstitution were investigated as predictor for clinical outcomes. Additionally, other immune reconstitution markers including CD3+, CD4+, B- and NK-cell counts were evaluated as a predictor for outcome. All patients were censored at the date of last contact.

Statistical analyses

Duration of follow-up was defined as the time from CBT to last contact or death. Factors considered as predictors for outcome included patient related variables (age, sex, cytomegalovirus [CMV] serostatus and Epstein-Barr virus [EBV] serostatus), disease variables (malignancy, primary immune deficiency [PID], bone marrow failure syndromes, or benign non-PID), donor related variables (HLA-disparity, CMV serostatus, EBV serostatus), treatment period (before or after median year of transplantation), ATG exposure measures (AUC before and after CBT) and CD4+ immune reconstitution. Immune reconstitution was considered as a time-varying predictor. Variables with a 2-sided p-value of < 0.05 in univariate analysis were considered as a predictor in multivariate analysis. Probabilities of survival were determined using the Kaplan Meier estimation; p-values were calculated using a two-sided log-rank test. Cox proportional hazard and logistic regression models were used. For the endpoints acute and chronic GvHD, NRM and RRM, Fine-Gray competing risk models were used. For finding optimal cut-off values for the primary outcome, receiving-operator-characteristic (ROC) curves were used. The cut-off with the maximum sum of sensitivity and specificity, and therefore the most accurate, was selected. Statistical analyses were performed using R version 3.2.3, with the packages cmprsk, survival, and ROCR.

RESULTS

Patients

A total of 137 patients with a median age of 7.4 (range 0.2-22.7) were included in this analysis (Table 1, and split for ATG exposure in table S1). Of these patients, 66 patients (48%) were included in a previous analysis[15]. Most (82%) patients received ATG as part of their conditioning regimen; ATG was omitted in 17/30 patients with AML and 6/20 patients with ALL. One patient (0.7%) was excluded due to the development of anti-ATG antibodies. Busulfan-based conditioning regimen was the most frequently used regimen (89%) either combined with fludarabine alone (67%) or with fludarabine and clofarabine (22%). The indication for CBT was a non-malignant disorder in 58% of the patients. Median follow-up was 36 months (0.3-131).

Main outcome of interest

Low exposure to ATG after infusion of the CB transplant was found to be the best predictor for successful CD4+ immune reconstitution. In multivariate (MV) analysis, chance on CD4+ IR was reduced 26% with every 10 points increase in AUC after CBT (hazard ratio (HR) 0.974, 95% confidence interval (CI) 0.962 - 0.986, p<0.0001; Table 2, Supplemental Table S2). Next, the optimal cut-off in exposure to ATG after CBT for CD4+ IR was investigated using ROC curves. The most optimal cut-off was found to be 16 AU*day/mL, with a specificity of 85% with a sensitivity of 65% (Supplemental Figure S1). Therefore, the patients were divided in groups of no ATG, low AUC after CBT exposure (<16 AU*day/mL) and high AUC after CBT (≥16 AU*day/mL). Omitting ATG resulted in 100% CD4+ IR, while patients that did receive ATG had lower chances of reaching IR (81±6%, p=0.008 and 33±6%, p<0.0001 in no ATG versus low AUC and versus high AUC, respectively; Figure 1). No other multivariate predictors for CD4+ IR were found (Supplemental Table S1). When investigating whether the optimal ATG exposure differed based on donor, recipient or

	Total
Number of patients (n)	137
Male sex [n(%)]	82 (60)
Age at transplant (years)	7.4 (0.2-22.7)
Patients receiving ATG [n(%)]	112 (82)
Diagnosis [n(%)]	
Malignancy	56 (41)
Acute Lymphoid	22 (16)
Acute Myeloid	30 (22)
Lymphoma	4 (3)
Primary Immune Deficiency	33 (24)
Bone Marrow Failure	7 (5)
Benign non-PID	41 (30)
Conditioning regimen [n(%)]	
Bu-Flu	92 (67)
Bu-Flu-Clo	30 (22)
TBI based	10 (7)
Cy-Flu	5 (4)
Matchgrade [n(%)]	
6/6 matched	55 (40)
5/6 matched	63 (46)
4/6 matched	18 (13)
3/6 matched	1 (1)
Follow-up (months) [mean (range)]	44 (0.2-143)

Table 1. Patient Characteristics

Variable	HR	95% CI	p	
Post-HCT AUC of ATG (continuous)				
CD4+ Immune Reconstitution	0.974	(0.962-0.986)	<0.0001	****
Event Free Survival	1.005	(1.001-1.009)	0.0071	**
Overall Survival	1.005	(1.001-1.009)	0.026	*
Non-relapse Mortality	1.005	(1.001-1.009)	0.028	*
Use of ATG (no ATG is reference)				
Acute GvHD grade 2-4	0.878	(0.353-2.185)	0.78	
Acute GvHD grade 3-4	0.268	(0.083-0.862)	0.027	*
Chronic Extensive GvHD	1.132	(0.137-9.383)	0.91	
Immune reconstitution (no IR is reference)				
Event Free Survival	0.264	(0.156-0.447)	<0.0001	****
Overall Survival	0.516	(0.279-0.955)	0.035	*
Non-relapse mortality	0.358	(0.154-0.829)	0.017	*
Relapse mortality in myeloid leukemia	0.134	(0.03-0.595)	0.008	**

Table 2. Multivariate analysis

transplant characteristics, no variables could be identified, indicating the optimal exposure after CBT is <16 AU*day/mL, irrespective of disease, age and HLA match-grade.

Other outcomes of interest according to ATG exposure after CBT

Event-free survival was comparable in patients not receiving ATG and having low ATG exposure after CBT, while those with high AUC after CBT ATG had a significantly lower EFS compared to each of the other 2 groups (Figure 2). However, patients not receiving ATG are not comparable to those that do: ATG is omitted only in AML and high-risk ALL. In multivariate analysis, high ATG exposure after CBT (HR 1.005, 95% 1.001-1.009, p=0.0071) and positive recipient CMV serostatus were multivariate predictors for inferior EFS (HR 2.01, 95% CI 1.113 - 3.64, p=0.021). Here, ATG exposure after CBT was introduced as a continuous covariate to use the full statistical power of the data and to minimize bias. Overall survival chances were comparably improved with lower ATG exposure after CBT (HR 1.005, 95% CI 1.001-1.01, p=0.022). This indicates that every 10 points increase in ATG exposure after CBT results in 5% lower survival probability. Causes of death in the three exposure groups can be found in table S3. RRM and relapse incidence was not impacted by ATG exposure, but the incidence of NRM was significantly reduced in patients with a lower ATG exposure after CBT (HR 1.005, 95% CI 1.001-1.009, p=0.036). A trend for lower incidence of viral reactivations was seen in no ATG and low ATG exposure (figure S2). GvHD was not impacted by exposure to ATG after CBT (p=0.38, p=0.21 and p=0.15 for grade 2-4 acute GvHD, grade 3-4 acute GvHD and extensive chronic GvHD, respectively).

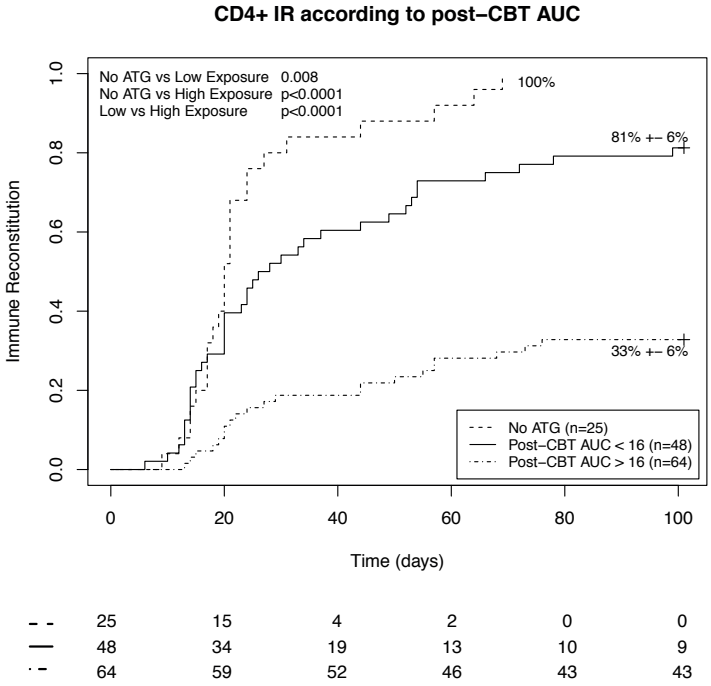

Figure 1. Cumulative incidence of CD4+ IR within 100 days according to exposure to ATG after CBT. Orange line: No ATG; Black line: Exposure to ATG after CBT < 20 AU*day/mL; Red line: Exposure to ATG > 20 AU*day/mL.

Other outcomes of interest according to use of ATG

No difference in GvHD incidence was found based on ATG exposure levels. Therefore, we investigated whether the use of ATG impacted GvHD. Here, we found that although the use of ATG did not predict the incidence of grade 2-4 GvHD (p=0.74), patients receiving ATG had a significantly lower incidence of grade 3-4 acute GvHD compared to those not receiving ATG (HR 0.27, 95% CI 0.08-0.86, p=0.027). No differences were found in incidence of chronic GvHD and graft failure according to the use of ATG, which may be due to the low number of events (5% and 11% for chronic GvHD and graft failure, respectively).

Other outcomes of interest in the context of CD4+ IR

Within the immune reconstitution markers, we found CD4+ IR to be the strongest and only predictor for the outcomes of EFS, OS, NRM and RRM.

Patients with successful early CD4+ IR at day +100 had better chances on EFS (HR 0.25, 95% CI 0.14-0.43, p<0.0001; Figure 3a). Besides CD4+ IR, negative CMV serostatus before transplantation was a multivariate predictor for improved chances on EFS. Overall survival was comparably improved with successful CD4+ IR (HR 0.40, 95% CI 0.21-0.77, p=0.0070, Figure 3b). Moreover, successful CD4+ IR lowered the chances on NRM (Figure 3c) and

RRM (Figure 3d), the latter only in myeloid leukemia's and in a small subgroup of patients. In the multivariate models for NRM and RRM, CD4+ IR was the only predictor (HR 0.28, 95% CI 0.12-0.67, p=0.004; HR 0.18, 95% CI 0.04-0.88, p=0.034, respectively). In patients

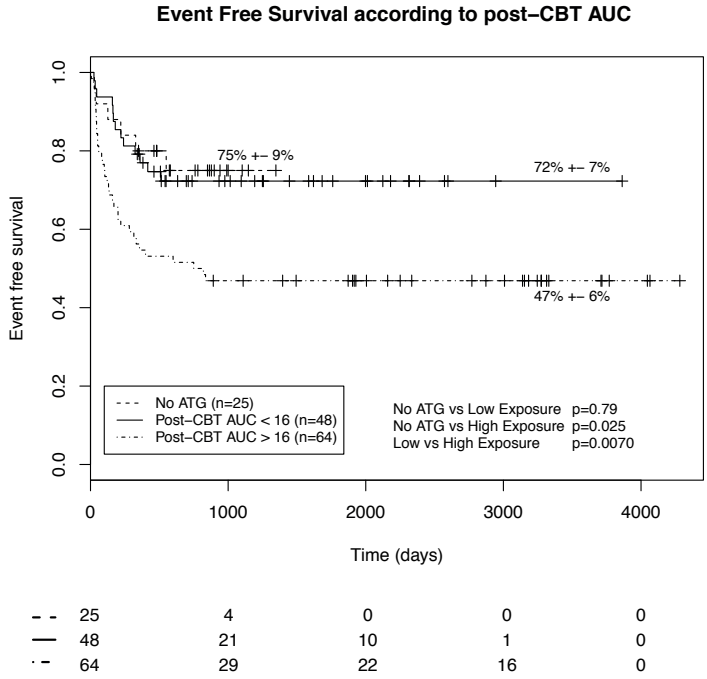

Figure 2: Event-free survival according to exposure to ATG after CBT. Orange line: No ATG; Black line: Exposure to ATG after CBT < 20 AU*day/mL; Red line: Exposure to ATG > 20 AU*day/mL.

not reaching CD4+ IR, infectious disease was the most common cause of NRM: 11/52 patients, compared to 5/85 patients with CD4+ IR. Other causes of NRM were comparable between the groups.

DISCUSSION

To our knowledge this is the largest pediatric single center study comprehensively investigating the relation between ATG exposure and CD4+ T-cell IR and clinical outcomes. We show that even very minimal exposure of ATG after CBT has a detrimental effect on early CD4+ immune reconstitution. We also found that both ATG exposure and CD4+ IR were predictors for clinical outcome, including EFS, OS, and NRM. The use of ATG was associated with a lower incidence of grade 3-4 acute GvHD but not grade 2-4 or chronic GvHD, the latter possibly due to low incidence. While recognizing the limitations of a retrospective cohort

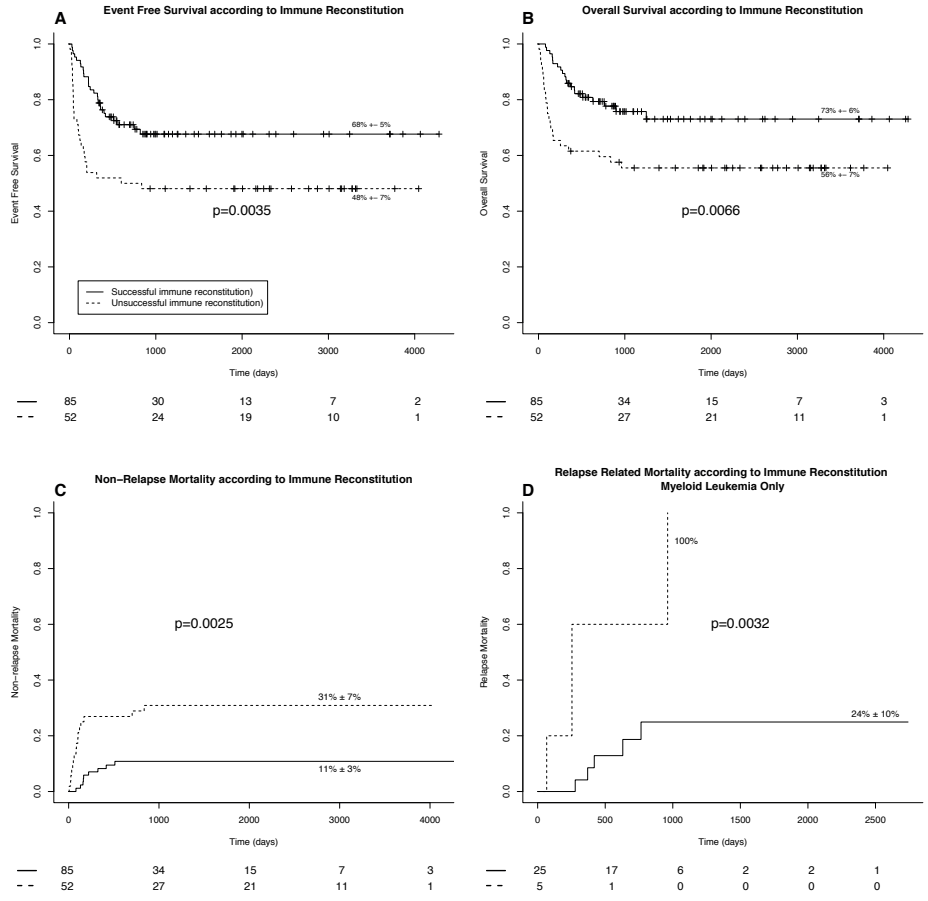

Figure 3. Event-free survival (Panel A), overall survival (Panel B) and non-relapse mortality (Panel C) according to successful CD4+ IR in all patients. Panel D: Relapse Related Mortality according to successful CD4+ IR in myeloid leukemia's only. Black lines: successful CD4+ IR; red lines: no successful CD4+ T-cell IR.

study, the strengths of this study include the homogeneous group of patients analyzed, e.g. conditioning regimens, GvHD-prophylaxis, supportive care. Additionally, the inclusion of consecutive patients with prospective data collection minimizes the risk for potential bias. Taken together, these results stress the importance to aim for optimal ATG exposure in pediatric cord blood transplantation; both for achieving early immune reconstitution and ensuring improved survival chances.

Outcomes of CBT have improved significantly over the last decade. In the nineties and early 2000's, lower engraftment rates were a significant disadvantage of CBT, however the current use of higher cell dosed units has significantly reduced this issue[4]. NRM by infectious causes however is still reported to be a serious limitation of CBT, while relapse incidence

on the other hand is suggested to be lower in CBT[4]. As timely immune reconstitution seems essential in preventing relapse- and non-relapse mortality, strategies to improve immune reconstitution to increase protection against viral disease and relapse of malignancy are warrented[7,15,27,31]. T-cell immune reconstitution has been associated with use, dose and timing of serotherapy, all indicating that a higher dose of serotherapy shortly before infusion of the graft is detrimental for timely and robust immune reconstitution[6,14,27,32,33]. Thus, in-vivo exposure of the graft-infused T-cells to serotherapy results in significant depletion of T cells and limits the exceptional proliferative capacity of CB T-cells as recently shown[15]. The present study confirms this: very low exposure to ATG has dramatic effects on T cell reconstitution potential, while no ATG shows exceptional IR. The IR potential in CBT is possibly even better than other stem cell sources as suggested before[27].

In the major landmark studies by Rocha et al. and Eapen et al. comparing cord blood to other stem cell sources, most cord blood patients received ATG[3,30]. Taking the results of the current study into account, when reducing in-vivo exposure of ATG to the graft in these studies the cord blood groups may have performed superior.

Moreover, the presented results are likely true in adult CBT, and potentially at a greater magnitude. Due to the higher body weights in adults compared to children, the number of T-cells per kilogram is lower. Additionally, from a pharmacokinetic perspective, ATG clearance is markedly higher in smaller (younger) children compared to older children, and is likely to be even lower in adults. Therefore, current dosages of ATG in adult CBT may result in very high ATG exposure after infusion of the graft, and fully deplete the even lower amount of graft-infused T-cells. This will negatively impact outcomes, both in terms of CD4+ IR and EFS in line with the results presented in this paper.

Both successful CD4+ IR and lower ATG exposure after CBT were associated with survival due to both lowering non-relapse and relapse mortality in AML. This is in line with previous studies, where CD4+ IR was associated with lower incidence of viral reactivations and relapse, and better survival chances[6,9,15,27,31]. Few studies however report on the association between ATG PK and survival, where PK is evaluated either as single concentration measurements or as exposure. Studies investigating single concentrations at a certain time-point could not find a correlation with survival, while actual exposure of ATG before and after transplantation was associated with a variety of outcomes[15,19–21,34]. This discrepancy may be due to a lower number of patients included in the concentration-studies, however ATG exposure (AUC) more likely harbors more information compared to single concentration samples and therefore is a stronger predictor[35]. Wide scale implementation of ATG PK as a variable in outcome following CBT depends on an assay for measuring ATG concentrations and skilled pharmacology staff. This should be achievable in larger centers.

In the current study, the use of ATG was found to lead to a lower incidence of grade 3-4 acute GvHD, which is in line with previous studies [6,36–38]. No correlation was found however between the use of ATG and grade 2-4 GvHD, extensive chronic GvHD and graft failure, which in previous work were related to exposure of ATG before transplantation. The absence of a correlation may be due to the relatively low incidence of chronic GvHD and graft-failure in this cohort (5% and 11% for extensive chronic GvHD and GF, respectively). Also the incidence of acute GvHD grade 2-4 was relatively low compared to previous studies where ATG was omitted[27]. This difference may be due to different GvHD-prophylaxis regimens: the current study used prednisolone while Chiesa et al. used mycophenolate mofetil[27].

Strategies for improved and predictable T-cell IR are warranted and may lead to improved survival chances following HCT. This is especially important in CBT, where even very low exposure after CBT has a major impact on CD4+ IR probability. Omission of ATG in a CBT-setting for all indications may contribute to improved IR, however is less feasible in immune-competent recipients in whom omitting ATG could potentially lead to more graft-failure or GvHD [6,27]. All chemo-naïve patients (e.g. benign disorders) received ATG in the current analysis. Therefore no inferences can be made on omitting ATG in these patients based on this study. Individualizing ATG is an alternative strategy: dosing is based on patient characteristics (e.g. weight and absolute lymphocyte count) aiming to reach the optimal exposure before and after CBT. The advantages of individualized dosing include optimal immune reconstitution while having protections against GvHD and graft-failure, thereby giving the best of both worlds. This approach is currently being investigated in a prospective clinical trial (Dutch trial register NTR4960). This individualized dosing regimen is relatively easy to use, also in centers not able to do ATG PK. In our opinion, both malignant and non-malignant indications can be treated with individualized ATG in a CBT-setting, while in AML, ATG can be omitted to add more safety. The T-cell repertoire in CBT following successful CD4+ IR may give an additional advantage over BM and PBSC[39]. Not only do CB derived T-cells mediate a more potent anti-leukemic effect[7], the high number of naïve cells make CBT a powerful platform for adjuvant cellular therapies (e.g. cell vaccinations)[40,41].

In conclusion, this study shows the importance of adequate targeting of ATG exposure after CBT to ensure early CD4+ T-cell reconstitution. By omitting ATG or using an individualized dosing and timing of ATG aiming for optimal target exposure, T-cell IR will improve. Our data indicate that promoting CD4+ IR will likely improve survival chances by lowering both relapse- and non-relapse mortality.

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