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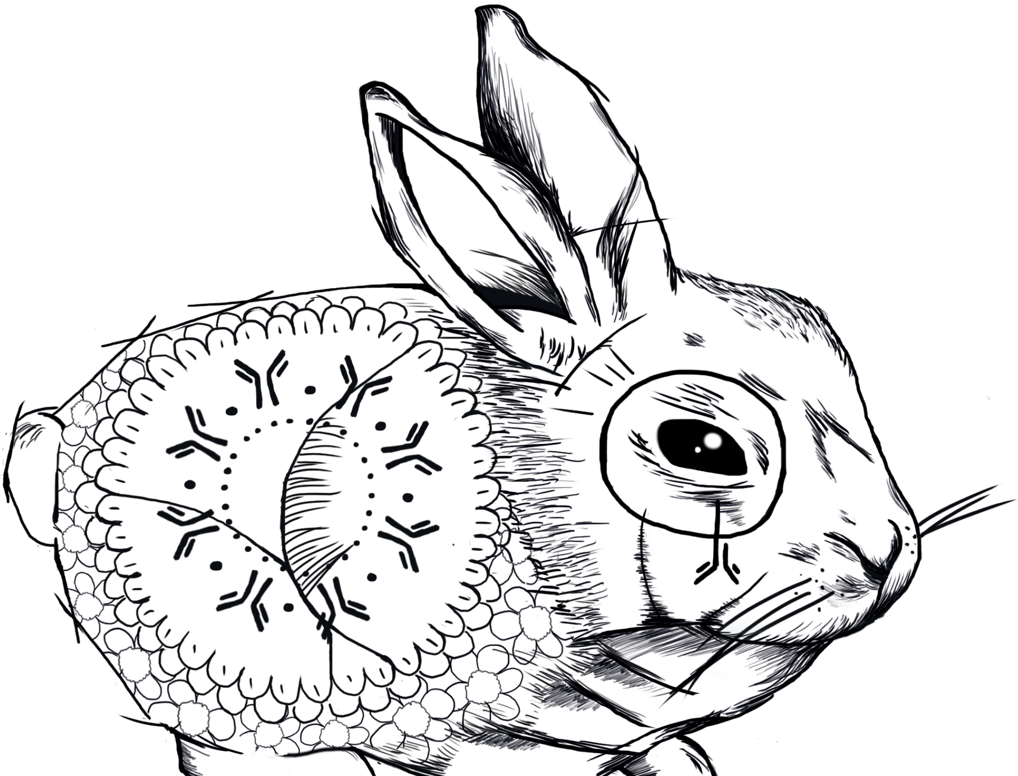

Chapter 8

Impact of alemtuzumab exposure on clinical outcomes of hematopoietic cell transplantation: are we overdosing children using I.V. dosing close to transplantation?

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To the editor,

Alemtuzumab (Campath®, Genzyme, MA, USA) is used as serotherapy in hematopoietic cell transplantation (HCT) to prevent graft-versus-host-disease (GvHD) and graft failure by in-vivo depletion of lymphocytes. Inclusion of alemtuzumab in the conditioning regimen significantly reduces the incidence of both acute and chronic GvHD[1–3]. Higher doses of alemtuzumab have been associated with delayed immune reconstitution (IR) by excessive lymphodepletion[4–6]. Poor IR could potentially lead to increased viral reactivations as well as less graft-versus-leukemia effect, thereby abrogating the beneficial effect on GvHD reduction.

A recent publication by Marsh and colleagues[7] in Blood described the impact of peri-transplant alemtuzumab concentrations on clinical outcomes. Low concentrations were associated with acute GvHD, while higher concentrations led to poor lymphocyte reconstitution and increased mixed chimerism.

We aim to evaluate these results in a larger cohort of children. We use a pharmacologically and methodologically stronger approach by calculating the alemtuzumab concentrations on the basis of a validated pharmacokinetic model[8]. This exactly estimates concentrations at the beginning of infusion of the graft rather than peri-transplant concentrations (±3 days) and eliminates some of the uncertainty incorporated in a single concentration measurement.

Children receiving their first HCT in the Leiden University Medical Center (Leiden, the Netherlands; LUMC) and Great Ormond Street Hospital (London, United Kingdom; GOSH) with alemtuzumab as part of the conditioning were included. Patients using other serotherapy drugs (anti-thymocyte globulin; ATG) within the same conditioning regimen were excluded. Data was collected after informed consent; ethical committee approval through trial numbers P01.028 (Leiden) and V0904 (London).

Alemtuzumab (Campath, Genzyme, USA) was dosed at 0.5 or 1 mg/kg (5 x 0.1-0.2 mg/kg/day) in most children. Details about the samples and alemtuzumab assay is available in the supplements. Conditioning regimens were given according to (inter)national protocols.

176 patients were included between January 2003 and July 2015 with a median age of 4.8 years (range 0.2-19 years; Table 1). Full description on the definitions and statistical approach can be found in the supplements. Fifty-four percent of patients received a cumulative dose of 1 mg/kg over 5 doses, 35% received a dose of <0.9 mg/kg. Median starting day was day -8 (-21 to -3). Immune deficiency was the most frequent indication for HCT. Median follow-up for surviving patients was 64 months (range 16.9-149).

	London	Leiden	Total
Number of patients (n)	125	51	176
Male sex (%)	64	67	65
Age (years)	4.0 (0.4-15)	8.1 (0.2-19)	4.8 (0.2-19)
Cumulative dose (mg/kg) (%)			
<0.9 mg/kg	36	29	35
0.9-1.1 mg/kg	50	65	54
>1.1 mg/kg	14	6	11
Starting day alemtuzumab (days before transplantation)	8 (5-21)	6 (3-16)	8 (3-21)
Number of samples [n (mean per patient)]	309 (2.5)	557 (10.9)	866 (4.9)
Diagnosis (%)			
Malignancy	14	41	22
Immune deficiency	65	37	57
Bone marrow failure	15	20	16
Metabolic disease	5	0	4
Benign hematology	1	2	1
Stem cell source (%)			
Bone marrow	35	65	44
Peripheral blood stem cells	65	27	54
Cordblood	0	8	2
Conditioning regimen (%)			
Reduced intensity	45	63	50
Chemotherapy-based	50	31	45
TBI-based	5	6	5
Follow-up surviving patients (months)	59 (17.3-130)	84 (16.9-149)	64 (16.9-149)

Shown as median (range) unless otherwise specified

Table 1. Patient characteristics

A clear difference in C_{graft} was observed compared to peri-transplant concentrations as reported by Marsh et al[7]. In our cohort, 6 patients (3%) had a very low C_{graft} < 0.155 µg/mL, while C_{graft} was very high (>4.36 µg/mL) in 38 patients (21%; Figure S1). In the Marsh report, of 18% and 8% had very low and very high concentrations, respectively[7]. Other groups were comparable. Patients were analyzed in groups with a C_{graft} < 1, 1-2, 2-3 and >3 µg/mL.

The incidence of grade 2-4 acute GvHD was impacted by C_{graft}. Patients with high C_{graft} showed the lowest incidence, while the three groups with lower C_{graft} performed worse (figure 1a). In multivariate analysis (MV), high C_{graft} was associated with a lower incidence of acute GvHD in both grades 2-4 and 3-4 (hazard ratio [HR] 0.79, 95% confidence interval

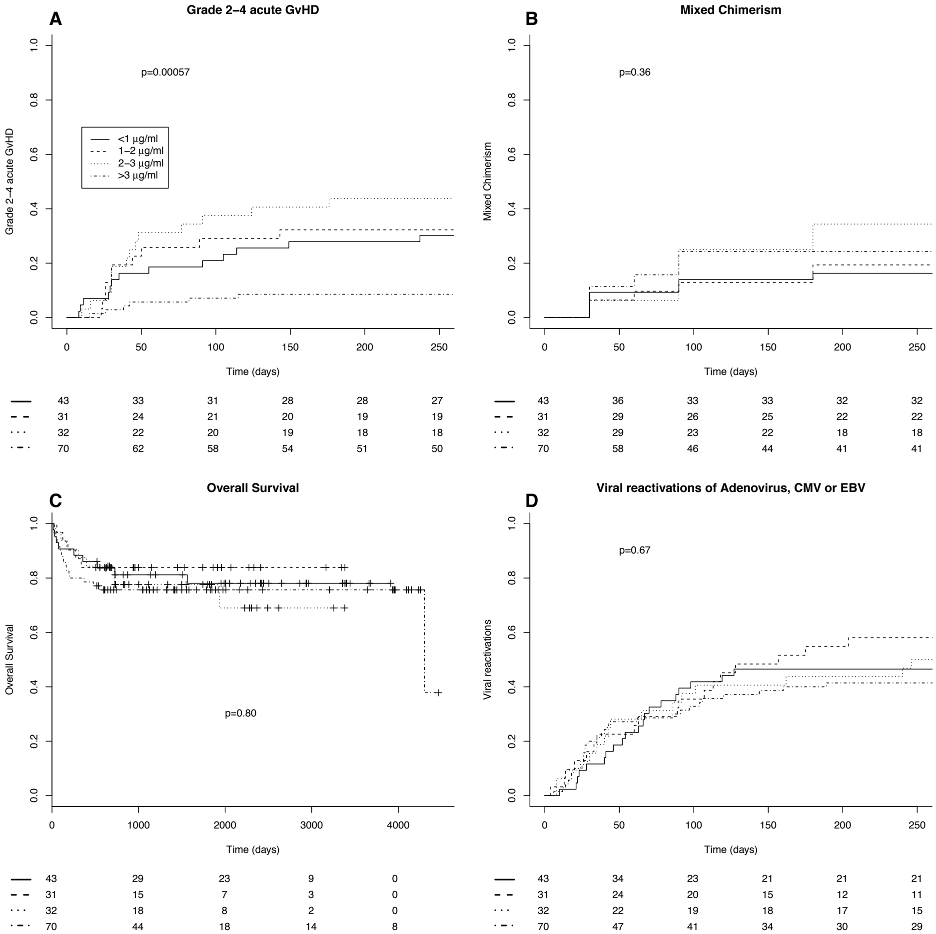

Figure 1. Cumulative incidence of acute GvHD (panel A), mixed chimerism (panel B) and viral reactivations (panel D) and Kaplan-Meier overall survival curve (panel C) in groups of alemtuzumab concentrations at time of graft infusion. Orange: <1 µg/mL; black: 1-2 µg/mL; red: 2- 3 µg/mL; blue: >3 µg/mL.

[CI] 0.67-0.93, p=0.0046 and HR 0.58, 95% CI 0.38-0.87, p=0.0081, respectively; Table S1 and S2). No other multivariate predictors were identified. Chronic GvHD was not impacted by C_{graft} (p=0.32 in MV analysis).

Mixed chimerism, defined as < 95% donor chimerism in 2 whole blood samples, did not differ between groups (figure 1b). In multivariate analysis, no significant impact of C_{graft} on mixed chimerism could be identified (HR 1.03, 95% CI 0.93-1.13, p=0.60).

Overall survival was not impacted by C_{graft}, both in univariate (fig 1c) and in multivariate analysis (HR 0.99, 95% CI 0.88-1.13, p=0.97). However, treatment after 2009 (median treatment year in this cohort) was a multivariable predictor for improved survival (p=0.046, Table S2).

We also investigated viral reactivations of Epstein-Barr virus (EBV), cytomegalovirus (CMV) and adenovirus, defined as a > 1000 viral copies/mL in 2 subsequent measurements. No difference in viral reactivations was found between groups of C_{graft} (HR 0.96, 95% CI 0.88-1.06, p=0.40; figure 3d).

Finally, the relation between C_{graft} and CD3+ T-cell counts was investigated. CD3+ immune reconstitution (IR) was defined as a CD3+ T-cell count >100 x 10^6/L in 2 samples before 100 days, as adapted from literature[10]. While there were significant differences between groups of C_{graft}, the distribution of CD3+ kinetics not ordered in terms of C_{graft} (figure S2a). This is reflected in the multivariate analysis, where only a trend was found between C_{graft} and CD3+ IR (HR 1.14, 95% CI 0.99-1.31, p=0.060). The absolute CD3+ count at day 100 was not significantly different between groups (p=0.064; figure S2b).

These data suggest that high alemtuzumab concentrations during graft infusion may reduce the incidence of acute GvHD. Other outcome parameters including survival, mixed chimerism, viral reactivation and T-cell recovery are not impacted by alemtuzumab concentrations.

Compared to previous the study by Marsh et al[7], the alemtuzumab concentrations in this cohort are relatively high. Part of this difference may be due to differences in underlying disease and conditioning regimen. The dosing and route of administration are most likely larger contributors to the difference in alemtuzumab concentrations. The alemtuzumab dose used by Marsh (1mg/kg, or fixed dose of 33 mg [<10 kg] or 48 mg [>10kg]) is generally higher than was used in this cohort (0.5-1 mg/kg in most patients). However, the starting day was more proximal (i.e. closer to graft infusion) in the current study, and 66% of patients received subcutaneous dosing in the Marsh-study[7]. Subcutaneous dosing leads to a high first-pass metabolism, and results in a slower release, both reducing alemtuzumab concentrations in blood[11]. Still, taking into account the higher C_{graft} in this cohort, the results are generally comparable. The most optimal therapeutic window has not been set convincingly.

From a pharmacological perspective, the most optimal dose for a drug shows the desired effects with minimal toxicity[12]. For alemtuzumab, the desired effect is the prevention of GvHD and mixed chimerism, while toxicities include poor T-cell reconstitution and subsequent viral reactivations[13]. In the current study, a moderate exposure-response effect can be identified for GvHD, not for mixed chimerism. Of note, stable mixed chimerism can be allowed in subgroups of patients. The C_{graft} however is not correlated to toxicity. It can be concluded that a vast majority of patients had a supratherapeutic alemtuzumab exposure. This is in line with the final conclusion by Marsh, who reports the optimal C_{graft} for alemtuzumab to be between 0.2-0.4 μg/mL[7].

Therapeutic drug monitoring (TDM) in combination with individualized dosing may be useful in assuring the most optimal alemtuzumab exposure[14,15]. However, the long time window (±10 days) between the infusions and the graft infusion, combined with the narrow therapeutic window during graft infusion, may complicate this approach. Still, taking into account the very slow clearance[16–18] and low lympholytic level[7] of alemtuzumab, approaches to target exposure will be difficult. Anti-thymocyte globulin has a faster clearance[19,20] and higher a lympholytic level[20], and therefore may be a more attractive therapeutic option to prevent GvHD and graft failure[21–24].

In conclusion, alemtuzumab concentrations at graft infusion after intravenous doses of 0.5-1 mg/kg starting 10-6 days before HCT results in supratherapeutic exposures. Dosing should be reduced, given earlier or administered subcutaneously.

REFERENCES

1. Perez-Simon JA, Kottaridis PD, Martino R, et al. Nonmyeloablative transplantation with or without alemtuzumab: Comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood*. 2002;100(9):3121-3127.
2. van Besien K, Kunavakkam R, Rondon G, et al. Fludarabine-Melphalan Conditioning for AML and MDS: Alemtuzumab Reduces Acute and Chronic GVHD without Affecting Long-Term Outcomes. *Biol Blood Marrow Transplant*. 2009;15(5):610-617.
3. Poire X, van Besien K. Alemtuzumab in allogeneic hematopoetic stem cell transplantation. *Expert Opin Biol Ther*. 2011;11(8):1099-1111.
4. Spyridonidis A, Liga M, Triantafyllou E, et al. Pharmacokinetics and clinical activity of very low-dose alemtuzumab in transplantation for acute leukemia. *Bone Marrow Transplant*. 2011;46(10):1363-1368.
5. Lane JP, Evans PT, Nademi Z, et al. Low-dose serotherapy improves early immune reconstitution after cord blood transplantation for primary immunodeficiencies. *Biol Blood Marrow Transpl*. 2014;20(2):243-249.
6. Rao K, Amrolia PJ, Jones A, et al. Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. *Blood*. 2005;105(2):879-885.
7. Marsh RA, Lane A, Mehta PA, et al. Alemtuzumab levels impact acute GVHD, mixed chimerism, and lymphocyte recovery following alemtuzumab, fludarabine, and melphalan RIC HCT. *Blood*. 2015;127(4):503-513.
8. Admiraal R, Jol-van der Zijde C, Furtado Silva J, et al. Population pharmacokinetics of alemtuzumab (Campath) in pediatric hematopoietic cell transplantation: towards individualized dosing to improve outcome. Submitted.
9. Rebello P, Hale G. Pharmacokinetics of CAMPATH-1H: assay development and validation. *J Immunol Methods*. 2002;260(1-2):285-302.
10. Admiraal R, van Kesteren C, Jol-van Der Zijde CM, et al. Association between anti-thymocyte globulin exposure and CD4+ immune reconstitution in paediatric haematopoietic cell transplantation: a multicentre , retrospective pharmacodynamic cohort analysis. *Lancet Haematol*. 2015;2(5):e194-e203.
11. Hale G, Rebello P, Brettman L. Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of. *Blood*. 2004;104(4):948-955.
12. Gabrielsson J, Weiner D. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, Third Edition. Vol 2001.; 2001.
13. Elter T, Molnar I, Kuhlmann J, Hallek M, Wendtner C. Pharmacokinetics of alemtuzumab and the relevance in clinical practice. *Leuk Lymphoma*. 2008;49(12):2256-2262.
14. Mould D. Why therapeutic drug monitoring is needed for monoclonal antibodies and how do we implement this? *Clin Pharmacol Ther*. 2016;99(4):351-354.
15. Mould DR, Green B. Pharmacokinetics and pharmacodynamics of monoclonal antibodies: concepts and lessons for drug development. *BioDrugs*. 2010;24(1):23-39.
16. Mould DR, Baumann A, Kuhlmann J, et al. Population pharmacokinetics-pharmacodynamics of alemtuzumab (Campath) in patients with chronic lymphocytic leukaemia and its link to treatment response. *Br J Clin Pharmacol*. 2007;64(3):278-291.

17. Morris EC, Rebello P, Thomson KJ, et al. Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. *Blood*. 2003;102(1):404-406.
18. Chakraverty R, Orti G, Orti G, et al. Impact of in vivo alemtuzumab dose before reduced intensity conditioning and HLA-identical sibling stem cell transplantation: pharmacokinetics, GVHD, and immune reconstitution. *Blood*. 2010;116(16):3080-3088.
19. Admiraal R, van Kesteren C, Jol-van der Zijde CM, 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(4):435-446.
20. Waller EK, Langston A a, 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(7):460-471.
21. Juliusson G, Theorin N, Karlsson K, Frödin U, Malm C. Subcutaneous alemtuzumab vs ATG in adjusted conditioning for allogeneic transplantation: influence of Campath dose on lymphoid recovery, mixed chimerism and survival. *Bone Marrow Transplant*. 2006;37(5):503-510.
22. Willemsen L, Jol-van der Zijde CM, Admiraal R, et al. Impact of Serotherapy on Immune Reconstitution and Survival Outcomes After Stem Cell Transplantations in Children: Thymoglobulin Versus Alemtuzumab. *Biol Blood Marrow Transplant*. 2015;21(3):473-482.
23. Park SH, Choi S-M, Lee D-G, et al. Infectious complications associated with alemtuzumab use for allogeneic hematopoietic stem cell transplantation: comparison with anti-thymocyte globulin. *Transpl Infect Dis*. 2009;11(5):413-423.
24. Veys P, Wynn RF, Ahn KW, et al. Impact of immune modulation with in vivo T cell depletion and myleoablative total body irradiation conditioning regimen on outcomes after unrelated donor transplantation for acute lymphoblastic leukemia in children. *Blood*. 2012;119(25):6155-6162.