# Association between anti-thymocyte globulin exposure and survival outcomes in adult unrelated haemopoietic cell transplantation: a retrospective, pharmacodynamic cohort analysis

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## Summary

**Background** Anti-thymocyte globulin (ATG) is used to prevent graft-versus-host disease (GvHD) after allogeneic haemopoietic cell transplantation (HCT). However, ATG can also cause delayed immune reconstitution of T cells, negatively affecting survival. We studied the relation between exposure to ATG and clinical outcomes in adult patients with acute leukaemia and myelodysplastic syndrome.

**Methods** We did a retrospective, pharmacokinetic-pharmacodynamic analysis of data from patients with acute lymphoid leukaemia, acute myeloid leukaemia, or myelodysplastic syndrome receiving their first T-cell repleted allogeneic peripheral blood stem cell HCT with ATG (thymoglobulin) as part of non-myeloablative conditioning from March 1, 2004, to June 1, 2015. Patients received a cumulative intravenous dose of 8 mg/kg divided over 4 days, starting on day –8 before HCT. Active ATG concentrations were measured using a validated bioassay and pharmacokinetic exposure measures (maximum concentration, concentration at time of infusion of the graft, time to reach a concentration of 1 arbitary unit [AU] per day/mL, area under the curve [AUC], and the AUC before and after HCT) were calculated with a validated population pharmacokinetic model. The main outcome of interest was 5-year overall survival, defined as days to death from any cause or last follow-up. Other outcomes were relapse-related mortality, non-relapse mortality, event-free survival, acute and chronic GvHD, and assessment of current and optimum dosing. We used Cox proportional hazard models and Fine-Gray competing risk models for the analyses.

**Findings** 146 patients were included. ATG exposure after HCT was shown to be the best predictor for 5-year overall survival. Optimum exposure after transplantation was determined to be 60–95 AU per day/mL. Estimated 5-year overall survival in the group who had optimum exposure (69%, 95% CI 55–86) was significantly higher than in the group who had below optimum exposure (32%, 20–51, p=0·00037; hazard ratio [HR] 2·41, 95% CI 1·15–5·06, p=0·020) and above optimum exposure (48%, 37–62, p=0·030; HR 2·11, 95% CI 1·04–4·27, p=0·038). Patients in the optimum exposure group had a greater chance of event-free survival than those in the below optimum exposure group (HR 2·54, 95% CI 1·29–5·00, p=0·007; HR for the above optimum group: 1·83, 0·97–3·47, p=0·063). Above-optimum exposure led to higher relapse-related mortality compared with optimum exposure (HR 2·66, 95% CI 1·12–6·31; p=0·027). Below optimum exposure increased non-relapse mortality compared with optimum exposure (HR 4·36, 95% CI 1·60–11·88; p=0·0040), grade 3–4 acute GvHD (3·09, 1·12–8·53; p=0·029), but not chronic GvHD (2·38, 0·93–6·08; p=0·070). Modelled dosing based on absolute lymphocyte counts led to higher optimum target attainment than did weight-based dosing.

**Interpretation** Exposure to ATG affects survival after HCT in adults, stressing the importance of optimum ATG dosing. Individualised dosing of ATG, based on lymphocyte counts rather than bodyweight, might improve survival chances after HCT.

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## Introduction

Allogeneic haemopoietic cell transplantation (HCT) is a potentially curative treatment option for high-risk or relapsed acute leukaemias and myelodysplastic syndrome (MDS). Immunological rejection of any residual tumour by donor-derived immune cells (graft-*vs*-leukaemia) should enable disease control, including lifelong antitumour immune surveillance.[1] Graft-versus-host disease (GvHD) is a severe complication of HCT, leading to substantial morbidity and mortality. As a strategy to prevent GvHD after HCT, anti-thymocyte globulin (ATG) was introduced to the conditioning regimens applied before HCT in the early 1980s.[2] Although use of ATG was associated with a decreased incidence of acute GvHD[3–5] and chronic GvHD,[4,6] the results of most studies have not shown a

## Research in context

### Evidence before this study

We searched PubMed on Sept 20, 2016, with no language restrictions, using a search term combining MeSH terms "bone marrow transplantation" and "antilymphocyte serum". We selected studies that compared anti-thymocyte globulin (ATG) versus no ATG, different doses of ATG, and different starting days of ATG before haemopoietic cell transplantation (HCT), and studies relating outcome to concentrations of active ATG. We identified three systematic reviews, seven randomised controlled trials, 12 clinical controlled trials, and four case series. Most studies investigated ATG versus no ATG, or compared two doses of ATG with each other. In four studies, ATG concentration or exposure was investigated as a predictor of outcome. In the concentration and exposure studies, high exposures to ATG were associated with poor immune reconstitution, and some studies showed a lower incidence of acute graft-versus-host disease (GvHD) with high exposures to ATG. One study investigating ATG exposures showed that low exposure after HCT led to improvements in immune reconstitution and overall survival. Results from most studies investigating the use or dosage of ATG showed no effects of ATG on overall. ATG is suggested to have a dose-related effect on immune reconstitution, whereby a lower dose leads to a better immune reconstitution, which translates to fewer viral reactivations. The incidence of acute and chronic GvHD was reduced when ATG was introduced to the conditioning regimen. Findings from a few studies have shown an effect of ATG on relapse and rejection. Although this literature review included clinical trials, and case series, these results are in line with those from published systematic reviews, which only included trials comparing ATG versus no serotherapy.
The relationship between ATG exposure and clinical outcome in adults has not yet been thoroughly investigated. The absence of differences in survival chances with different doses in most other studies might be explained by the high variability of ATG exposures.

### Added value of this study

In this study, we showed that there is an optimum ATG exposure after HCT. This optimum exposure is associated with higher survival probability through lower transplantation-related mortality and lower relapse-related mortality. Too low exposure is associated with more GvHD and total relapse mortality, whereas too high exposure is associated with more relapse. Survival chances after non-myeloablative conditioning in adults might be improved by individualised dosing of ATG. This hypothesis needs to be investigated in a prospective study.

### Implications of all the available evidence

Inclusion of ATG in the conditioning regimen reduces the incidence of acute and chronic GvHD. However, over-exposure and under-exposure to ATG seems to lead to higher mortality. Individualised dosing aiming for optimum exposure in all patients will contribute to optimising efficacy while preventing toxicity of ATG. Although this might increase survival after HCT, this outcome needs to be validated in prospective studies.

survival advantage with ATG addition.[5–7] One of the potential reasons for the absence of an advantage could be that ATG-induced, in-vivo T-cell depletion of the graft is unpredictable and can result in delayed or absent early T-cell immune reconstitution.[5,8,9] Poor immune reconstitution abrogates the graft-versus-leukaemia effect and antiviral activity, resulting in increased relapse mortality and non-relapse mortality.[10]

In this delicate balance between preventing GvHD and timely T-cell immune reconstitution, ATG has a pivotal role as a T-cell depleting antibody of host and donor T cells. Investigators of several dose-effect studies have tried to determine the optimum dose of ATG;[11–14] however, a major drawback of these studies is that there is high interpatient variability in the pharmacokinetics of ATG. Therefore, the use of standard dosing for all patients, without taking exposure into account, renders the results difficult to interpret.[15,16] Furthermore, because patients are exposed to ATG before and after transplantation due to its long half-life of 5–14 days,[17–19] the timing of ATG is an additional important variable,[5] and actual exposure should be correlated with outcome rather than dose. Population pharmacokinetic modelling can be used to describe pharmacokinetics and determine individual exposure, and is the standard for reporting pharmacokinetic data according to current US Food and Drug Administration[20] and European Medicines Agency guidelines.[21] Although ATG has been used since the early 1980s, its pharmacokinetics have not been thoroughly described.[17,19,22–25]

We have described previously[22] that ATG exposure affected clinical outcomes in paediatric receivers of HCTs receiving either bone marrow or cord blood after myeloablative conditioning. High exposure to ATG after HCT was associated with a poor immune reconstitution probability and low survival, whereas high exposure before HCT was associated with less GvHD and less graft failure than was low exposure before HCT. No studies have investigated the optimum exposure to ATG in adult recipients of HCT using mobilised peripheral blood stem cells (PBSC) after non-myeloablative conditioning.[26,27] Therefore, we aimed to assess whether ATG exposure affected clinical outcomes in this non-myeloablative PBSC setting. To achieve this aim, the available pharmacokinetic model for ATG in children and young adults was expanded and validated for adults. We subsequently did a retrospective cohort analysis of consecutive patients to relate different exposure measures of the pharmacologically active fraction of

ATG (hereafter referred to as ATG) to various clinical outcomes of HCT, such as GvHD, relapse, and survival.

## Methods

### Study design and patients

We did a retrospective, pharmacokinetic-pharmacodynamic analysis of data from patients with acute lymphoid leukaemia (ALL), acute myeloid leukaemia (AML), or MDS receiving their first HCT between March 1, 2004, and June 1, 2015, within the adult blood and marrow transplantation unit at the University Medical Centre (Utrecht, Netherlands). Only patients receiving a T-repleted PBSC graft with ATG (thymoglobulin) as part of non-myeloablative conditioning, and a matched or mismatched unrelated donor graft, were included. No restrictions applied in terms of remission status or comorbidities. Clinical data and serum samples for ATG concentration measurements were collected prospectively; consecutive patients were included. Minimal follow-up for surviving patients was 6 months. Patients were included and data were collected after written informed consent was acquired. Ethical committee approval of the University Medical Centre Utrecht was given through trial number 11/063.

### Procedures

Patients underwent a non-myeloablative conditioning regimen containing a cumulative intravenous dose of 8 mg/kg ATG divided over 4 days, starting on day –8 before HCT, fludarabine 90 mg/m² (day –3, –2, and –1) and 200 cGy total body irradiation on day 0. The actual dose of ATG was rounded upwards to 25 mg so that patients received only full vials. Clemastine, paracetamol, and 100 mg prednisolone were given intravenously before ATG infusion. GvHD prophylaxis consisted of cyclosporin A and mycophenolate mofetil. Start dose of cyclosporin A was 4·5 mg/kg per day intravenously until day +120 (target trough levels 200–350 mg/L). Thereafter, cyclosporin A was tapered if no GvHD was present. Patients received 15 mg/kg per day mycophenolate mofetil (maximum of 3 g/day) until day +84, also followed by tapering in the absence of GvHD.[28] Ciprofloxacin and fluconazole were given as selective gut decontaminants, and trimethoprim–sulfamethoxazole and valaciclovir were used for infectious prophylaxis until 12–15 months after HCT. GvHD prophylaxis, gut decontaminants, and infectious prophylaxis were started intravenously and switched to oral medication upon discharge.

To describe ATG pharmacokinetics from young children to adult patients, an adult dataset for ATG was combined with a previously published dataset of children and young adults.[15] In the adult population, samples were collected weekly after HCT, while samples during ATG infusions were available for 35 patients. The complete development and validation of the population pharmacokinetic model is in the appendix (pp 3–7).

After development of the population pharmacokinetic model, full concentration–time curves were calculated for each individual patient. Based on these, individual pharmacokinetic exposure measures could be determined. The pharmacokinetic exposures of interest included the maximum concentration ($C_{max}$), concentration at time of

| | Patients (n=146) |
|---|---|
| Sex | |
| Men | 84 (58%) |
| Women | 62 (42%) |
| Lymphocyte count before first anti-thymocyte globulin dose (× $10^9$/L) | 0·7 (0·3–1·1) |
| Age at transplantation (years) | |
| <20 | 7 (5%) |
| ≥20–40 | 46 (32%) |
| >40 | 93 (64%) |
| Disease stage at haemopoietic cell transplantation | |
| Early | 88 (60%) |
| Intermediate | 30 (21%) |
| Late | 28 (19%) |
| Time interval between diagnosis and haemopoietic cell transplantation | |
| <12 months | 102 (70%) |
| ≥12 months | 44 (30%) |
| Patient donor sex mismatch | |
| Female donor, male recipient | 23 (16%) |
| All other combinations | 123 (84%) |
| EBMT risk score | |
| 1–2 | 32 (22%) |
| 3 | 46 (32%) |
| 4 | 24 (16%) |
| 5 | 28 (19%) |
| 6–7 | 16 (11%) |
| Cumulative dose of anti-thymocyte globulin (mg/kg) | 8·0 (7·5–8·4) |
| Starting day of anti-thymocyte globulin (days before haemopoietic cell transplantation) | 8 (7–8) |
| Number of blood samples taken per patient | 4 (3–5) |
| CD3 T cells infused (× $10^6$/kg) | 1946 (366–2856) |
| Diagnosis | |
| Myeloid leukaemia | 74 (51%) |
| Myelodysplastic syndrome | 36 (25%) |
| Lymphoid leukaemia | 36 (25%) |
| Stem-cell source | |
| Peripheral blood stem cells | 146 (100%) |
| Donor relation | |
| Unrelated | 146 (100%) |
| Conditioning regimen | |
| Fludarabine–total body irradiation | 146 (100%) |
| Match grade | |
| Matched | 111 (76%) |
| Mismatched | 35 (24%) |
| Follow-up (months) | 37·0 (7·6–65·0) |

Data are n (%) or median (IQR). EBMT (European Society for Bone Marrow Transplantation) risk score according to Gratwohl and colleagues.[33]

***Table 1*: Patient characteristics**

See Online for appendix

infusion of the graft ($C_{HCT}$), time to reach a concentration of 1 arbitary unit (AU) per day/mL ($T_{C<1}$),[18] the area under the curve (AUC), and the AUC before and after HCT (appendix p 27).

To study the most predictive pharmacokinetic measure for the main outcome of interest (5-year overall survival), models were selected based on the lowest Akaike Information Criterion (AIC), a criterion to select the best predicting model; in this case, the proportional hazard model. Once the model was developed we identified a range of ATG exposures that resulted in lowest relapse-related mortality and therapy-related mortality.

After the determination of the most predictive pharmacokinetic exposure measure and the subsequently determined optimum range of exposures, available ATG dosing regimens were assessed for target attainment. 1000 individuals were included in each bodyweight baseline lymphocyte count treatment regimen combination. Investigated regimens included current local dosing (8 mg/kg over 4 days, starting day –8), the EBMT protocol[29] (7·5 mg/kg over 3 days, starting day –3), and a recently published regimen in adult reduced intensity PBSC[30] (4·5 mg/kg, starting day –2). Additionally, an optimum dosing regimen was to be designed and subsequently assessed using the same approach. Groups of patients were selected based on the predictors for pharmacokinetics. Concentration–time profiles were simulated using the validated pharmacokinetic model, incorporating 1000 virtual patients in each group, while taking into account full interindividual variability. For each group, median exposure after HCT was compared with the optimum therapeutic window.

## Outcomes

The main outcome of interest was 5-year overall survival, defined as days to death from any cause or last follow up. Other outcomes of interest were non-relapse mortality and relapse-related mortality, which were defined as days to death from any cause other than relapse and days to death due to relapse, respectively, or last follow-up. Event-free survival was defined as the days to death, relapse, graft failure, or last follow-up, whichever occurred first, while relapse incidence was defined as time to relapse or last follow-up. Acute and chronic GvHD were classified according to the Glucksberg[31] and Shulman[32] criteria. GvHD scoring according to National Institutes of Health criteria was not possible because of the retrospective character of the data. Graft failure was defined as non-engraftment or secondary graft rejection. Because we were interested in the predictive power of the various pharmacokinetic exposure measures, we related the outcomes of interest with these measures.

## Statistical analysis

We defined duration of follow-up as the time from HCT to last contact or death. Patients were censored at the date of last contact. Factors considered as predictors for outcome included patient variables (age, sex, Epstein-Barr virus and cytomegalovirus serostatus, and European Society for Bone Marrow Transplantation [EBMT] risk score according to Gratwohl and colleagues[33]), disease variables (ALL, AML, MDS), donor-related variables (HLA disparity, and Epstein-Barr virus and cytomegalovirus serostatus), year of treatment (before or after median year of HCT), and ATG exposure measures.

We determined probabilities of survival using the Kaplan-Meier estimation and calculated p values using a two-sided log-rank test. Cumulative incidences were calculated with the method of Gray, and 95% CIs for survival probabilities and cumulative incidence were calculated using the Greenwood formula. Variables with a p value less than 0·05 from univariate analysis were included as a predictor

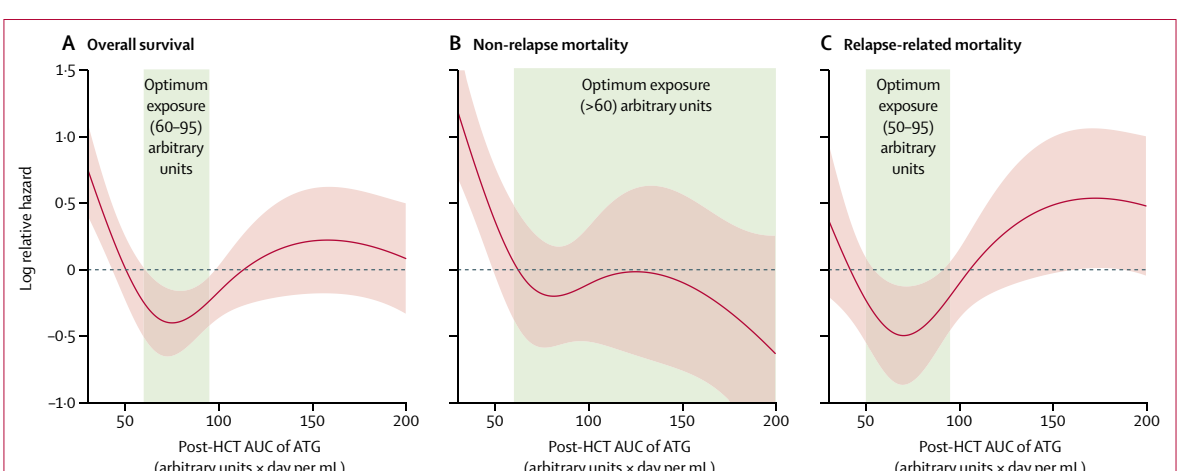


***Figure 1*: Overall survival (A), non-relapse mortality (B), and relapse-related mortality (C) risk according to anti-thymocyte globulin (ATG) exposure after haemopoietic cell transplantation (HCT)**

Blue dotted line occurs where log relative hazard=0. Pink shaded areas represent 95% CI for log relative hazard. Green panels represent optimum exposure range. AUC=area under the curve. Log relative hazards for relapse incidence according to ATG exposure after HCT are in the appendix (p 31).

in multivariate analysis. For the endpoints overall survival and event-free survival, we used Cox proportional hazard models; for the endpoints of non-relapse mortality, relapse-related mortality, and transplantation-related mortality, relapse and acute and chronic GvHD, we used Fine-Gray competing risk models.[34] We did the statistical analyses using R version 3.2.4, with the cmprsk, survival, and rms packages.

We assessed current ATG dosing regimens and a novel absolute lymphocyte count-based nomogram thymoglobulin; groups of patients with bodyweights of 50–100 kg and an absolute lymphocyte count before the first infusion of ATG of $0{\cdot}1–2{\cdot}0\times10^9$/L were simulated for three current thymoglobulin intravenous dosing regimens (appendix p 16).

## 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.

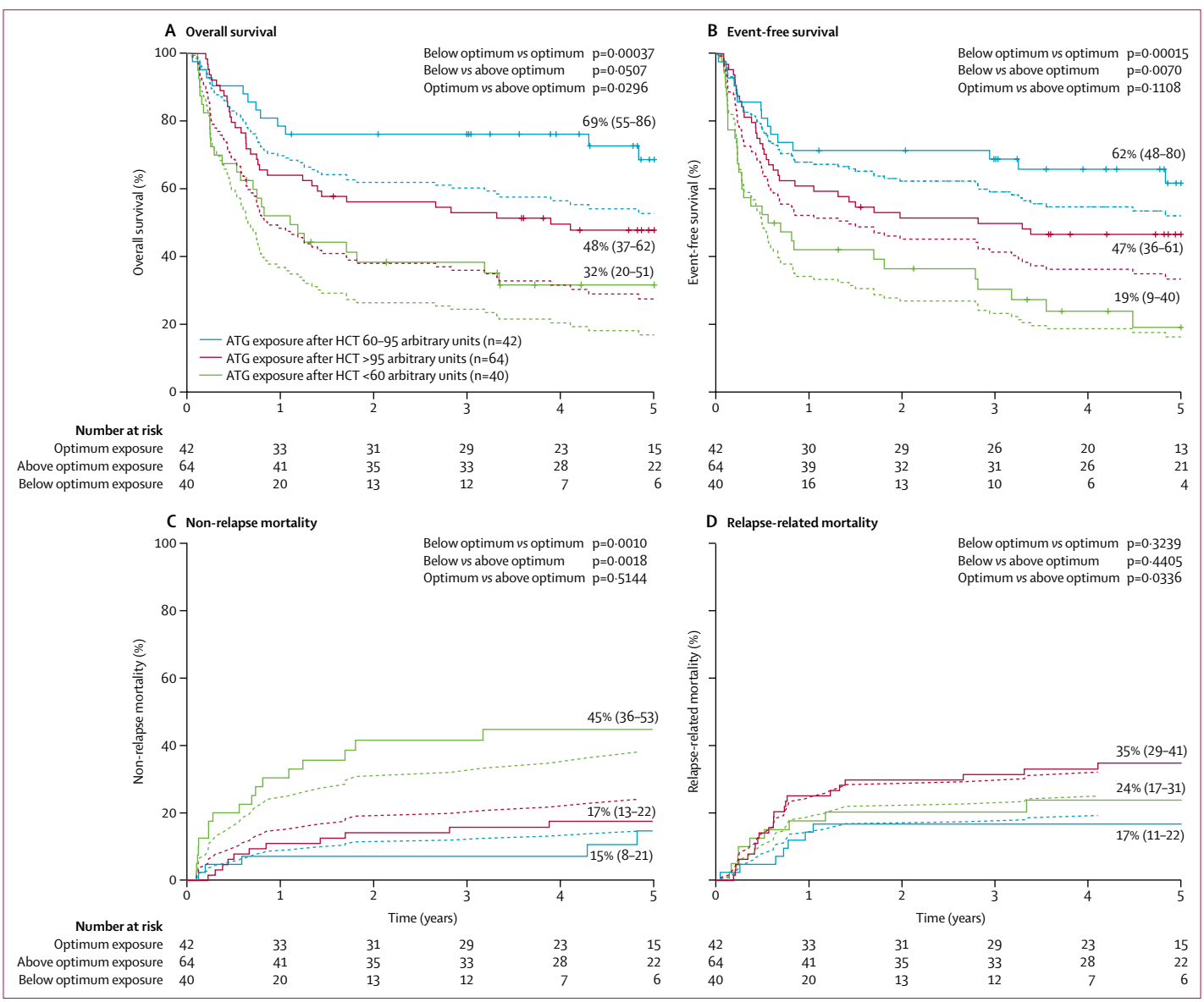


***Figure 2*: Estimations of clinical outcomes according to anti-thymocyte globulin (ATG) exposure after haemopoietic cell transplantation (HCT)**
Data are unadjusted (solid lines) and adjusted (dashed lines) estimations (percentage [95% CI]) of (A) overall survival, (B) event-free survival, (C) non-relapse mortality, and (D) relapse-related mortality. Adjusted estimations are to be interpreted as the expected outcomes if all exposure groups were the same, on average, with respect to all multivariate predictors (diagnosis [all], age [overall survival, event-free survival, non-relapse mortality], and EBMT [European Society for Bone Marrow Transplantation] risk score [overall survival and event-free survival]). p values are derived from the two-sided log-rank test. Overall clinical outcomes are in the appendix (p 30).

## Results

146 patients were included; 74 (51%) with AML, 36 (25%) with ALL, and 36 (25%) with MDS as indication for HCT (table 1). Median age at HCT was 50 years (IQR 32–59); 111 patients (76%) received a 10/10 matched graft. Median follow-up of all patients was 37·0 months (IQR 7·6–65·0).

A population pharmacokinetic model was developed (appendix pp 6, 7) that accurately described concentration data and was extensively validated (appendix pp 24, 25). In the model, bodyweight was shown to be a predictor for ATG clearance for bodyweight less than 50 kg. Above this weight, no increase in clearance occurred with increasing bodyweight (appendix p 22). Absolute lymphocyte counts before the first dose of ATG (baseline lymphocytes) also predicted clearance, which is in line with the pharmacological properties of ATG. A greater number of lymphocytes harbour more targets for ATG binding, leading to increased clearance (appendix p 22). ATG exposure measures could be accurately calculated for all patients using the validated pharmacokinetic model.

| | Hazard ratio (95% CI) | p value |
|---|---|---|
| **Overall survival** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 2·41 (1·15–5·06) | 0·020 |
| Above optimum ATG exposure | 2·11 (1·04–4·27) | 0·038 |
| **Event-free survival** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 2·54 (1·29–5·00) | 0·0070 |
| Above optimum ATG exposure | 1·83 (0·97–3·47) | 0·063 |
| **Non-relapse mortality** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 4·36 (1·60–11·88) | 0·0040 |
| Above optimum ATG exposure | 1·64 (0·58–4·62) | 0·35 |
| **Relapse mortality** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 1·45 (0·55–3·83) | 0·46 |
| Above optimum ATG exposure | 2·66 (1·12–6·31) | 0·027 |
| **Relapse incidence** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 1·28 (0·57–2·86) | 0·55 |
| Above optimum ATG exposure | 1·79 (0·89–3·61) | 0·11 |
| **Incidence of grade 2–4 graft-versus-host-disease** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 1·45 (0·76–2·75) | 0·26 |
| Above optimum ATG exposure | 0·79 (0·42–1·51) | 0·48 |
| **Incidence of grade 3–4 graft-versus-host-disease** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 3·09 (1·12–8·53) | 0·029 |
| Above optimum ATG exposure | 1·07 (0·35–3·24) | 0·91 |
| **Incidence of chronic graft-versus-host-disease** | | |
| Optimum ATG exposure | 1·00 (ref) | .. |
| Below optimum ATG exposure | 2·38 (0·93–6·08) | 0·070 |
| Above optimum ATG exposure | 0·93 (0·37–2·36) | 0·88 |

Adjusted multivariate analyses were done using a Cox proportional hazard model and Fine-Gray competing risk models. ATG=anti-thymocyte globulin.

***Table 2*: Multivariate analyses of survival, relapse, and graft-versus-host-disease with anti-thymocyte globulin exposure after haemopoietic cell transplantation**

Exposure to ATG after HCT was shown to be the best predictor for overall survival. The AIC for exposure after HCT was lowest (appendix p 10), indicating the best fit of the Cox proportional hazard model.

To assess the most optimum range of exposure to ATG after HCT, the hazard ratio (HR) for overall survival, non-relapse mortality, and relapse-related mortality was plotted against ATG exposure after HCT (figure 1). The optimum AUC after HCT (ie, optimum exposure) for overall survival was determined to be between 65 and 90 AU per day/mL; below this threshold increased risk for non-relapse mortality, and both below and above this threshold increased the chance of relapse mortality. Estimated 5-year overall survival after optimum ATG exposure (69%, 95% CI 55–86) was significantly higher than in the groups below optimum exposure (32%, 20–51; p=0·00037) and above optimum exposure (48%, 37–62; p=0·030; HR 2·41, 95% CI 1·15–5·06, p=0·020 and HR 2·11, 95% CI 1·04–4·27, p=0·038 for below and above optimum exposures, respectively) (figure 2, table 2, appendix pp 11–14, causes of death are shown on p 17). In multivariate analysis, older age (>58 years) was also a predictor for worse outcome (appendix pp 13, 14).

Other outcomes of interest are in table 2 and figure 2. Relapse-related mortality was higher in patients with above optimum exposure after HCT than in those with optimum exposure after HCT. Patients with a below optimum exposure had an increased risk for non-related mortality compared with those with optimal exposure. Patients with above optimum exposure did not have significantly different results compared with those with optimum exposure in terms of non-relapse mortality. Patients in the optimum exposure group had a higher chance of event-free survival than did those given below or above optimum exposure. The incidence of grade 3–4 GvHD was higher in patients with below optimum exposure than in those in the optimum exposure group. No significant differences were noted between above optimum exposure and optimum exposure for this outcome. Below optimum exposure increased grade 3–4 acute GvHD compared with optimum exposure but not chronic GvHD (appendix p 29).

Results of the simulated absolute lymphocyte count values were in line with the actual lymphocyte counts of included patients (median $0·72\times10^9$/L, IQR 0·3–1·1). The dosing regimen used in our centre is representative of that used in most Dutch centres and affiliated centres participating in HOVON studies.[28] The regimen showed high variability in ATG exposure after HCT; median exposure was on target in 33% of the groups (figure 3). Practices from other centres using thymoglobulin with

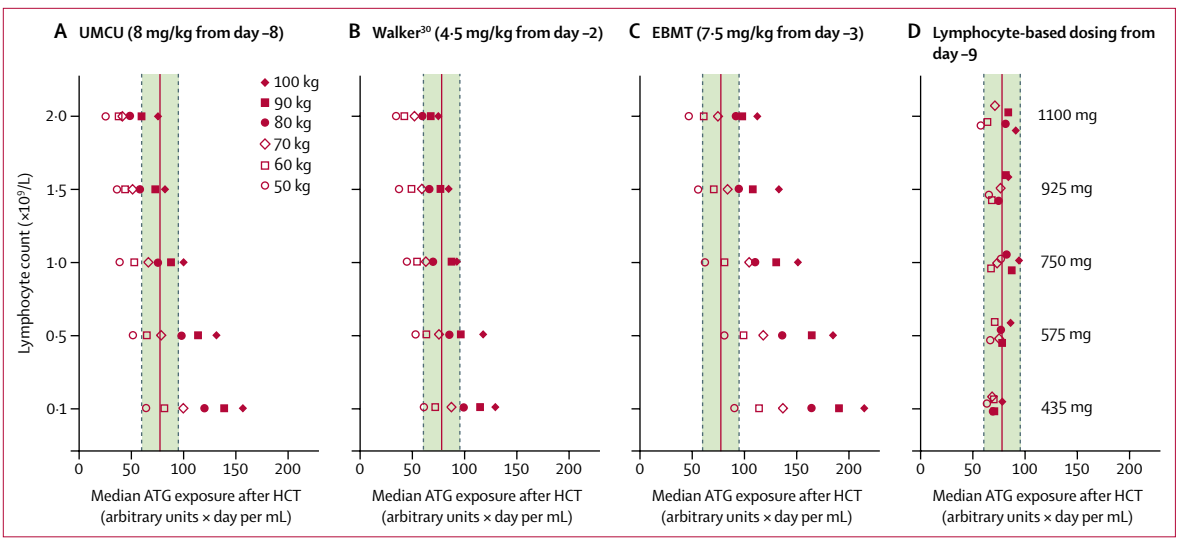


***Figure 3*: Anti-thymocyte globulin (ATG) exposure after haemopoietic cell transplantation (HCT) for different lymphocyte or bodyweight groups**
Data show median ATG exposure after transplantation with currently used ATG dosing regimens. Dosing regimen based only on lymphocyte count, given over 4 days, starting on day –9. Absolute cumulative dose is given for each lymphocyte level. (A) Dosing regimen used for the current cohort: cumulative dose of 8·0 mg/kg over 4 days, starting day –9 (UMCU=University Medical Centre Utrecht). (B) Dosing regimen according to Walker and colleagues:[30] cumulative dose of 4·5 mg/kg, 0·5 mg/kg on day –2, 2 mg/kg on day –1, and 2 mg/kg on day +1. (C) Dosing regimen proposed by European Bone Marrow Transplant Society (EBMT): cumulative dose of 7·5 mg/kg over 3 days, starting day –3. (D) Dosing regimen based only on lymphocyte count, given over 4 days, starting on day –9. Absolute cumulative dose is given for each lymphocyte level. Symbols depict bodyweights; open circles: 50 kg; open squares: 60 kg; open diamonds: 70 kg; filled circles: 80 kg; filled squares: 90 kg; filled diamonds: 100 kg. Green sections represent optimum exposure. Dotted lines represent the upper and lower limits of optimum exposure. Solid lines represent the mean upper and lower limits of optimum exposure.

different timings and doses (0·5 mg/kg on day –2, 2·0 mg/kg on day –1, and 2 mg/kg on day +1)[30] showed an optimal target attainment in 53% of groups (figure 3B), and the current EBMT recommended dose (7·5 mg/kg in 3 days, starting day –3) of thymoglobulin for unrelated donors[29] led to an optimum target attainment in 30% of groups only (figure 3C). Based on the developed pharmacokinetic model, the optimum dosing should be based on absolute lymphocyte count, because ATG clearance in patients (>50 kg) was not affected by weight. When targeting to the optimum ATG exposure after HCT, cumulative intravenous ATG dosage is calculated using the following formula:

$$\text{Cumulative dose} = 400 + 350 \times \text{lymphocyte count (in } 10^9/\text{L)}$$

This cumulative dose should be given intravenously over 4 days starting on day –9. Simulations of this dosing regimen led to optimal exposure in 97% of groups (figure 3); higher than any of the regimens analysed in the current study.[28–30]

## Discussion

To our knowledge, this is the first study investigating the pharmacokinetics and pharmacodynamics of ATG in a large, consecutive cohort of adult patients receiving PBSC after non-myeloablative conditioning for acute leukaemias and MDS. We aimed to determine the therapeutic window of ATG in this setting. Taking into account the limitations of a retrospective study, the data show that exposure to ATG after HCT affects survival as well as acute GvHD. There seems to be an optimum window of exposure to ATG after HCT (60–95 AU per day/mL); lower exposure increased the chance of mortality, mostly associated with GvHD, while an exposure above the optimum was associated with more relapse-related mortality. The pharmacokinetic model showed that absolute lymphocyte count was the only relevant predictor for ATG pharmacokinetics in adults. Therefore, absolute lymphocyte count-based dosing would result in achieving optimum AUC in more than 95% of the simulated patient groups. This might subsequently result in higher survival chances after non-myeloablative conditioning HCT in patients receiving PBSC.

The importance of T-cell immune reconstitution after HCT is increasingly recognised. The use of ATG, more particularly exposure of the graft to ATG, has been associated with poor immune reconstitution early after HCT.[5,13,18,22] In the early phase after HCT, until thymic output, patients depend on graft-infused T cells undergoing peripheral expansion.[35] Therefore, in-vivo depletion of these T cells might result in prolonged T-cell lymphopenia, leaving patients vulnerable to relapse and viral reactivations.[2] Restoration of thymic function can be hampered by age, GvHD, chemotherapy, and steroids.[35] A limitation of this study was that few data were available for immune reconstitution shortly after HCT. The increased relapse mortality after high ATG exposure, however, suggests that one of the causes of relapse after HCT might be poor immune reconstitution, as was

shown in other studies.[22,36,37] It was not possible to score chronic GvHD according to NIH criteria due to the retrospective character of the data, which makes comparison with other studies difficult.

Higher concentrations of ATG following infusion of the graft have been associated with a lower incidence of GvHD, although these studies only investigated single concentrations.[19,25] However, in a more comprehensive study investigating ATG pharmacodynamics in children, too low ATG exposure before HCT was associated with higher chances of GvHD, whereas exposure after HCT did not affect GvHD.[22] This finding contrasts with our results in adults, where ATG concentrations before HCT did not affect clinical outcome, and a very low exposure to ATG after HCT was associated with an increased incidence of grade 3–4 acute GvHD. These differences might be because most patients already had a very high exposure to ATG before HCT and we consequently had no power to address the question of levels of exposure before HCT and clinical outcome. However, calculation of the exposure before HCT might be somewhat less accurate in view of the lower number of concentration samples available before HCT. Additionally, the profound effect of high ATG exposure after HCT on GvHD in this analysis presumably reflects the at least one log higher numbers in T cells used in PBSC, compared with cord blood and bone marrow.[38] The very low-intensity conditioning regimen might have further contributed to this effect.

The determined therapeutic window for ATG in this setting might not apply to myeloablative conditioning or other cell sources. The ATG pharmacokinetic model, however, which included adult and paediatric patients receiving different cell sources and conditioning regimens, is generally applicable, because age, stem-cell source, and conditioning regimen did not affect ATG pharmacokinetics. Additionally, although literature shows a relationship between chronic GvHD and relapse incidence,[39,40] only incidence on relapse-related mortality, not chronic GvHD, was increased by above optimum ATG exposure.

The clearance of ATG in adults is not affected by bodyweight above 50 kg. By contrast with current practice, ATG should therefore not be dosed in mg/kg, but rather as a fixed dose based on absolute lymphocyte count before ATG. This finding is shown by simulation studies: the currently used weight-based dosing regimens for ATG result in poor optimum target attainment of 30–53%. Additionally, lymphocyte-depleting chemotherapy is given before ATG in two of the regimens, leading to an even higher chance for overexposure, especially in the EBMT regimen. An absolute lymphocyte count-based dosing regimen leads to optimum exposure in 97% of patients. However, this proposed dosing nomogram should be assessed in a prospective study. Moreover, the dosing regimen is only valid for thymoglobulin, because the various ATG preparations are not biosimilar.

In conclusion, our data showed that survival after HCT is highly affected by ATG exposure after HCT. By using a dosing regimen that aims for optimum ATG exposure, which can best be achieved using an absolute lymphocyte count-based dosing, outcomes following HCT could be improved, resulting in higher survival chances.

#### Contributors

RA and JJB designed the study. RA, JJB, SN, CvK, CAJK, GF, and LV analysed data and wrote the manuscript. MAdW and JK designed the research, included patients, and wrote the manuscript. EJP, RAPR, RGMB, and MCM included patients and critically appraised the manuscript. SVB analysed data and wrote the manuscript. All authors reviewed and approved the final version of the manuscript. RA and JJB had full access to all data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

#### Declaration of interests

JK is scientific co-founder and chief scientific officer of Gadeta BV. All other authors declare no competing interests. CvK and RA are paid through the same project funds by the Rational Pharmacy Program of the Netherlands Organization for Health Research and Development.

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