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### **Citation**

Admiraal, R., Nierkens, S., Bierings, M. B., Bredius, R. G. M., Vliet, I. van, Jiang, Y. L., ... Boelens, J. J. (2022). Individualised dosing of anti-thymocyte globulin in paediatric unrelated allogeneic haematopoietic stem-cell transplantation (PARACHUTE): a single-arm, phase 2 clinical trial. *The Lancet Haematology*, 9(2), E111-E120.  
doi:10.1016/S2352-3026(21)00375-6

Version: Publisher's Version

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**Note:** To cite this publication please use the final published version (if applicable).



# Individualised dosing of anti-thymocyte globulin in paediatric unrelated allogeneic haematopoietic stem-cell transplantation (PARACHUTE): a single-arm, phase 2 clinical trial

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## Summary

**Background** Anti-thymocyte globulin, which is used in the conditioning of haematopoietic stem-cell transplantation (HSCT) to prevent graft-versus-host disease (GVHD) and graft failure, has highly variable pharmacokinetics. Overexposure to anti-thymocyte globulin leads to poor CD4<sup>+</sup> T-cell immune reconstitution, which is associated with inferior overall survival. We hypothesised that individualised anti-thymocyte globulin dosing would promote CD4<sup>+</sup> immune reconstitution, while still preventing GVHD and graft failure.

**Methods** We report the results of a prospective, single-arm, phase 2 clinical trial done at the University Medical Center Utrecht and the Princess Máxima Center for Pediatric Oncology (Utrecht, Netherlands) to investigate individualised dosing of anti-thymocyte globulin for unrelated allogeneic HSCT in paediatric patients. Anti-thymocyte globulin dosing was based on bodyweight, absolute lymphocyte counts before the first dose, and the stem-cell source, with cumulative doses ranging from 2–10 mg/kg. Patients younger than 18 years receiving a first HSCT with a T-cell repleted graft for any indication and a Lansky/Karnofsky performance status of at least 70% were eligible for inclusion. The primary endpoint was CD4<sup>+</sup> immune reconstitution ( $>0.05 \times 10^9$  CD4<sup>+</sup> T-cells per L twice within 100 days [ $\pm 3$ ] after transplantation). The primary endpoint needed to be met in 38 of 53 evaluable patients (no death, relapse, or graft failure before day 100). Toxicity was registered according to Common Terminology Criteria for Adverse Events criteria version 4.0. The study is registered with the Dutch Trial Register, NL4836.

**Findings** Between July 1, 2015, and Aug 22, 2018, 58 patients were included in the study, of whom 51 were evaluable for the primary endpoint. Median follow-up was 25.6 months (IQR 15.0–37.0) and median age was 7.4 years (IQR 2.8–13.2). 29 (50%) of 58 patients were female. CD4<sup>+</sup> immune reconstitution was reached in 41 (80%, 95% CI 67–90, in survival analysis) of 51 evaluable patients, hence the study met its primary endpoint. There was no difference in CD4<sup>+</sup> immune reconstitution between patients who received different stem-cell sources (87% [95% CI 61–96] in cord blood, 77% [54–89] in bone marrow [ $p=0.62$ ]). The most common grade 3–5 adverse events were infections (32 [50%] patients had grade 3, two [3%] patients had grade 4, and seven [11%] patients had fatal events) and immunological disorders (seven [11%] patients had grade 3, three [5%] patients had grade 4, and five [8%] patients had fatal events). Two (3%) of 64 patients died of GVHD, which might be indirectly related to the intervention.

**Interpretation** Individualised dosing of anti-thymocyte globulin led to a significant improvement in early CD4<sup>+</sup> immune reconstitution without increasing GVHD and graft failure incidence. Promotion of early CD4<sup>+</sup> immune reconstitution by individualising anti-thymocyte globulin dose might improve outcomes of allogeneic HSCT.

**Funding** Sanofi.

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## Introduction

Allogeneic haematopoietic stem-cell transplantation (HSCT) is a potentially curative treatment option for some malignant and non-malignant disorders. Major complications of the procedure include graft-versus-host disease (GVHD) and graft failure. Rabbit anti-thymocyte globulin, a polyclonal rabbit-derived antibody that depletes lymphocytes, including T cells, was introduced to the HSCT conditioning regimen in the 1980s to prevent GVHD and transplant rejection.<sup>1</sup> However,

inclusion of anti-thymocyte globulin led to unpredictable and poor T-cell reconstitution after HSCT, potentially leading to decreased graft-versus-leukaemia effect and life-threatening infectious complications.<sup>2–4</sup> Although the incidence of GVHD and graft failure decreased after inclusion of anti-thymocyte globulin, no clear benefit in survival was shown.<sup>5–8</sup> The lower mortality by prevention of GVHD and graft rejection might be cancelled out by increased mortality due to delayed T-cell immune reconstitution in patients with high anti-thymocyte

*Lancet Haematol* 2022; 9: e111–20

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### Research in context

#### Evidence before this study

We searched PubMed with MeSH terms “Hematopoietic Stem Cell Transplantation” and “antilymphocyte serum”. We selected studies that compared anti-thymocyte globulin with no anti-thymocyte globulin, different doses of anti-thymocyte globulin, different starting days of anti-thymocyte globulin before transplantation, and studies relating concentrations of pharmacologically active anti-thymocyte globulin to outcomes, published before Sept 15, 2021, with no language restrictions. We identified 40 studies, of which 16 were done (partly) in a paediatric population. Nine randomised controlled trials, 12 controlled clinical trials, and 21 case series were available. 29 studies compared different doses of anti-thymocyte globulin or no anti-thymocyte globulin with a fixed dose of anti-thymocyte globulin. Seven studies investigated anti-thymocyte globulin concentrations or exposure as a predictor for clinical outcome. Four studies investigated CD4<sup>+</sup> immune reconstitution related to anti-thymocyte globulin exposure. The studies investigating use of different dosages of anti-thymocyte globulin (includes all randomised controlled trials) reported conflicting findings for overall survival—most found no effect, one showed poorer overall survival with anti-thymocyte globulin, and two showed improved overall survival with anti-thymocyte globulin. The study reporting poorer overall survival when using anti-thymocyte globulin reported an interaction with absolute lymphocyte count before anti-thymocyte globulin dosing; patients with a high absolute lymphocyte count seemed to benefit from anti-thymocyte globulin, whereas those with low absolute lymphocyte count showed inferior survival. In the studies investigating anti-thymocyte globulin concentrations or exposures, high exposures to anti-thymocyte globulin were associated with poor immune reconstitution of CD4<sup>+</sup> T cells. In studies comparing different doses of anti-thymocyte globulin, immune recovery was improved in patients receiving lower

doses. Both high anti-thymocyte globulin exposure after graft infusion and unsuccessful CD4<sup>+</sup> reconstitution were associated with decreased survival chances. Reduced incidence of acute and chronic graft-versus-host disease (GVHD) was observed when using anti-thymocyte globulin and when using higher doses. All four studies investigating successful CD4<sup>+</sup> immune reconstitution (using the same definition as the current study) showed improved overall survival and lower therapy-related mortality.

#### Added value of this study

The current study prospectively validated the hypothesis that optimal exposure to anti-thymocyte globulin (resulting from individualised dosing) leads to a significant improvement in early CD4<sup>+</sup> T-cell reconstitution. Despite administering lower doses in a selection of patients with earlier administration relative to graft infusion, no increased incidences of GVHD or graft failure occurred. This study underscores the importance of optimal anti-thymocyte globulin exposure to minimise toxicity (T-cell recovery) while still being active (preventing GVHD and graft failure).

#### Implications of all the available evidence

The use of anti-thymocyte globulin leads to a reduced incidence of acute and chronic GVHD, and overall survival is not impacted, probably caused by hampered T-cell recovery and subsequent viral reactivations and relapse. This delicate balance might explain the conflicting results on whether use of anti-thymocyte globulin affects overall survival. The current study showed that individualising anti-thymocyte globulin led to improved T-cell recovery without increasing the incidence of GVHD. The effect of individualised anti-thymocyte globulin dosing on overall survival needs to be addressed in a subsequent trial.

globulin exposure after HSCT.<sup>9–12</sup> Early CD4<sup>+</sup> immune reconstitution of more than 50 cells per  $\mu$ L within 100 days at two consecutive timepoints is a reliable predictor for outcomes, such as non-relapse mortality, overall survival, GVHD, and viral reactivation in various transplantation settings (cord blood, T-cell replete, T-cell deplete, paediatric, and adult).<sup>11–16</sup>

Several studies, some randomised, have tried to investigate the optimal dose of anti-thymocyte globulin in both children and adults.<sup>6–8,17,18</sup> These studies in general did not show an optimal dose for anti-thymocyte globulin, considering an optimal balance between the prevention of GVHD and graft failure, and promotion of early T-cell reconstitution. These trials used a fixed weight-based dose of anti-thymocyte globulin. Given the high interpatient variability in pharmacokinetics of anti-thymocyte globulin, fixed dosing is expected to lead to unpredictable and highly variable exposure, mainly caused by the non-linear relationship between anti-thymocyte globulin elimination

and both bodyweight and absolute lymphocyte count on the first day of treatment.<sup>19</sup> Besides variable exposure, the exposure–response relationship (pharmacodynamics) of anti-thymocyte globulin suggests that the timing of treatment (relative to graft infusion) and stem-cell source are also important factors to consider.<sup>11,13</sup>

The ideal dose of anti-thymocyte globulin yields a high degree of protection against GVHD and graft failure, and at the same time allows infused T cells to repopulate through peripheral expansion, to protect against viral disease and relapse.<sup>20,21</sup> Therefore, our group has developed a population pharmacokinetics model for anti-thymocyte globulin.<sup>19</sup> Such models are considered the gold standard for reporting pharmacokinetics according to guidelines from the US Food and Drug Administration and European Medicines Agency. In subsequent pharmacodynamics studies, higher exposure to anti-thymocyte globulin after HSCT was associated with hampered early CD4<sup>+</sup> recovery, whereas higher exposure

before HSCT was associated with lower graft failure and lower rates of GVHD.<sup>11,22</sup> On the basis of these pharmacokinetic<sup>19</sup> and pharmacodynamic<sup>11,22</sup> data, we developed an individualised dosing nomogram.

We aimed to prospectively validate this individualised dosing nomogram for anti-thymocyte globulin in children,<sup>11</sup> and to increase the probability of reaching CD4<sup>+</sup> T-cell reconstitution within 100 days after HSCT while maintaining protection against GVHD and graft failure.

## Methods

### Study design and participant

This trial, called the prospective analysis of an individualised dosing regimen of anti-thymocyte globulin in children undergoing HSCT: reducing toxicity and improving efficacy (PARACHUTE), was an open label, non-randomised, single-centre, single-arm, phase 2 trial that used a Simon two-stage design. Patients were recruited at the University Medical Center Utrecht (Utrecht, Netherlands) and the Princess Máxima Center for Pediatric Oncology (Utrecht, Netherlands). The latter centre was opened halfway through the study as a nationwide paediatric oncology referral centre, and the bone marrow transplant unit was moved from the University Medical Center Utrecht to the Princess Maxima Center. Patients younger than 18 years receiving their first T-cell repleted unrelated HSCT for any indication with anti-thymocyte globulin as part of the conditioning regimen with a Lansky/Karnofsky performance status of at least 70% were eligible for the study. We did not exclude any comorbidities in patients who were eligible for transplantation. We excluded those who received serotherapy 3 months before this HSCT, and those not in morphological complete remission, receiving ex-vivo T-cell depleted grafts, pregnant or breastfeeding or not willing to use adequate contraceptive methods, those with acute or chronic infections for which all forms of immune suppression are contraindicated, cardiac ejection fraction of 30% or less, and those who did not provide informed consent or were screen failures (full inclusion and exclusion criteria are presented in the appendix p 12). Post-pubertal female patients received contraception because of the teratogenic effect of many of the administered therapies. HSCT is a second-line treatment for most indications other than inborn errors in metabolism or immunity and bone marrow failure syndromes, for which it might be a first-line treatment.

Secondary objectives in our study were compared with a historical cohort of patients treated with standard doses of anti-thymocyte globulin as per the study protocol. As such, we selected a subset of consecutive paediatric patients (ages were 0·2–19·2 years) from a previously published study<sup>11</sup> treated at the University Medical Center Utrecht and the Leiden University Medical Center (Leiden, Netherlands) between April 1, 2004, and April 1, 2012, who received a cumulative dose of 10 mg/kg ( $\pm 1$ ) over 4 days starting 5 days ( $\pm 1$ ) before their first allogeneic HSCT. No

restrictions in terms of stem cell source or conditioning regimens were used. There were no major changes in treatment protocols between historical controls and study patients including donor hierarchy, conditioning regimens including therapeutic drug monitoring, GVHD prophylaxis, infectious prophylaxis, and all nursing protocols.

In previous studies,<sup>11,13</sup> outcome in terms of overall survival in teenage patients who received regular doses of anti-thymocyte globulin was poor, mainly due to viral reactivations, and hence there was a high medical need to improve outcome. We observed that these patients had extremely high anti-thymocyte globulin exposures with no or very slow CD4<sup>+</sup> recovery, which is in line with the hypothesis that high anti-thymocyte globulin exposure leads to poor CD4<sup>+</sup> recovery and could result in an increase in HSCT-related complications. Our aim in the current study was to prospectively validate that precision dosing improved CD4<sup>+</sup> immune reconstitution. In preparation for this prospective study, and in discussion with the institutional ethics committee, we therefore decided to include the historical control group rather than a prospective control group. Given the absence of major changes in our protocols for HSCT, the historical controls are highly comparable to the study cohort.

The medical ethics committee of the University Medical Center Utrecht reviewed and approved the trial. The trial was conducted according to the Declaration of Helsinki. Patients were enrolled after giving written informed consent. During the trial, there were no amendments that affected trial recruitment or conduct, and all amendments were approved before implementation. The full study protocol is supplied in the appendix (pp 16–86).

### Procedures

Patients received intravenous anti-thymocyte globulin per individualised dosing nomogram (actual bodyweight, baseline absolute lymphocyte count, and graft source [cord blood; appendix p 10, and bone marrow or peripheral blood stem cells; appendix p 11]). The dosing regimen was based on anti-thymocyte globulin (thymoglobulin; Genzyme; Cambridge, MA, USA; the most used type of anti-thymocyte globulin) population pharmacokinetic<sup>19</sup> and pharmacodynamic analyses<sup>11,13</sup> in a myeloablative setting in children. Cumulative dose in the nomogram varied between 2–10 mg/kg and was given over 1–4 consecutive days. The main goal of the individualised dosing was to achieve optimal anti-thymocyte globulin exposure as identified in previous studies.<sup>13,19</sup> To minimise the exposure to anti-thymocyte globulin after graft infusion, the first dose was given on day –9 before HSCT. We wanted to compare individualised anti-thymocyte globulin with the recommended dose in most paediatric protocols—fixed weight-based cumulative dose of 10 mg/kg from day –5 before HSCT. Patients not receiving the intended dose of anti-thymocyte globulin (90–110% of planned dose) were removed from the study.

See Online for appendix

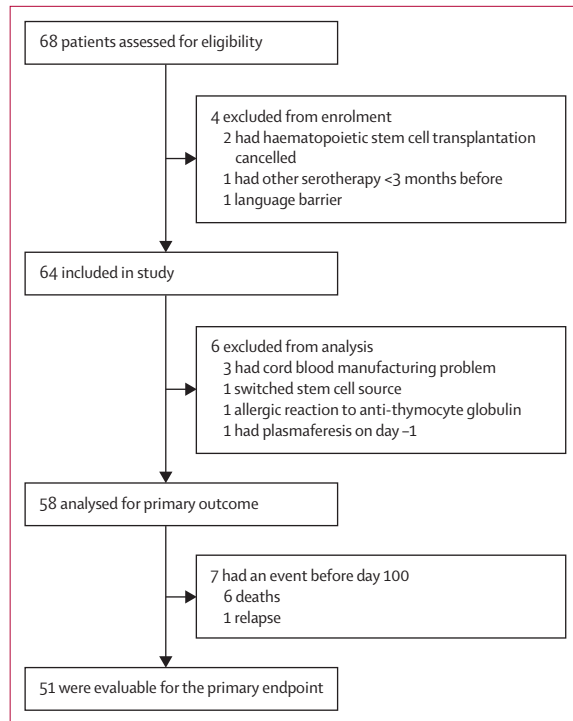


Figure 1: Trial profile

Conditioning regimens were given according to national and international protocols. Busulfan was targeted with therapeutic drug monitoring to reach an area under the curve (AUC) of 75–95 mg/h/L. Patients with severe aplastic anaemia and Fanconi's anaemia received reduced intensity conditioning. Selective gut decontamination, infection prophylaxis, and GVHD prophylaxis were given according to local protocols as described previously.<sup>11</sup> GVHD prophylaxis consisted of ciclosporin, with therapeutic drug monitoring to reach trough levels of 150–250 µg/L, combined with methotrexate 10 mg/m<sup>2</sup> on days 1, 3, and 6 after infusion (bone marrow) or prednisolone 1 mg/kg (cord blood).

A high-resolution CT-scan of the thorax was made before conditioning in all patients. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Routine blood was taken three times per week for cell counts and extensive chemistry, with viral loads, therapeutic drug monitoring, and galactomannan once a week. Additional blood was taken on indication. Conditioning regimens (except the anti-thymocyte globulin dosing), supportive care, and transplantation team did not change over time (enrolment trial and historical cohort). Adverse events were directly reported to the study team and were monitored weekly.

Minimum follow-up was 12 months. Briefly, visits were planned weekly during the first 3 months, and after this every 3 weeks until 6 months, followed by every 3 months up to 2 years (appendix p 56). Assessments focussed on

laboratory tests, HSCT-related complications, and adverse events.

Adverse events were graded according to the common terminology criteria for adverse events (CTCAE) version 4.0. Given the nature of the procedure of allogeneic HSCT, where numerous grade 1–2 adverse events are to be expected, we chose to only collect and report adverse events of grade 3 and worse as per the study protocol. Adverse events that are not clearly mentioned in the CTCAE were graded according to the clinical descriptions (medically significant or [prolonging] hospitalisation or limiting self-care for grade 3, life threatening for grade 4, and death for grade 5 events). We recorded adverse events in all patients at every visit, including those excluded from the activity analyses.

### Outcomes

The primary endpoint was CD4<sup>+</sup> immune reconstitution, defined as a CD4<sup>+</sup> T-cell count of at least  $0.05 \times 10^9$  cells per L at two consecutive measurements within 100 days ( $\pm 3$ ) after HSCT. Early CD4<sup>+</sup> immune reconstitution was chosen as a primary endpoint as this was found to be a reliable predictor (in different centres and transplantation settings) for transplantation outcomes such as overall survival, non-relapse mortality, viral reactivation, and GVHD.<sup>11,15,16,22</sup>

Secondary endpoints included overall survival, event-free survival, therapy-related mortality, relapse, acute and chronic GVHD, graft failure, and viral reactivations of cytomegalovirus, adenovirus, Epstein Barr virus, and engraftment.

Overall survival was defined as the time from transplantation to last follow-up or death. Event-free survival was defined as survival from HSCT to last contact or an event defined as graft failure, relapse, or death. All surviving patients were censored at date of last contact. Therapy-related mortality was defined as from causes other than relapse of a malignancy. Acute GVHD (grade 2–4 and grade 3–4) was classified according to the Glucksberg criteria<sup>24</sup> and chronic GVHD (moderate–severe *vs* no–mild) was classified according to the National Institutes of Health criteria.<sup>25</sup> Graft failure was defined as non-engraftment (ie, autologous reconstitution) or graft-rejection (ie, secondary loss of donor chimerism). Non-engraftment was defined as failure to reach recovery of neutrophils 60 days after HSCT and engraftment was defined as a neutrophil count greater than  $0.5 \times 10^9$  cells per L with use of granulocyte colony-stimulating factor within 40 days. We were also interested in relating CD4<sup>+</sup> immune reconstitution to overall survival and therapy-related mortality in a post-hoc analysis because this measure was previously found to be a reliable predictor of both outcomes. The trial protocol included prospective validation of the population pharmacokinetic model and exploratory lymphocyte subset monitoring as secondary endpoints, which will be reported in future studies.

### Statistical analysis

We hypothesised that individualised dosing of anti-thymocyte globulin would result in an increase in CD4<sup>+</sup> immune reconstitution. The sample size was determined on the basis of the Simon two-stage design for phase 2 clinical trials.<sup>23</sup> In a previously described cohort of 258 patients,<sup>11</sup> the proportion of patients who reached immune reconstitution within day 100 after HSCT was 62% (null hypothesis  $p \leq 0.60$ ), and an increase in successful immune reconstitution of 20% was considered desirable (alternative hypothesis  $p \geq 0.80$ ). The Simon two-stage design with the smallest expected sample size was used with a probability of early termination of 69.2%. The actual power was 90.1% (target at 90%) and the actual  $\alpha$  was 4.3% (target at 5%). We estimated that a total of 38 (72%) of 53 evaluable patients needed to meet the primary endpoint—13 (68%) of 19 patients in the first stage and 25 (74%) of 34 patients in the second stage. After finishing the first stage, a safety analysis was done alongside the activity evaluation. Patients not evaluable for early T-cell reconstitution because of death, relapse, or graft failure before reaching day 100 were replaced to ensure the study was sufficiently powered for the primary endpoint. Patients with major protocol deviations in terms of anti-thymocyte globulin dosing or exposure or graft selection were excluded.

The primary endpoint was assessed only in evaluable patients and was analysed as per the Simon two-stage approach, and as cumulative incidence of CD4<sup>+</sup> immune reconstitution both in the intervention group only and compared with historical controls. Secondary endpoints were evaluated in all patients without major protocol violations; toxicity was evaluated in all patients including those with major protocol violations. For secondary endpoints, as per the study protocol, the individualised dosing regimen was compared with historical controls. All patients in both the study group and historical controls, including those with early events making them not evaluable for the primary endpoint, were considered for the secondary endpoints.

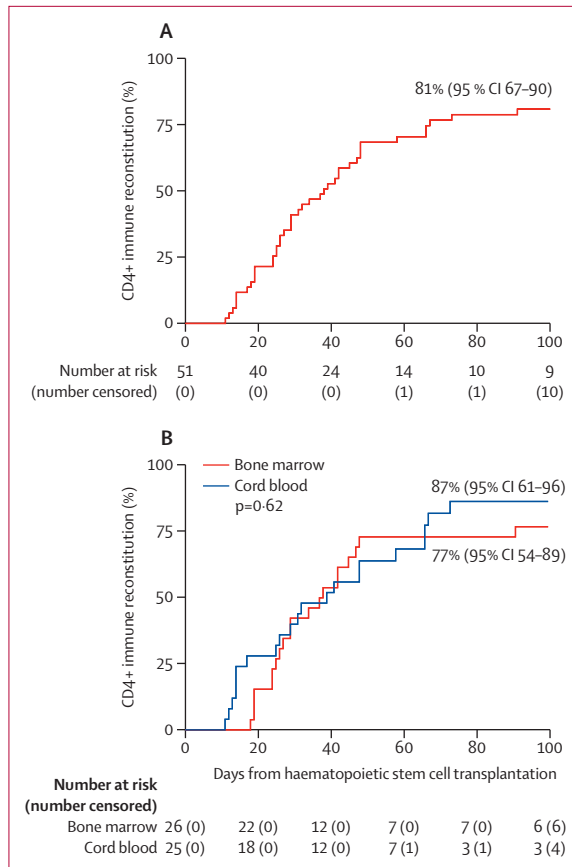
Duration of follow-up was defined as the time from HSCT to the last assessment for patients alive at time of analysis, or to death. To identify predictors for survival outcomes, Cox proportional hazards models were fitted for all secondary endpoints. Potential predictors associated with the recipient (age, sex, patient and donor viral serology status of cytomegalovirus and Epstein-Barr virus, and underlying disease) and the transplantation (human leukocyte antigen disparity and stem-cell source) were considered. Cumulative incidence curves were estimated for CD4<sup>+</sup> immune reconstitution, therapy-related mortality, relapse, acute and chronic GVHD, graft failure, and viral reactivations in a competing risk setting. Hazard ratios (HRs) were calculated using either Cox proportional hazard models or multivariable competing risk models using Fine-Gray tests. Fine-Gray competing risk

	Historical control (n=100)	Study patients (n=58)	p value
Age at HSCT, years	6.1 (2.0–11.6)	7.4 (2.8–13.2)	0.83
Sex	..	..	0.32
Male	59 (59%)	29 (50%)	..
Female	41 (41%)	29 (50%)	..
Starting day before HSCT	5 (5–5)	9 (9–9)	<0.0001
Cumulative dose of anti-thymocyte globulin, mg/kg			
All patients	10.0 (10.0–10.0)	8.7 (6.4–10.0)	<0.0001
Only bone marrow or peripheral blood stem cells	10.0 (10.0–10.0)	9.1 (8.0–10.0)	0.00059
Only cord blood	10.0 (10.0–10.0)	7.8 (5.3–10.0)	<0.0001
Diagnosis	..	..	0.0013
Malignancy	43 (43%)	20 (34%)	..
Immune deficiency, haemophagocytic lymphohistiocytosis, or autoimmune disease	24 (24%)	8 (14%)	..
Bone marrow failure	7 (7%)	18 (31%)	..
Metabolic or benign haematology	26 (26%)	12 (21%)	..
Stem-cell source	..	..	0.028
Bone marrow	47 (47%)	28 (48%)	..
Peripheral blood stem cells	10 (10%)	0	..
Cord blood	43 (43%)	30 (52%)	..
Cytomegalovirus status	..	..	0.91
Patient + or –, donor +	24 (24%)	15 (26%)	..
Patient –, donor –	43 (43%)	24 (41%)	..
Patient +, donor –	29 (29%)	19 (33%)	..
Unknown	4 (4%)	0	..
Epstein-Barr virus status	..	..	0.046
Patient + or –, donor +	44 (44%)	17 (29%)	..
Patient + or –, donor +	16 (16%)	10 (17%)	..
Patient + or –, donor +	24 (24%)	23 (40%)	..
Unknown	16 (16%)	8 (14%)	..
Human leukocyte antigen mismatch	..	..	0.24
Fully matched	53 (53%)	37 (64%)	..
Partially matched	47 (47%)	21 (36%)	..
Follow-up, months	133.5 (115.0–152.0)	25.6 (15.0–37.0)	<0.0001
Treatment year	2008 (2006–2009)	2016 (2015–2018)	<0.0001

Data are median (IQR) or n (%). HSCT=haematopoietic stem-cell transplantation.

**Table 1: Patient characteristics**

regressions were used for multivariable analyses. Considered competing events were death from other causes (therapy-related mortality, relapse, GVHD, and viral reactivations) and death, relapse, or graft failure before 100 days (CD4<sup>+</sup> immune reconstitution). Clinical outcomes were also analysed in patients with or without successful



**Figure 2: Primary endpoint of CD4<sup>+</sup> immune reconstitution in all evaluable patients (A) and in all evaluable patients according to stem-cell source (B)** p value from part B is derived from the two-sided log-rank test.

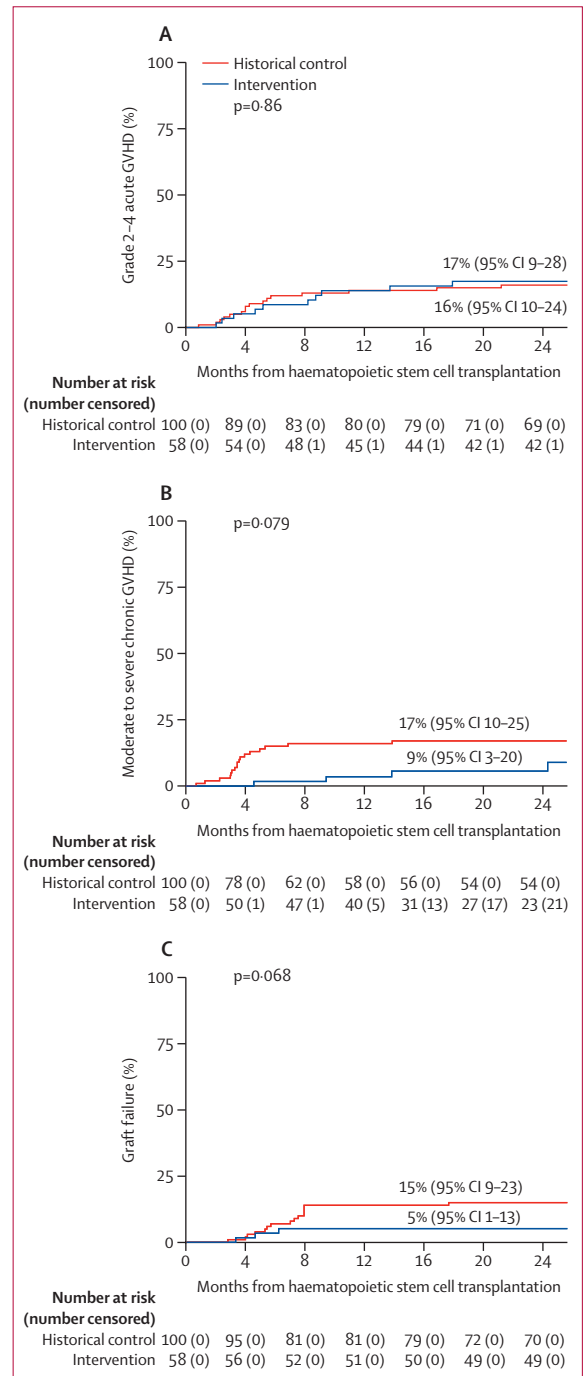
CD4<sup>+</sup> immune reconstitution. Gray's test was used for testing differences in cumulative incidence functions. We calculated probabilities of event-free survival and overall survival using the Kaplan-Meier method; a two-sided log-rank test was used for univariable comparisons. Variables in univariable analyses with a p value less than 0.1 were selected for multivariable analyses on the basis of the relatively small population; a p value less than 0.05 was considered statistically significant. Statistical analyses were done using SAS version 9.4 and R version 1.3.1093. A Data Safety Monitoring Board oversaw the safety of the study as described in the study protocol. The study is registered in the Dutch Trial Register, NL4836.

**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.

**Results**

Between July 1, 2015, and Aug 22, 2018, 68 patients were assessed for eligibility (figure 1). Four patients were excluded from enrolment. Six patients were later excluded



**Figure 3: Cumulative incidence curves for acute GVHD (A) and chronic GVHD (B) and graft failure (C) in all treated patients and in patients from the historical cohort**

p-values are derived from the competing risk analysis (Fine-Gray test) as a univariate analysis. GVHD=graft-versus-host disease.

from the analysis (appendix p 13). Seven (12%) of the remaining 58 patients had an event 100 days after HSCT and were unevaluable for the primary endpoint (figure 1). For the primary endpoint analyses, the final cohort consisted of 51 rather than 53 patients, which was accepted

	Grade 3	Grade 4	Grade 5
<b>Infectious</b>			
BK virus cystitis	2 (3%)	0	0
Candida sepsis	0	0	1 (2%)
Catheter-related infection	6 (9%)	0	0
Cytomegalovirus reactivation	1 (2%)	0	0
Epstein-Barr virus reactivation	1 (2%)	0	0
Febrile neutropenia	6 (9%)	0	0
Fungal pneumonia	0	0	1 (2%)
Influenza	1 (2%)	0	0
Lung infection	7 (11%)	1 (2%)	0
<i>Pneumocystis jirovecii</i> pneumonia	0	0	2 (3%)
Sepsis	0	1 (2%)	3 (5%)
Skin infection	2 (3%)	0	0
Upper respiratory tract infection	1 (2%)	0	0
Urinary tract infection	2 (3%)	0	0
Viral gastro-enteritis	3 (5%)	0	0
<b>Immune system disorders</b>			
Allergic reaction (to anti-thymocyte globulin)	1 (2%)	0	0
Allergic reaction (to liposomal amphotericin B)	1 (2%)	0	0
Auto-immune cytopenia	1 (2%)	0	0
Auto-immune thrombocytopenia	1 (2%)	0	0
Capillary leak syndrome	0	0	1 (2%)
Cytokine release syndrome	1 (2%)	0	0
Graft failure	0	4 (6%)	0
Graft-versus-host disease	1 (2%)	2 (3%)	2 (3%)
Immune dysregulation	1 (2%)	0	0
Progression of underlying disease (lung disease, immunodeficiency, chromosome breakage syndrome)	0	0	1 (2%)
Secondary haemophagocytic lymphohistiocytosis	0	0	1 (2%)

(Table 2 continues in next column)

as the primary endpoint was already met with this cohort. The cumulative dose of anti-thymocyte globulin in the trial group ranged from 2.3–10.0 mg/kg (table 1). Median follow-up time in the study was 25.6 months (IQR 15.0–37.0) and median age was 7.4 years (IQR 2.8–13.2; table 1). 29 (50%) of 58 patients were female. As per the study protocol, we did not collect ethnicity or racial data in the trial.

The trial met the primary endpoint in reaching the desired improvement in CD4<sup>+</sup> immune reconstitution. 15 (79%) of the 19 evaluable patients in the first stage of the study reached successful CD4<sup>+</sup> immune reconstitution. At study conclusion, 41 (80%) of 51 patients reached CD4<sup>+</sup> immune reconstitution. When analysing CD4<sup>+</sup> immune reconstitution over time, we observed an incidence of

	Grade 3	Grade 4	Grade 5
(Continued from previous column)			
<b>Benign, malignant, and unspecified neoplasms</b>			
Post-transplantation lymphoproliferative disorder	1 (2%)	0	0
Relapsed leukaemia	0	3 (5%)	2 (3%)
<b>Gastro-intestinal</b>			
Dehydration	1 (2%)	0	0
Feeding problems	4 (6%)	0	0
Gastro-intestinal haemorrhage	2 (3%)	0	1 (2%)
Gastro-oesophageal reflux disease	1 (2%)	0	0
<b>Metabolism and nutrition</b>			
Hypokalemia	0	1 (2%)	0
<b>Cardiac disorders</b>			
Pericardial effusion	3 (5%)	0	0
<b>Hepatobiliary disorders</b>			
Hyperbilirubinaemia	2 (3%)	0	0
Veno-occlusive disease	1 (2%)	0	0
<b>Respiratory, thoracic, and mediastinal disorders</b>			
Bronchiolitis obliterans	2 (3%)	0	0
<b>Nervous system disorders</b>			
Seizure	1 (2%)	0	0
Neuralgia	1 (2%)	0	0
<b>Renal and urinary disorders</b>			
Acute kidney injury	0	1 (2%)	0
No grade 1–2 adverse events were collected as per protocol specifications. All grade 3, 4 and 5 adverse events are listed. 64 patients were evaluated for adverse events.			

**Table 2: Overview of adverse events**

successful CD4<sup>+</sup> immune reconstitution of 81% (95% CI 67–90), with half the population reaching CD4<sup>+</sup> immune reconstitution at 38 days after HSCT (figure 2A). The incidence of CD4<sup>+</sup> immune reconstitution was 77% (95% CI 54–89) in patients receiving a bone marrow graft and 87% (61–96) in patients receiving a cord blood transplantation (p=0.62; figure 2B).

For the 58 patients enrolled on the trial, 75% (95% CI 61–84; 43 patients) attained successful CD4<sup>+</sup> immune reconstitution compared with 51% (41–60; 52 patients) of 100 control patients in a competing risk setting (p=0.00030; appendix p 2). In multivariable analysis, individualised anti-thymocyte globulin dosing (HR 2.07, 95% CI 1.35–3.19; p=0.00090), metabolic or benign haematology (1.86, 1.03–3.35; p=0.039), and cytomegalovirus serostatus (0.52, 0.29–0.91; p=0.023) were the only significant predictors for CD4<sup>+</sup> immune reconstitution (appendix p 14).

Individualising the dose of anti-thymocyte globulin did not affect grade 2–4 acute GVHD (HR 1.07, 95% CI 0.49–2.34; p=0.86; figure 3A) nor grade 3–4 acute GVHD (1.09, 0.36–3.33; p=0.87; appendix p 3). The incidence of moderate to severe chronic GVHD showed was not significantly different between the historical



control and the trial group (HR 0.45, 95% CI 0.16–1.25; multivariable  $p=0.13$ ; figure 3B). The incidence of graft failure was also not statistically different in the trial group versus the historical group (HR 0.34, 95% CI 0.10–1.17; multivariable  $p=0.086$ ; figure 3C).

Overall survival at 3 years was 81% (95% CI 71–92) in the trial group versus 66% (57–76) in the control group ( $p=0.11$ ; appendix p 4). 11 (19%) of 58 patients died in the study group whereas 36 (36%) of 100 patients died in the control group. No multivariable significant predictors for overall survival were identified. When evaluating overall survival in different stem cell sources, the difference between treatment groups was larger in the bone marrow or peripheral blood recipients versus the cord blood recipients (93% [95% CI 84–100] vs 71% [60–83]; HR 0.24, 95% CI 0.06–1.00;  $p=0.038$ ; appendix p 5). Event-free survival showed similar results to overall survival, with an estimate of 72% (95% CI 60–85) in the trial group versus 57% (47–68) in the control group (HR 0.65, 95% CI 0.37–1.17;  $p=0.15$ ; appendix p 6). Relapse incidence was similar between treatment groups (study group 27%, 95% CI 9–49 vs control group 28%, 15–42; HR 0.92, 0.33–2.53;  $p=0.89$ ; appendix p 7).

The incidence of Epstein-Barr virus reactivations was lower in the trial group (3.5%, 95% CI 0.6–10.7%) compared with the control group (20%, 13–28; HR 0.17, 95% CI 0.04–0.77;  $p=0.022$ ; appendix p 8). The incidence of adenovirus reactivations was 5.1% (95% CI 1.3–13.1) in the trial group versus 16% (9.6–23.9) in the control group (HR 0.43, 95% CI 0.14–1.31;  $p=0.13$ ; appendix p 8). By contrast, the incidence curves for cytomegalovirus overlapped (ie, there was no difference; appendix p 8).

In the post-hoc analyses, we evaluated the predictive power of CD4<sup>+</sup> immune reconstitution on overall survival in both the trial group and control group combined and found that, irrespective of treatment group, successful CD4<sup>+</sup> immune reconstitution led to superior overall survival (HR 0.36, 95% CI 0.19–0.67;  $p=0.0012$ ; appendix p 9). This result was mainly due to lower therapy-related mortality after successful CD4<sup>+</sup> immune reconstitution; the risk of therapy-related mortality was 5-times higher in the group that did not reach CD4<sup>+</sup> immune reconstitution (HR 0.19, 95% CI 0.08–0.47;  $p=0.00029$ ) and similar for the historical group (cumulative incidence 12%, 95% CI 4–27 vs 40%, 26–52) and trial group (cumulative incidence 7%, 2–17 vs 43%, 16–67).

83 adverse events were reported—58 were grade 3, 10 were grade 4, and 15 were fatal (grade 5; table 2; data are disaggregated by sex in the appendix p 15). Infections and immunological disorders were frequently observed, accounting for 41 (49%) of 83 and 15 (18%) of 83 adverse events, respectively. Some adverse events were directly related to anti-thymocyte globulin, including a severe allergic reaction to anti-thymocyte globulin, which led to discontinuation of treatment. No patients required a dose reduction. Two (3%) of 64 patients died of GVHD, which might be indirectly related to the intervention and is to be

expected in a HSCT procedure. No suspected unexpected serious adverse reactions occurred using the summary of product characteristics as a reference. Moreover, the observed adverse events were mostly in line with what is to be expected in a population of patients who have received allogeneic HSCT.

## Discussion

To our knowledge, this is the first prospective trial to investigate fully individualised dosing based on population pharmacokinetic and pharmacodynamic modelling. We found that CD4<sup>+</sup> immune reconstitution reached the desired 20% improvement in the primary endpoint per Simon two-stage design, which was also found in a comparison with a historical cohort group. In a multivariate analysis, individualised anti-thymocyte globulin dosing was the main predictor of immune reconstitution. Individualised dosing did not impact GVHD and graft failure probability, and viral reactivations (Epstein-Barr virus and adenoviruses) were lower in the individualised dosing group. In a post-hoc analysis, we found that CD4<sup>+</sup> immune reconstitution within 100 days was an excellent predictor for overall survival and therapy-related mortality. Patients without CD4<sup>+</sup> immune reconstitution had a 5-times higher risk of therapy-related mortality compared with those with CD4<sup>+</sup> immune reconstitution at 100 days.

Historically, early recovery of neutrophils and thrombocytes have been the most important cellular markers of transplantation success, which are met by most patients. However, early recovery of T cells is hampered in a large proportion of patients, mainly impacting therapy-related mortality. The results of our trial show that individualising the dose of anti-thymocyte globulin on the basis of population pharmacokinetic models led to predictable and early T-cell recovery, with significantly improved CD4<sup>+</sup> immune reconstitution. Studies in adults and children have shown the importance of early CD4<sup>+</sup> T-cell recovery, using the same definition as in this trial, with improved overall survival, reduced therapy-related mortality, lower incidence of viral reactivations and markedly lower GVHD-related mortality.<sup>11,13–15,26,27</sup>

The main concern while designing this study was that a lower dose of anti-thymocyte globulin might increase the incidence of GVHD or graft failure. In the trial group, patients received anti-thymocyte globulin at 9 days before HSCT in a dose that resulted in low exposure of anti-thymocyte globulin after transplantation, which led to less in-vivo T-cell depletion. In patients with relatively low clearance of anti-thymocyte globulin (ie, children with higher bodyweight and those with low absolute lymphocyte count before the first dose of anti-thymocyte globulin), the dose had to be markedly reduced compared with historical, fixed weight-based dosing, to reach sufficiently low exposure after HSCT. This change resulted in a median dose reduction of 13%, ranging up to 78%, compared with traditional dosing. We did not observe an increase in incidence of GVHD nor graft

failure. Therefore, the results suggest that dose individualisation of anti-thymocyte globulin is safe despite a substantial dose reduction in certain patients.

Although this is the first trial to our knowledge to investigate individualised dosing of anti-thymocyte globulin using early T-cell reconstitution as the primary outcome measure, some studies are available that investigated the role of anti-thymocyte globulin in clinical outcome parameters without applying individualised dosing.<sup>6–8,17,18</sup> No prospective studies were available that investigated the pharmacokinetics of anti-thymocyte globulin or its association with T-cell reconstitution. The available studies showed a decrease in both acute and chronic GVHD when comparing adult patients who received a fixed dose of anti-thymocyte globulin compared with adults who did not receive anti-thymocyte globulin. The effect of the use of anti-thymocyte globulin on overall survival is unclear—three studies found no effect,<sup>7,17,18</sup> one study showed improved overall survival after anti-thymocyte globulin, and another study found decreased overall survival in the anti-thymocyte globulin group in patients who received a total body irradiation-based myeloablative regimen.<sup>8</sup> Notably, the latter study found an association between absolute lymphocyte count before the first dose of anti-thymocyte globulin and overall survival; survival was severely impaired only in those patients with low absolute lymphocyte count receiving anti-thymocyte globulin. This observation could be due to higher anti-thymocyte globulin exposures after HSCT, due to decreased clearance of anti-thymocyte globulin. The impact of the use of anti-thymocyte globulin on outcomes is potentially affected by many variables, including conditioning intensity, stem-cell source, T-cell content of the graft, lymphocyte counts in the recipient and use of granulocyte colony-stimulating factor early after transplantation.<sup>16,28</sup> Our previous work reduces these variables to a clinically applicable dosing nomogram, which was prospectively evaluated in this study.

We recognise the use of a historical cohort for the secondary endpoint as an important limitation of this study, given that supportive care is hard to standardise over longer periods of time. When designing the study, there was strong retrospective evidence that fixed bodyweight-based dosing of anti-thymocyte globulin would lead to overexposure in patients with low baseline lymphocytes or higher bodyweight.<sup>19</sup> This observation is in line with a ground rule of developmental pharmacology—children over 12 years are prone to overdosing using weight-based dosing.<sup>29</sup> We showed that overexposure to anti-thymocyte globulin led to delayed or poor CD4<sup>+</sup> reconstitution, which is associated with worse overall survival, and associated with increased viral reactivations and therapy-related mortality.

In conclusion, individualised dosing of anti-thymocyte globulin on the basis of population pharmacokinetic and pharmacodynamic modelling resulted in a significant improvement in early T-cell reconstitution. Dose

individualisation did not lead to an increase in GVHD or graft failure. Promotion of early CD4<sup>+</sup> immune reconstitution by individualising anti-thymocyte globulin dose might improve outcomes of allogeneic HSCT. With the success of this trial, a randomised phase 3 trial, powered for the endpoint of overall survival, would give a more definite conclusion on the added value of individualised anti-thymocyte globulin dosing.

#### Contributors

All authors designed the trial, and were responsible for patient inclusion, data collection, and patient registration. RA, YJ, ML-Y, CAL, and J-JB analysed the data and prepared the manuscript. All authors reviewed the manuscript and vouch for the accuracy and completeness of the data and analyses. RA, CL, YJ, and ML-Y had access to the raw data. RA and CAL have verified the data. All authors had access to the statistical reports, and all had final responsibility for the decision to submit for publication.

#### Declaration of interests

CAL reports honoraria for a lecture from Genzyme (related to this topic). J-JB reports honoraria from BlueRock, AvroBio, Race Oncology, Medexus, and Omeros for consulting (not related to this topic) and from Sanofi (related to this topic), and participation on a data safety monitoring board for Advanced Clinical. All other authors declare no competing interests.

#### Data sharing

No data can be shared, since patients did not give consent for data sharing.

#### Acknowledgments

The trial was funded by an unrestricted research grant from Sanofi.

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