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

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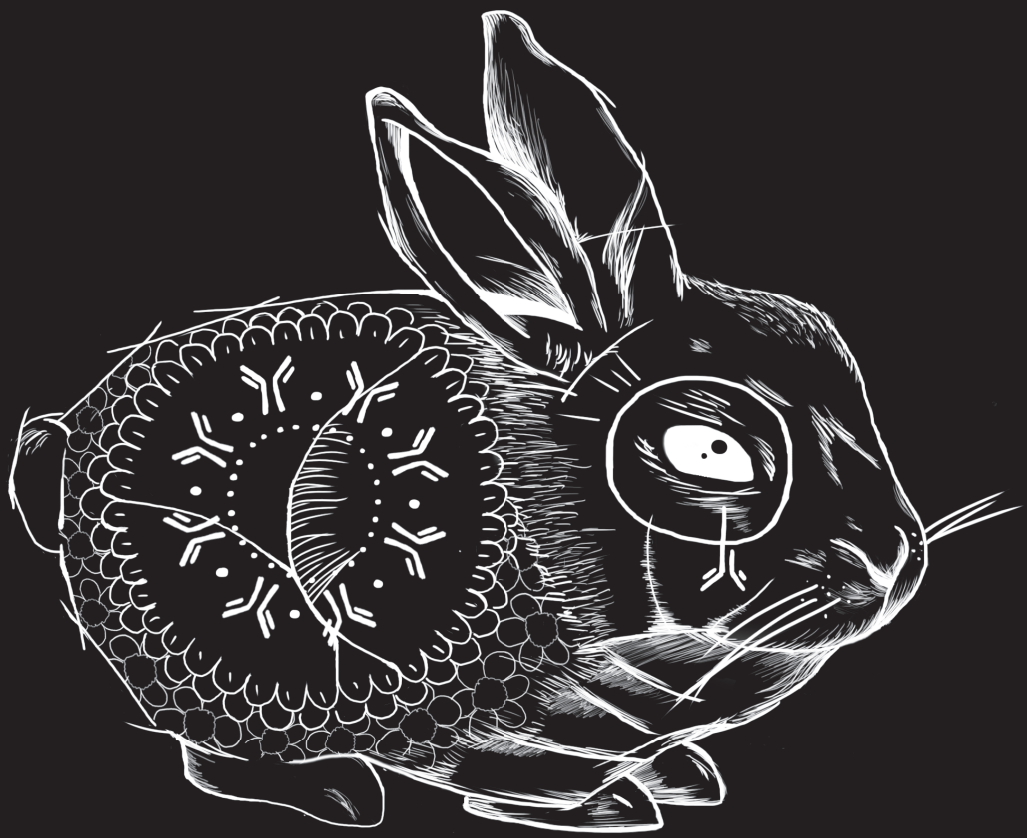
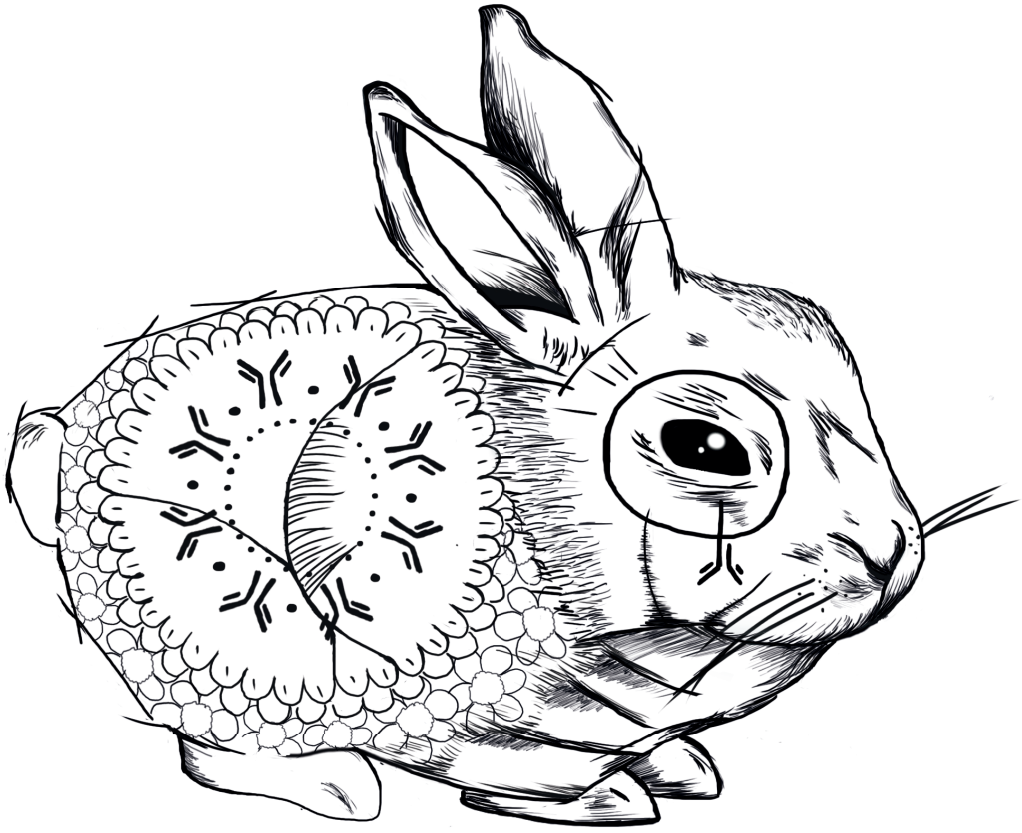


PART IV

Immune Reconstitution Predicting Clinical Outcome



Chapter 9

Leukemia-free Survival in Myeloid Leukemia, but not in Lymphoid Leukemia, is Predicted by Early CD4+ Reconstitution following Unrelated Cord Blood Transplantation in Children: a Multicenter Retrospective Cohort Analysis

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Hematopoietic cell transplantation (HCT) is a potentially curative option for chemotherapy-resistant leukemia. In addition to either high-dose chemotherapy or TBI, the advantages of HCT include clearance of residual disease by GvL effect.[1] This should lead to a durable CR.

Unrelated umbilical cord blood (CB) has been increasingly used as an alternative cell source during the last decades. However, T-cell immune reconstitution (IR) in CB can be delayed when compared with matched unrelated donors or sibling transplants,[2] which may be caused by the lower number of infused T cells combined with a potentially higher susceptibility of CB T cells to serotherapy (for example, anti-thymocyte globulin; ATG).[3] Sufficient IR has subsequently been related to a reduced relapse incidence, indicating that reduction of in vivo lymphodepletion of the graft is of utmost importance.[4] For this reason, some centers decided to remove ATG from the conditioning regimen in malignant indications.[5]

Here, we aim to investigate CD4+ T-cell IR as a predictor of leukemia-free survival (LFS), being the main outcome of interest. Children receiving a cord blood transplantation (CBT) in two dedicated CB transplantation centers, the University Medical Center Utrecht (UMCU) in Utrecht, The Netherlands, and Great Ormond Street Hospital in London, UK, were included. In both centers, CB was the preferred cell source when a matched sibling donor was not available. IR was defined as reaching a CD4+ T-cell count of 50×106 cells/L within 100 days after CBT in two consecutive measurements that was previously described as being the best predictor.[3,6] We also took the effect of exposure to ATG on T-cell reconstitution into account, as ATG may significantly hamper CD4+ IR. Patients were divided into three groups: no ATG, low exposure to ATG after CBT (<20 arbitrary units (AU)days/mL) and high exposure (>20 AU×days/mL). We also related CD4+ IR to other outcomes of interest: overall survival (OS), non-relapse mortality (NRM) and GvHD.

The conditioning regimens in this cohort were given according to (inter)national protocols, mostly consisting of myeloablative busulfan-based regimens. The majority of patients did not receive ATG: only those patients treated at the UMCU before 2013 and those with ALL treated after 2013 received ATG. ATG was given at a cumulative dose of 10 mg/kg over 4 consecutive days, starting from day −5 in patients treated before 2009, while patients treated from 2009 onwards received the same dose of ATG starting from day −9. From 2010 onwards, patients with a body weight 430 kg received a lower cumulative dose of ATG (7.5 mg/kg), and a 50% dose reduction was given when pre-conditioning lymphocyte counts were less than 300×10^6/L.

Patients received infection prophylaxis including gut decontamination and GvHD prophylaxis according to local protocols. GvHD prophylaxis consisted of cyclosporin A aiming at a trough level of 200–250 µg/L (therapeutic drug monitoring controlled), combined with

prednisolone 1 mg/kg (Utrecht, The Netherlands) or mycophenolate mofetile (London, UK).

We included a total of 87 consecutively treated children between 1 January 2004 and 1 August 2014 (Table 1). Of these patients, 40 percent (n=35) of children had lymphoid leukemia (acute lymphoid leukemia), 55% (n=48) of patients suffered from myeloid leukemia (acute and chronic myeloid leukemia myelodysplastic syndrome) and 4 patients (5%) had a lymphoma. This cohort includes 30 patients who have been described in a previous report.[3] Forty-one percent of the treated children received ATG in the conditioning regimen. The median follow-up for surviving patients was 48 months (range 9.4–139 months).

	GOSH	UMCU	Total	
Number of patients (n)	33	54	87	
Transplants [n(%)]				p=0.08
First transplants	29 (88)	51 (94)	80 (92)	
Second transplants	4 (12)	1 (2)	5 (6)	
Third transplants	0 (0)	2 (4)	2 (2)	
Male sex [n(%)]	19 (58)	35 (65)	54 (62)	p=0.65
Age at transplant (years)	5.4 (0.7-12.7)	9.7 (1-18.1)	8 (0.7-18.1)	p=0.6
Patients receiving ATG [n(%)]	0 (0)	36 (67)	36 (41)	p<0.001
Diagnosis [n(%)]				p=0.11
Lymphoid leukemia	10 (30)	25 (46)	35 (40)	
Myeloid leukemia	22 (67)	26 (48)	48 (55)	
Other malignancies	1 (3)	3 (6)	4 (5)	
CR status [n(%)]				p=0.00022
No CR	9 (27)	0 (0)	9 (10)	
CR 1	10 (30)	18 (33)	28 (32)	
CR 2	14 (42)	36 (67)	50 (57)	
Remission status [n(%)]				p=0.0029
Refractory disease	11 (33)	6 (11)	17 (20)	
CR, MRD positive	9 (27)	34 (63)	43 (49)	
CR, MRD negative	13 (39)	14 (26)	27 (31)	
Conditioning regimen [n(%)]				p=0.0018
Busulfan based	17 (52)	40 (74)	57 (66)	
Treosulfan based	9 (27)	1 (2)	10 (11)	
TBI based	6 (18)	13 (24)	19 (22)	
Other	1 (3)	0 (0)	1 (1)	
Follow-up (months) [mean (range)]	34 (1.2-81)	32 (0.2-139)	32 (0.2-139)	p=0.73

Table 1. Patient characteristics. GOSH: Great Ormond Street Hospital; UMCU: University Medical Center Utrecht; ATG: Anti-thymocyte globulin; CR: Complete Remission; MRD: Minimal Residual Disease; TBI: Total Body Irradiation.

When analyzing the data, we found a strong correlation between ATG exposure after CBT and probability of CD4+ IR (Kaplan–Meier estimated incidence of 89±4%, 50±18%, and 32±9% in patient with no ATG, low exposure and high exposure, respectively, P<0.0001). Successful CD4+ IR resulted in a higher probability of LFS (hazards ratio (HR) 0.44, 95% confidence interval (CI) 0.23–0.84, P=0.013); when subdividing leukemia's, this effect was only observed in myeloid leukemia's (HR 0.24, 95% CI 0.10–0.61, P=0.003, figure 1a), while in lymphoid leukemia's, LFS is not significantly influenced by CD4+ IR (figure 1b). In multivariate analysis, CD4+ IR was found to be a significant predictor of LFS (HR 0.44, 95% CI 0.23–0.84, P=0.013), along with CR status.

Corresponding with findings for LFS, OS was higher in patients reaching successful CD4+ IR (HR 0.32, 95% CI 0.16–0.64, P=0.0014). This difference was found only in children with myeloid leukemia (HR 0.16, 95% CI 0.06–0.45, P=0.00044), while in lymphoid leukemia, patients with or without successful CD4+ IR performed similar. In a multivariate model, CR status was also identified as a predictor of OS. In the whole cohort, NRM was lower in patients who achieved early CD4+ IR (HR 0.20, 95% CI 0.06–0.65, P=0.0072; Figure 1C), with no other multivariate predictors found then CD4+ IR. Relapse incidence in myeloid leukemia was significantly reduced by successful CD4+ IR (HR 0.31, 95% CI 0.10–0.95, P=0.041), while CD4+ IR did not predict relapse incidence in lymphoid leukemia's. The incidence of GvHD, both acute and chronic, was not impacted by CD4+ IR.

Taking into account the limitations of a retrospective cohort study and potential center differences, these data suggest successful CD4+ IR, resulting from no or low ATG exposure after CBT, improve LFS in myeloid leukemia. In lymphoid leukemia's, however, CD4+ IR did not predict LFS. NRM was reduced by successful CD4+ IR in all patients. These results

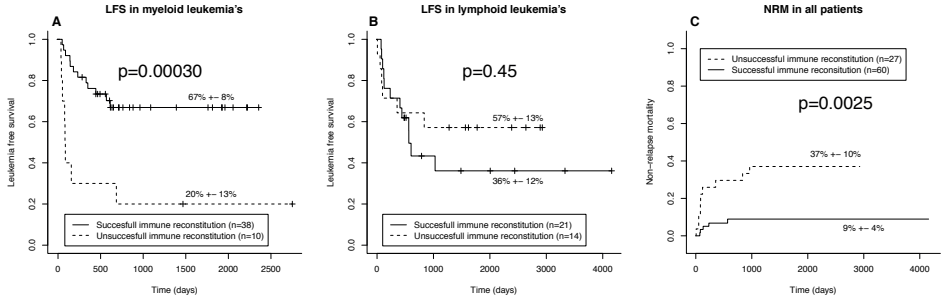

Figure 1. Kaplan–Meier curves of leukemia-free survival (LFS) according to successful CD4+ T-cell reconstitution in myeloid leukemia's (a) and lymphoid leukemia (b); non-relapse mortality (NRM) according to successful CD4+ T-cell reconstitution in all patients (c). Solid lines: successful CD4+ reconstitution, dashed lines: unsuccessful CD4+reconstitution.

stress the importance of achieving an early immune reconstitution in order to generate a GvL effect, especially in myeloid leukemia, and reduce the NRM for all patients.

CB as emerging alternative cell source shows promising results in terms of LFS,[7] anti-leukemic effect[8] and reduction of viral reactivations[5] as compared with bone marrow. Outcome may, however, be further improved by promoting or achieving predictable early immune reconstitution. Timely reconstitution of CD4+ T cells has been shown to be associated with higher survival chances.[3] Other studies comparing conditioning without ATG in a CB-setting as compared with ATG showed mixed results in terms of OS, NRM and relapse; either having no effect,[5] a lower incidence of relapse but not OS,[9] or lower incidence of NRM and OS, but not relapse.[10]

These data suggest a difference in susceptibility between myeloid leukemia and lymphoid leukemia to early CD4+ T-cell reconstitution in terms of relapse. The mechanism behind this difference is not clear, although this finding seems to be in line with the observed effect of donor-lymphocyte infusions, which is suggested to be stronger in AML compared with lymphoid leukemias.[11] Also in a recent CIBMTR study, no difference in relapse and LFS were seen in children with ALL with or without ATG in the conditioning regimen.[12] It can be speculated that this difference in GvL effect in favor of myeloid leukemia may be due to the TCR repertoire, which may harbor more targets to myeloid blasts compared with lymphoblasts. In addition, ATG itself may be able to target residual lymphoblasts, and therefore high exposure to ATG in ALL may have anti-leukemic effects itself. This may potentially result in neutralizing the beneficial effect of low ATG exposure after CBT on NRM. In vitro studies have shown tumor lysis by ATG on tumor cell lines.[13]

This study stresses the importance of early immune reconstitution to prevent relapse and NRM after CBT. Omission of serotherapy seems a feasible option in a CBT setting for malignancies. However, omission of serotherapy is associated with an increased incidence of GvHD.[14,5] Another strategy to prevent GvHD may be to optimize mycophenylate mofetil dosing. With recent understanding of the pharmacokinetics and pharmacodynamics of ATG in pediatric CBT,[3,15] individualized dosing (and timing) of ATG may yield benefits over omission of ATG.

On the basis of this study, designing a conditioning regimen that facilitates early CD4+ IR after CBT may optimize LFS in AML and reduce NRM for all CBT recipients. Moreover, early and predictable CD4+ IR opens the possibility for adjuvant immunotherapies, which depend on a functional adaptive immune system. All together, optimizing early immune reconstitution is likely to improve outcomes in patients receiving a CBT transplant for malignant indications.

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