The impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune-reconstitution and clinical outcome

Caroline A. Lindemans 1*, Robert Chiesa3*, Persis J. Amrolia3, Kanchan Rao3, Olga Nikolajeva3, Arianne de Wildt1, Corinne E. Gerhardt1, Kimberly C. Gilmour4, Marc Bierings1, Paul Veys3, Jaap J Boelens1,2

1. Pediatric Blood and Bone Marrow Program, University Medical Center Utrecht, The Netherlands
2. U-DANCE, Tumorimmunology, Lab Translational Immunology, University Medical Center Utrecht, The Netherlands
3. Bone Marrow Transplantation Department, Great Ormond Street Hospital, London, UK
4. Immunology Department, Great Ormond Street Hospital, London, UK
* Joint first author

Short title: The impact of ATG in unrelated CB-transplantation

Correspondence:
C. A. Lindemans, MD, PhD
UMC Utrecht
KC03.063.0
PO BOX 85090
3508 AB Utrecht
The Netherlands
c.a.lindemans@umcutrecht.nl
Key points
1) For good immune-reconstitution and less viral reactivations, omit thymoglobulin in cord blood transplants.
2) As omission of thymoglobulin is associated with higher aGVHD rates further improvement of outcome may require individualized dosing.

Abstract
In vivo T-cell-depletion with Thymoglobulin (ATG) might contribute to the delayed immune reconstitution observed after unrelated umbilical cord blood transplantation (UCBT). We studied the impact of early, late and no ATG on immune reconstitution and outcome.

127 Children receiving UCBT in London or Utrecht were included into 3 groups: late ATG (day -5 to 0, n=48), early ATG (day -9 to -5, n= 33) and no ATG (n=46). The no ATG group received MMF+CsA as GVHD- prophylaxis, while the ATG groups both received CSA+prednisone. Endpoints studied were survival, immune-recovery, infections and GvHD.

The probability of survival was similar in all groups: 71% +/- 8% (no ATG), 68% +/- 9% (early ATG) and 61% +/- 7% (late ATG). CD3+, CD4+ and CD4+ naïve T-cell-counts were significantly higher (p<0.001) in the no ATG group at 1,2,3,6, and 12months post-UCBT. In the no ATG group significantly less viral reactivations (p=0.021) were noted. A higher probability of severe acute GvHD(31%) was found in the no ATG group compared to 18% (p =0.018) for early ATG and 5% (p<0.001) for late ATG. This was not associated with more chronic GvHD.

The findings of improved immune-reconstitution, associated with lower viral reactivations, albeit at the cost of increased rates of acute GvHD (but not cGvHD), suggest that omitting ATG may be important to prevent viral reactivations.
Introduction

Umbilical cord blood (UCB) is increasingly used as an alternative donor stem cell source for treating patients with leukemia, inborn errors of metabolism, immune deficiencies and bone marrow failure syndromes. The use of UCB donors has important advantages, such as: (1) prompt availability (HLA-typed and banked); (2) less stringent HLA-matching criteria, with a donor being available for >95% of the patients; (3) lower probability of graft-versus-host disease (GvHD), while maintaining a powerful graft-versus-leukemia (GvL) effect. However, the use of UCB is also associated with a prolonged neutropenic period and higher rates of graft failure, particularly for UCB units with low cell numbers (<3x10^7 total nucleated cells/kg). Moreover, compared to matched sibling and matched unrelated donor (MUD) hematopoietic cell transplantation (HCT), patients undergoing UCBT experience a delayed T-cell reconstitution, with an increased risk of opportunistic infections during the first three months post transplant. This is a significant concern in patients with ongoing bacterial/fungal infection at the time of transplantation and/or in patients with a history of multiple viral reactivations.

One explanation for the delayed T-cell reconstitution in UCBT recipients may be the use of in vivo T-cell depletion (thymoglobulin or alemtuzumab) in the conditioning regimen. Thymoglobulin (ATG) doses of 10mg/kg are usually given in the UCBT setting where, on average, a log less T-cells are infused compared to a bone marrow donor graft. Importantly, in some pharmacokinetic studies, thymoglobulin can be detected in the recipient’s plasma up to 25-60 days after HCT. In recent years, some transplant centers have started to omit ATG in the cord blood setting. Comparative analyses have however not been published previously. In line with our hypothesis, our group (Chiesa et al.) recently reported that in the absence of ATG, using Cyclosporin A and MMF as GVHD prophylaxis, an excellent immune recovery can be observed following unrelated UCBT. From these data, it can be concluded that the intrinsic immune recovery after UCBT in children can be very rapid, and that early T-cell reconstitution in
this context is thymus-independent and driven by the peripheral expansion of naïve cord blood lymphocytes infused with the graft. Moreover, it was observed that UCB naïve T-lymphocytes appear to undergo a rapid conversion to a memory/effector phenotype in response to viral/antigenic stimuli within the first 2 months post transplant\textsuperscript{12,13} and that a long term diverse immune repertoire is achieved\textsuperscript{14}. Nevertheless, a drawback of omitting ATG prior to UCBT may be an increased incidence of GvHD or graft rejection which may negatively impact on survival.

For the first time we report the role and timing of ATG on immune reconstitution and clinical outcome in children undergoing unrelated UCBT. Despite the heterogeneity of this population, these data may provide better guidance to future decisions on the use of ATG (or other serotherapy) in UCBT protocols.
Methods

Setting and study population

All patients who received an unrelated UCBT at either the University Medical Center (UMC) Utrecht or at Great Ormond Street Hospital (GOSH), London were included in this study. Patients were divided in 3 groups: those who received ATG late (between day -5 to 0) in the conditioning regimen, those who received ATG early (between day -9 to -5), and those who received no ATG. Patients without ATG were transplanted in London between April 2008 and December 2011, while patients given ATG were transplanted in Utrecht between 2004 and November 2011 (late ATG 2004-2009, early ATG 2009-2011). The ATG used was Thymoglobulin at a total dose of 10mg/kg; except for patients with active hemophagocytic lymphohistiocytosis who received a double dose and patients with a CD3+ T-cell count <300x10e6/L (before conditioning) who received a dose reduced by 50%. All immune reconstitution data were collected prospectively when patients were enrolled for HCT after written informed consent was obtained in accordance with the Declaration of Helsinki and institutional ethical committee approval for sample and data collection.

Transplantation details, conditioning and supportive care.

Patients’ and transplant’s characteristics are summarized in Table 1. Children were conditioned with either a myeloablative or a reduced intensity conditioning (RIC) regimen prior to UCBT. For patients receiving i.v. busulfan-containing regimens in the UMC Utrecht, therapeutic drug monitoring was used to target to a myeloblative AUC of 85-95 mg*h/L.

GvHD-prophylaxis consisted of cyclosporine-A (CsA) supplemented with prednisolone 1 mg/kg at the UMC Utrecht or with Mycophenolate Mofetile (15mg/kg TDS) at GOSH. CsA was administered from 2 or 3 days pre-UCBT, with a targeted trough level of 150-250 ng/mL.

All patients received recombinant human granulocyte colony-stimulating factor (G-CSF): 5 ug/kg/day at GOSH from day+14 until neutrophil recovery (1 x10e9/L)
and 10 ug/kg/day at the UMC Utrecht from day+7 until neutrophil recovery (2x10e9/L).

Antimicrobial prophylaxis consisted of ciprofloxacin (from start of the conditioning regimen until neutrophil recovery), acyclovir (from start of the conditioning until CD4+ T-cell recovery for VZV-positive patients) and itraconazole (GOSH) or voriconazole (UMC Utrecht) from the start of conditioning until neutrophil recovery and no steroid treatment. *Streptococcus viridans* prophylaxis consisted of cefazolin (from day 0) at UMC Utrecht, while patients treated in London received vancomycin prophylaxis (from day +1) from year 2010. Prevention of *Pneumocystis jiroveci* pneumonia included cotrimoxazole from myeloid-recovery, 3 weekly until at least 6 months (UMC Utrecht) or a CD4+ T-cell count >300x10e6/L (GOSH) and absence of chronic GvHD/immunosuppressive treatment.

### Donor selection and processing of the graft

Umbilical cord blood (UCB) units were obtained from (inter-)national cord blood banks. UCB units were required to be HLA matched to the patient at 4/6 or greater for HLA A-, HLA- B- and HLA-DRB1-antigens (HLA-A, -B serological typing and HLA-DRB1 high resolution allele typing). The minimum total nucleated cell (TNC) dose required for the transplant was 2.5, 3 and 5x10e7 NC/kg in case of a 6/6, 5/6 or 4/6 HLA-matched UCB-unit, respectively. Children who did not meet these criteria received a double UCBT: both CB units were required to be a 4/6 match with each other and the patient and having a minimal cumulative cell dose of 4x10e7 NC/kg.

In GOSH, prior to transplantation, full allelic typing of HLA A, B, C, DRB1 and DQB1 antigens was performed on the cord sample and from April 2010 units with more than three allelic mismatches were not selected.

### Post UCBT follow-up

*Engraftment and chimerism*: myeloid recovery was defined as the first of three consecutive days with an absolute neutrophil count exceeding 0.5x10e9/L, while
platelet recovery was defined as an unsupported platelet count of 20x10^9/L (TBC20) and 50x10^9/L (TBC50). Chimerism studies were performed by short tandem repeat variability on peripheral blood samples from the first signs of engraftment (lymphocyte count >0.4x10^9/L) and repeated every 2-4 weeks until >95% UCB chimerism was observed. Mixed chimerism was defined as the present of >5% recipient DNA.

**Immune-reconstitution**: Subset analysis CD3+, CD4+ and CD4+ naïve, B and NK-cell numbers were prospectively measured during post-transplant follow-up at 1, 2, 3, 6 and 12 months post-UCBT. To do so, all patients were included within immune-reconstitution studies approved by the respective institutional review boards and written informed consent was obtained from patients' parents or legal guardians in each case according to the declaration of Helsinki. Cell counts were performed using an automated cell counter. CD3-FITC, CD45-PerCP, CD19-APC or CD3-FITC, CD8-PE, CD45-PerCP, CD4-APC (MultiTEST, BD) containing reagent was added to a Facs tube containing a known quantity of beads, followed by 100uL of (EDTA-) whole blood and incubated for 10 minutes at room temperature. Red bloods cells were subsequently lysed for 10 minutes with FACS Lysing solution (BD). Samples were acquired using a FACS-Calibur and analyzed using BD software. The following lymphocyte subsets were counted: CD3+ T-lymphocytes, CD3+CD4+ and CD3+CD8+ T-lymphocytes, CD19+ B-lymphocytes, CD3-/CD16+CD56+ natural killer (NK), CDRA+CD27+ naïve T-lymphocytes, CDRA-CD27+ memory T-lymphocytes and CDRA+CD27-effector T-lymphocytes.

**Antimicrobial monitoring**: as standard of care viral (EBV, CMV, adenovirus) qDNA loads were weekly monitored in the peripheral blood using quantitative polymerase chain reaction.

**Remission status**: in GOSH bone marrow studies were standard post engraftment. In the UMC Utrecht these were not routinely performed, only in case of poor engraftment or evidence of disease relapse in the peripheral blood. Relapse was based on hematological, immunological and pathological confirmation of disease recurrence.
Post-transplant complications were defined similarly in both centers. Death without underlying disease progression was considered transplant-related. Toxicity was scored according to National Cancer Institute Toxicity Criteria. The diagnosis of aGvHD was made clinically, and confirmed pathologically with skin, mucosal or liver biopsy whenever possible. Grading of aGvHD was performed according to the Seattle criteria with the caveat of late aGVHD to occur after day 100, as described by the NIH consensus guidelines\textsuperscript{15,16}. Chronic GVHD was defined according to NIH guidelines.

The definition for non-infectious lung-injury has been the range Idiopathic pneumonia syndrome (IPS) and bronchiolitis obliterans syndrome (BOS), and only those where no infectious cause could be established (by respiratory viral PCR and bacterial cultures of tracheal secretions, imaging, aspiration of localized lesions identified with imaging, and/or post-mortem pathology results if available). IPS was defined as acute bilateral infiltrates with cough, dyspnea and hypoxemia in the absence of infection. BOS was defined as typical HRCT-changes such as bronchial wall thickening, air trapping and mosaic attenuation and if pulmonary function testing could be performed a decrease of $\geq 20\%$ in FEV1, again in the absence of signs of infection.

**Endpoints:**

Primary endpoints were: Overall survival (defined as survival from UCBT to death or last contact), Event-free survival (defined as survival time from the HSCT to relapse of malignant disease, death or graft failure), non-engraftment (defined as a lack of neutrophil recovery to $\geq 0.5 \times 10^9$/l), secondary graft failure (transient engraftment followed by a progressive decline of donor cells after transplantation with a requirement for a second transplant), relapse of malignant disease, and non-relapse mortality (NRM: death not due to underlying malignant disease). Secondary endpoints were immune reconstitution as measured by absolute numbers of CD3+ and CD4+ T-cells at 1, 2, 3, 6 and 12 months post UCBT (early immune reconstitution defined as at least 300x$10^6$/L CD3+ T-cell count...
within 2 months post UCBT); aGVHD II-IV and III-IV, cGvHD, viral reactivation of CMV, EBV or adenovirus, multiple viral infections and opportunistic infection.

**Statistical analyses**

The data were retrospectively analyzed. Differences in patients’ characteristics and in immune reconstitution parameters were assessed using Kruskal-Wallis tests for continuous data and $\chi^2$-tests for categorical data. The outcome parameters used for comparison between the three groups were (event free-) survival, relapse, non-relapse mortality, immune-recovery, graft failure, acute and chronic GVHD-incidence.

To analyze risk factors for outcomes in the whole cohort, we considered patient-related factors (median age at transplant, gender), the disease (malignant/non-malignant), use of ATG (+timing), donor-factors (HLA-disparity, median collected and infused total nucleated cell and CD34+cell doses), SCT number ($1^{st}/2^{nd}/3^{rd}$) and conditioning regimen (myeloblative/RIC). Analyses of the associations between the various time-dependent variables (the various main survival endpoints and aGVHD) were performed using Cox proportional hazards models. Univariate predictors of outcome that were statistically significant ($P$-value $\leq$ 0.10) were selected for multivariate Cox proportional hazards models. Results are expressed as hazard ratios (HR) and their corresponding 95% confidence intervals (95%CI).

For analyses of dichotomous outcome parameters the univariate and multivariate logistic regression analyses were used. Dichotomous outcomes (e.g., viral reactivation: yes/no) were used as dependent variables and predictors as independent variables. Univariate predictors of outcome that were statistically significant ($P$-value $\leq$ 0.10), were selected for multivariate logistic regression analysis. Results are expressed as odds ratios (OR) and their corresponding 95%CI. CIs not including 1 ($P$-value $\leq$ 0.05) were considered statistically significant. Statistical analysis was performed using SPSS version 19.0.
Results

Patients, donors and transplant characteristics
A total of 127 patients were included; 48 in the late ATG group, 33 in the early ATG group and 46 in the no ATG group (Table 1). No difference in ATG dose was found between the two ATG groups. RIC regimes were more common in the no ATG group.

Indication for UCBT was different amongst the treatment groups: there was a larger proportion of children with primary immunodeficiencies in the no ATG group, while there was a higher proportion of metabolic diseases in the late ATG and early ATG groups (Table 1). This difference resulted in a lower median age at transplantation (p=0.006) and a higher UCB-CD34+/kg cell dose in the no ATG group (Table 1). Although cord blood selection rules differ between the two centers, HLA-disparity ended up not being different between centers (p=0.49).

Engraftment and survival
The CI for neutrophil engraftment within 60 days post-UCBT was 95%±4% for the no ATG group, 97%±3% for the early ATG and 90%±4% for the late ATG group. A more rapid myeloid and platelet recovery was observed in the early ATG group (Table 1, Fig.1B : the median time to neutrophil recovery was 21 days in the no ATG, as opposed to 17 days in the early ATG (p = 0.01) and 21 days (NS) in the late ATG group. Non-engraftment rates were low, with no statistically significant difference amongst the 3 groups (no ATG= 4%, early ATG= 3% and late ATG= 10%; p=NS). Equally, secondary graft failure rates were not statistically different: 2%, 3% and 11% respectively.

The 3-year estimated probability of overall survival was similar in the three groups (no ATG= 71%±8%, early ATG= 68%±9% and late ATG= 61%±7%). Non-relapse mortality, relapse rate and event-free survival were also not significantly different. The 3-year estimate probability of event-free survival was 61%±8% for the no ATG group, 68%±8% for the early ATG group and 55%±7% for the late ATG group (Fig. 1A).
In multivariate analysis only the use of a mismatched 4/6 or 5/6 UCB unit (HR 4.2, 95% CI 1.1-15.8, p=0.033) was found to be a predictor for lower EFS. The use of ATG was not. For NRM none of the variables were found to be significant predictors.

Immune reconstitution

Immune reconstitution data are available for 85-88% of the timepoints 1,2,3,6 and 12 months post UCBT. Using multivariate analysis the only predictor for early immune-reconstitution after UCBT was the omission of ATG from conditioning regimen (No ATG: OR 57 with a 95% CI 12-256, p<0.001). We found that CD3+, CD4+ and CD4+ naïve T-cell numbers were a log higher in the no ATG group compared to the early ATG and late ATG groups at 1,2,3,6 and 12 months post-UCBT (p<0.001, Fig. 2B). The median CD3+ T-cell count in the no ATG group at 1,2,3,6 and 12 months post UCBT was 340x10e6/L, 720x10e6/L, 535x10e6/L, 940x10e6/L, and 1860x10e6/L respectively (Fig. 2B).

Interestingly, patients in the early ATG group had significantly higher CD3+, CD4+ and CD4+ naïve T-cell counts at 1 and 2 months post-UCBT, when compared to patients in the late ATG group (1 month: p=0.02, 2 months: P=0.036, 3 months p=0.13, Fig. 2). There was no difference in NK- and B-cell recovery amongst the 3 groups: normal NK cell numbers were observed in all groups 1 month post UCBT and normal B-cells were observed 2 months post UCBT (Fig.2A)

Transplant-related complications

Viral reactivations: the no ATG group showed a lower number of episodes of viral reactivations (p=0.021) compared to patients who received ATG (Fig.3), while no difference was observed between the early ATG and late ATG groups. There was also a lower number of deaths from viral infections (p=0.002) in the group with no ATG compared to the groups that received ATG. Specifically for EBV: preemptive rituximab therapy in a dose of 375 mg/m2 was administered to 10
patients (2 in the no ATG group, 2 in the early ATG group and 6 in the late ATG group). However, clinically apparent post-transplant lymphoproliferative disorder was diagnosed only in 1 additional patient, who recovered after treatment and has good immune reconstitution and quality of life. Four of the 11 patients treated for EBV died. Neither of them died directly of EBV-infection. One died of aGVHD, another of invasive fungal infection and two of toxicity-related multi-organ failure and GVHD. All four were in the late ATG group.

The number of patients with opportunistic (including fungal) infections was not different for the three groups (p=0.24).

GvHD and non-infectious lung-injury

With multivariate analysis, both no ATG (HR 8.2, 95% CI 3.2-22.0, p<0.001) and early ATG (OR 3.9 95%CI 1.5-10.5, p=0.005) were independent predictors for aGVHD as opposed to the late ATG group.

A higher incidence of grade II-IV (p=0.003) and grade III-IV aGVHD (p=0.018) was observed in the no ATG group (Fig.1C) compared to the early ATG group. Patients in the early ATG group experienced a significantly higher incidence of grade II-IV aGVHD compared to patients in the late ATG group (44% vs. 14%, p=0.009). However, no difference was found for grade III-IV aGVHD (18% vs 5%, p=0.15) between the 2 ATG groups (data not shown). Despite the higher rate of aGVHD in the no ATG and early ATG groups, the incidence of cGVHD was not statistically different amongst the 3 groups: 12%±6% in the no ATG group, 11%±6% in the early ATG group and 28%±8% in the late ATG group (Fig. 1D).

The lowest incidence of non-infectious lung injury was found to be in the early ATG group (17±7%), but this was not significantly different to the other 2 groups (no ATG group: 37%±15% p=0.42, late ATG-group: 27%±7% (p=0.19); (data not shown).
Discussion

In this study we demonstrate that the use of *in vivo* T-cell depletion prior to UCBT significantly affects the early and late post-transplant T-cell reconstitution. It cannot be ruled out that differences between the HCT platforms in the two centers (e.g. GVHD-prophylaxis and conditioning regimen) contributed to the better immune reconstitution and higher GVHD rate observed in the *no-ATG* group. However, the hypothesis that ATG is important, is supported by the finding, that early rather than late administration of ATG also facilitated a better T-cell recovery within the first 2 months post UCBT using the same transplant platform. Furthermore, a similar observation has also been done for double UCBT without ATG by Sauter et al., showing a steady immune recovery, a low infection related mortality after day 120, but a higher GVHD risk compared to patients that received ATG prior to double UCBT.

In this manuscript we show that children transplanted without ATG as part of their conditioning regimen, experienced a remarkably quick CD3+ cell recovery early after UCBT, with a median T-cell-count of 720x10^6/L only two months after transplant. This is in contrast to those patients treated with early or late ATG, where a median CD3+ T-cell-count of <100x10^6/L was achieved at the same time point. The lymphocyte recovery was CD4+ biased as previously described, and the reason for this is currently unknown. Similarly, other studies have shown a comparable pattern of poor early T-cell recovery after UCBT, in the context of *in vivo* T-cell depletion.

The improved immune reconstitution in the group not receiving ATG prior to transplantation was associated with a lower incidence of reactivation of EBV, CMV and/or Adenovirus. Various reports have shown that UCBT-recipients are at increased risk of viral reactivation due to the delayed immune recovery, and early initiation of pre-emptive antiviral therapy is frequently used to prevent viral disease, using a lower cut-off qDNA-value in CB-recipients. Although we did not find the MAC/RIC regimen to be a predictor in this cohort; ATG used in association with RIC regimens has been shown to further increase the risk of
viral reactivation and specifically also the risk of EBV PTLD\textsuperscript{25,26}. Overall, EBV PTLD was well manageable in this cohort with preemptive treatment. Only one patient developed PTLD and she recovered completely.

The observed reduced incidence of viral reactivations in the \textit{no ATG} group is likely the result of not depleting the UCB-graft of lymphocytes, which although naïve, have the capacity of rapidly differentiating into the effector/memory phenotype in response to antigen stimulation even early after UCBT\textsuperscript{8,9,11,27}. It could be speculated that an early specific immune reconstitution post-UCBT may also lead to a more powerful graft-versus leukemia effect, as suggested in other studies\textsuperscript{2,11,28,30}. The number of patients with malignant disease in each group in this study was too small and disease-type and conditioning too heterogeneous to assess this.

In our pediatric population of children with mixed malignant and non-malignant indications, ATG was not required for engraftment as the CI of neutrophil engraftment at day 60 was similar between groups (Fig.1B). In contrast with others we found HLA disparity to be a predictor for EFS but not cell dose. This can be explained by the strict “cell dose rules” that were applied and the low median weight of the studied cohort leading to a high median cell dose in this study of 7.3 (0.8-33.2) \times 10^7 NC/kg after thawing.

The clinical benefit of an early immune reconstitution in the \textit{no ATG} group has to be balanced against the higher incidence of moderate to severe aGvHD observed in this cohort. Interestingly, this was not associated with a higher incidence of chronic GvHD or non-relapse mortality. Historically UCBT has been associated with a lower incidence of aGvHD compared to MUD HCT, as shown in various comparison studies. This study puts that in perspective, showing that the incidence of aGvHD in UCBT is very much influenced by \textit{in vivo} T-cell-depletion. Rates of aGvHD reported in this study are higher than previously published by other authors: e.g. Verneris et al reported aGvHD (grade II-IV) rates of 29% for single UCBT and 48% for double UCB transplants\textsuperscript{12,13,31}. A more recent and large Eurocord study showed similar GVHD incidences in single and double UCBT\textsuperscript{15,16,32}. MacMillan reported that the omission of ATG prior to double
UCBT did not influence mortality and led mainly to an increased incidence of grade II skin GvHD\textsuperscript{10,33}. The aGvHD rates in our cohort may however also be influenced by the selection of primary immunodeficiency patients in the no ATG group. Such children may be more at risk for aGvHD due to ongoing infections and tissue inflammation, Importantly however, a higher rate of aGvHD did not lead to a higher incidence of cGvHD, viral reactivations, or higher NRM.

In the most commonly used UCBT-protocols, particularly with “late” administration of ATG we most likely administer an “overdose” of ATG causing severe \textit{in vivo} depletion of the graft, resulting in absent or very late immune reconstitution. In fact there are centers that therefore use lower doses than 10 mg/kg in their conditioning regimen, especially in adults. It has recently been shown for adults that ATG as high as 8 mg/kg pre-transplant can have a deleterious influence using peripheral blood stem cells as the stem cell source in RIC setting, increasing the risk of relapse compared to the dose of 6 mg/kg\textsuperscript{34}. Potentially, various patient/donor-dependent variables such as weight, lymphocyte-count prior to UCBT and age influence ATG PK/PD\textsuperscript{35}. Improving outcome after UCBT further may therefore require a more individualized approach to ATG timing and dose, adapted to indication, immunological status and infectious co-morbidities of the patient. This can possibly be achieved by implementing pharmacokinetic and pharmacodynamic (PK/PD) modeling techniques for antibody-agents such as ATG. In a joint project between two pediatric blood and bone marrow transplantation units in the Netherlands (Utrecht and Leiden), ATG-PK/PD studies are currently being performed in over 300 patients. This will be the basis for the development of a worldwide applicable dosing algorithm (e.g. based on weight, age)\textsuperscript{8}. Other more novel GVHD-prevention strategies such as HDAC inhibitors, proteasome-inhibitors, antibodies targeting IL-21 or vascular adhesion molecules), B-cell targeting or Treg and MSC-infusions may also be among the options for diminishing GVHD-risk post UCBT without ATG\textsuperscript{36}. 
An early specific and diverse immune reconstitution is crucial for a good outcome after transplantation, since relapse and non-relapse mortality (due to e.g. acute-GvHD and viral reactivation) are the most important limiting factors\textsuperscript{37}. Currently, the improved immune reconstitution, associated with lower viral reactivations, albeit at the cost of increased rates of aGVHD (but not cGvHD), suggests that omitting ATG in CBT may be suitable for children treated for malignancy, or children at risk of viral reactivation, while children with otherwise uncomplicated non-malignant disorders may benefit from reduced aGVHD with inclusion of early ATG in the conditioning regimen.

**Authorship**

C.L. conducted the research, analyzed the data and wrote the paper, R.C. conducted the research, analyzed the data and wrote the paper, P.A, K.R, K.G and O.N. contributed to the UK data and the design of immune-reconstitution studies. C.G. and A.W. contributed to the Utrecht data. P.V. and J.B. performed research, analyzed data and wrote the paper and are involved in the design of immune reconstitution studies.

Authors have no conflict of interest to declare.

**Acknowledgements**

C.L. is supported by a clinical fellowship from the Dutch Cancer Society (KWF Kankerbestrijding).
References


13. Cantó EE, Rodríguez-Sánchez JLJ, Vidal SS. Naive CD4+ cells from cord blood can generate competent Th effector cells. Transplantation.


Figure legends

Table 1. Basic characteristics
Basic characteristics of the patients, which have been divided in a no ATG group, a group who received ATG early in the conditioning regimen and a group receiving ATG late in the conditioning. Non-parametric tests have been performed. A p-value of < 0.05 is considered significant. (ns= non-significant.)

Fig. 1 ATG in cord blood transplants does not influence EFS and engraftment, however does lead to more aGVHD. Kaplan-meier survival curve and Cox proportional hazards analysis of the (A) probability of Event-free survival (EFS), (B) probability of neutrophil engraftment: neutrophil count of 0.5 x 10^9/L for at least 3 days in the first 60 days post SCT (C) probability of aGVHD gr. II-IV (D) probability of cGVHD. A p-value of < 0.05 is considered significant. (NS= non-significant.)

Patients represented as received no ATG; received ATG early; _______ received late ATG.

Fig. 2 ATG affects post-SCT T-cell immune-reconstitution
(A) The panel represents a comparison of CD3+ cells numbers, B cell numbers and NK cell numbers for the three groups, at 2 months post SCT and 6 months post SCT. (B) represents the logarithmic evaluation of CD3+, CD4+, CD4+ naive cell counts in time post SCT (months).

Fig. 3 Lower incidence of viral reactivation in Cord blood transplants without ATG. The incidence of viral reactivation of Adenovirus, CMV or EBV is compared for each of the three groups in panel (A) and for with and without ATG in panel (B). Mann Whitney U tests have been performed. A p-value of < 0.05 is considered significant. (NS= non-significant.)
## Table 1

<table>
<thead>
<tr>
<th>SEROTHERAPY</th>
<th>NO ATG</th>
<th>EARLY ATG</th>
<th>LATE ATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M/F</td>
<td>28/18</td>
<td>20/13</td>
<td>22/26</td>
</tr>
<tr>
<td>- Malignancy</td>
<td>21</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>- Immunodeficiency (SCID)</td>
<td>24 (17)</td>
<td>10 (2)</td>
<td>12 (2)</td>
</tr>
<tr>
<td>- Bone marrow failure</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>- Metabolic Disease</td>
<td>1</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>- Refractory autoimmunity</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Age median (range)</td>
<td>1.8 (0.1-12.2)</td>
<td>5.5 (0.1-22.7)</td>
<td>2.3 (0.2-21.2)</td>
</tr>
<tr>
<td>SCT number 1st/2nd/3rd</td>
<td>42/4/0</td>
<td>32/1/0</td>
<td>45/2/1</td>
</tr>
<tr>
<td>Myeloablative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BU iv(TDM)/CY</td>
<td>1</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>- BU iv(TDM)/FLU/(Clo)</td>
<td>2</td>
<td>27 (+2)</td>
<td></td>
</tr>
<tr>
<td>- Treo 42/FLU150</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>- Treo 42/CY120</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BEAM</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- TBI based 12-14.4 GY</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>- BU/CY/Vp16(TDM)</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>- BU/CY 120/Mel140</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>- oral Bu 16/CY</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Treo 36/FLU 150</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Treo 36/CY 200</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- BU3/8;CY1/5;FLU3/8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>- BU3/8; FLU 3/8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>- FLU/CY 1/5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO conditioning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- rATG Genzyme 10 mg/kg</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>- rATG Genzyme 5 mg/kg</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>- rATG Genzyme 20 mg/kg (HLH)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NC/kg x 10e7 median (range)</td>
<td>8.1 (2.6-17.7)</td>
<td>5.8 (0.8-33.2)</td>
<td>7.3 (0.8-28.0)</td>
</tr>
<tr>
<td>CD34+/kg x 10e5 median (range)</td>
<td>3.4 (0.4-29.6)</td>
<td>1.4 (0.2-9.5)</td>
<td>2.2 (0.2-9.7)</td>
</tr>
<tr>
<td>- 6/6</td>
<td>18</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>- 5/6</td>
<td>25</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>- 4/6</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Single/Double CBU</td>
<td>42/4</td>
<td>30/3</td>
<td>47/1</td>
</tr>
</tbody>
</table>
Fig. 1
Fig. 2
Fig. 3

Panel A: Comparison of viral reactivation among different ATG categories (no, early, late). The p-values are marked with NS (not significant) for no significant difference and p=0.032 and p=0.068 for significant differences.

Panel B: Comparison of viral reactivation between ATG categories (−, +). The p-value is p=0.021 for a significant difference.
The impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune-reconstitution and clinical outcome

Caroline A. Lindemans, Robert Chiesa, Persis J. Amrolia, Kanchan Rao, Olga Nikolajeva, Arianne de Wildt, Corinne E. Gerhardt, Kimberly C. Gilmour, Marc Bierings, Paul Veys and Jaap J. Boelens