Efficacy of two different doses of rabbit anti-T-lymphocyte globulin to prevent graft-versus-host disease in children with haematological malignancies transplanted from an unrelated donor: a multicentre, randomised, open-label, phase 3 trial

Results of an open label, randomized trial comparing two different dosages of rabbit anti-lymphocyte globulin in children with haematological malignancies transplanted from an unrelated donor

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Summary

Background. Although rabbit anti-human-T-lymphocyte globulin (ATLG) is largely used for preventing immune-mediated complications in patients given allogeneic haematopoietic stem cell transplantation (HSCT) from an unrelated donor (UD), the optimal dosage of this drug in children is still undefined.

Methods. We conducted an open-label, randomized trial comparing two different dosages of ATLG (30 vs 15 mg/Kg, over 3 days) in children (aged 0-18) with haematological malignancies transplanted from a UD, selected using high-resolution typing for HLA-class I/II loci. All patients received a myeloablative regimen and cyclosporine-A plus short-term methotrexate as post-transplantation graft-versus-host disease (GvHD) prophylaxis. The study (NCT00934557) aimed at testing whether a higher dose of ATLG was superior to a lower dose for prevention of grade II-IV acute GvHD. Secondary end-points included cumulative incidence (CI) of chronic GvHD, non-relapse mortality (NRM), disease recurrence, overall survival (OS) and event-free survival (EFS).

Findings. From 01/2008 to 09/2012, 84 and 88 transplanted children allocated to the 30 and the 15 mg/kg group were included in this analysis. The 100-day CI of grade II-IV acute GvHD in the high- and low-ATLG group was 29% and 36%, respectively (P=0·26). The CI of NRM was 19% and 9% in the high- and low-ATLG group, respectively (P=0·09). CI of disease recurrence was 17%, being 14% and 20% in the low- and high-ATLG arm, respectively (P=0·254). The 5-year OS probability was 70% for the whole study population; it was 78% and 62% for the low- and high-ATLG patients, respectively (P=0·045). The 5-year EFS was 77% and 61% for children given low- and high-dose ATLG, respectively (P=0·028). In multivariable analysis, high-dose ATLG correlated with lower EFS and higher NRM.

Interpretation. Children with haematological malignancies transplanted from UDs selected through high-resolution HLA-typing benefit from the use of low-dosage ATLG in comparison to high-dosage ATLG.

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INTRODUCTION

Acute and chronic graft-versus-host disease (GVHD) remain major complications of allogeneic hematopoietic stem cell transplantation (HSCT), being associated with an increased risk of non-relapse mortality (NRM) and reduction in quality of life.[1-3] Anti-T-lymphocyte globulin (ATLG) has been widely used for prevention of acute and chronic GVHD in patients given allogeneic HSCT. In particular, in the last years, different groups have shown in randomized clinical trials that the addition of ATLG to GVHD prophylaxis is able to decrease the risk of both acute and chronic GVHD in adults receiving either HSCT from an unrelated donor (UD),[4-7] or peripheral blood stem cell (PBSC) transplantation from an HLA-identical sibling.[8] However, recent findings have suggested that use of ATLG could increase the risk of relapse and infections, potentially hampering the benefits of better GVHD prevention.[9] In fact, ATLG administration has been demonstrated to be associated with delayed T-cell reconstitution, including that of *naïve* and memory T lymphocytes, and with an increased risk of infectious complications and post-transplant lymphoproliferative disease.[10-12]

Rabbit ATLG is the preferred agent for approaches of GVHD prevention through serotherapy in HSCT. However, two diverse rabbit ATLG preparations are available for clinical use in patients given allogeneic HSCT and their efficacy can be markedly different and have an impact on outcome, since they differ substantially in potency and dosing, because of different preparation methods.[13]

None of the previously published trials has focused on a paediatric population. We hypothesized that fine tuning of GVHD prophylaxis and, in particular, of the dosage of ATLG administered before allogeneic UD-HSCT, could play an important role on the outcome of children. In order to try to define the better dose of rabbit ATLG, we conducted a prospective, multicentre, clinical trial comparing two different doses of the drug (i.e 15 mg vs. 30 mg/Kg) in

children affected by haematological malignancies transplanted with either bone marrow (BM) or PBSC from a UD selected using high-resolution molecular typing for class I and II HLA loci. We selected to compare 30 mg/kg versus 15 mg/kg of ATLG instead of the dose of 60 mg/kg used in UD-HSCT recipients in the trial published by Finke et al.,[6] because children are less prone to develop GVHD than adults.[3, 14] Support to this choice is also given by a retrospective analysis showing that 30 mg/kg of ATLG in single dose were equally effective for preventing GVHD than 60 mg/kg administered over three days in adults with haematological malignancies transplanted from an unrelated volunteer.[15] In that trial, the 2-year NRM was lower for the ATLG-30 mg/kg group (12% versus 33%, P=0·02), mainly because of a higher incidence of fatal infections in the ATLG-60 mg/kg group.[15]

PATIENTS and METHODS

Study design

This is a multicentre, open-label, phase 3 study enrolling children with haematological malignancies given allogeneic HSCT from a UD and randomly assigned to receive ATLG at a total dose of either 30 mg/kg or 15 mg/kg, over 3 days, namely from day -4 to day -2. The study was sponsored by the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and was conducted in collaboration with the HSCT Working Group of the Italian Association for Paediatric Haematology and Oncology (AIEOP). The institutional review board/ethical committee of each participating centre approved the study; written informed consent was obtained from patient parents or their legal guardians before enrolment. The trial was registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT00934557).

Inclusion criteria were: i) diagnosis of acute leukaemia in morphological complete remission (CR) or Philadelphia-positive (Ph+) chronic myelogenous leukaemia (CML) in chronic phase, non-Hodgkin lymphoma (NHL) in CR or myelodysplasia; ii) age at transplantation between 0 and 18 years; iii) a life expectancy of at least three months; iv) the availability of a UD selected using high-resolution molecular typing of HLA-A, B, C and DRB1 loci, with no more than two allelic disparities, or one antigenic difference; v) use of BM-derived stem cells or G-CSF-mobilized PBSC. Main exclusion criteria were: i) previous allogeneic or autologous HSCT; ii) use of cord blood as stem cell source; iii) treatment with rabbit ATLG in the previous 3 months before transplantation; iv) previous history of allergic reactions to ATLG; v) absence of signed informed consent.

To avoid imbalance between the two arms, before randomization, patients were stratified according to the degree of compatibility with their donor, the source of hematopoietic stem cells employed (BM vs. PBSC) and the disease risk category (standard- versus high-risk, SR vs. HR). Details on the stratification criteria are reported in the supplementary file.

Randomization and transplant procedure

Patients were randomly assigned to either of the two arms with a 1:1 ratio, according the stratification criteria reported above. The randomization code was generated by nQuery Advisor, with the use of a block randomization plan and a block size of four.

All patients were given a myeloablative regimen, chosen according to the disease and the policy of each participating centre (for further details on the conditioning regimens used see also Table 1).

GVHD prophylaxis

GVHD prophylaxis consisted of the combination of Cyclosporine-A, administered intravenously at an initial dose of 3 mg/kg/day in two divided doses, starting from day -7 until the drug could

be administered orally at a dose of 6 mg/kg/day, and short-term methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days 3, 6, and 11 after transplantation, respectively). Rabbit ATLG (Grafalon® kindly provided by FRESENIUS/NEOVII) was given on day -4, -3 and -2 at dose of either 10 or 5 mg/kg/day, according to the result of randomization.

Study endpoints and definition of outcomes

The primary endpoint of the study was 100-day cumulative incidence of grade II-IV acute GVHD in the two randomization arms. The sample size was calculated based on expected 100-day rate of grade II-IV acute GVHD of 25% in the high-ATLG group (30 mg/kg) and 50% in the low-ATLG group (15 mg/kg). We estimated a sample size of 160 patients (80 patients in each group) considering a power of 0.80 and alpha equal to 0.05 to reject the null hypothesis in a log-rank test model. All transplanted patients were evaluated for acute GVHD occurrence (evaluated at day +100 after transplantation), whereas those surviving more than 100 days were evaluated for chronic GVHD. Severity of acute GVHD and chronic GVHD was assessed according to the Glucksberg and the modified Seattle criteria (for categorization of chronic GVHD as clinically limited or extensive), respectively.[16, 17] In estimating GVHD occurrence, both rejection and NRM were considered competing events.[18] Grade I (i.e. skin-only) acute GvHD was homogeneously treated with topical steroids or low-dose (i.e. less than 1 mg/Kg) systemic steroids. Grade II-IV acute was initially treated with steroids; patients failing to respond to steroids were given a variety of different second-line therapies.

Secondary endpoints included incidence of NRM and disease recurrence and probability of event-free survival (EFS) and overall survival (OS) in the two arms. We also evaluated neutrophil and platelet engraftment, considering both rejection and death as competing events

and the composite endpoint of chronic GVHD–free and relapse-free survival.[19] Definitions of the different endpoints are reported in the supplementary file.

Statistical analysis and study oversight

Data on patients randomized in the study were collected in the data warehouse of the AIEOP HSCT group. Both primary and secondary end-points were assessed by the two statisticians of the group (CR and MZ). Data were analyzed as of March 1st, 2016. Acute and chronic GVHD, rejection, engraftment, OS, EFS, NRM and relapse incidence were estimated from the date of transplantation to the date of an event or last follow-up. Probabilities of EFS and OS were calculated according to the Kaplan and Meier method. Acute GVHD and chronic GVHD, NRM and relapse were calculated as cumulative incidence curves in order to adjust the estimates for competing risks. All results were expressed as probability or cumulative incidence (%) and 95% confidence interval (95% CI).[20, 21]

The significance of differences between EFS and OS was estimated by the log–rank test (Mantel–Cox), while Gray's test was used to assess, in univariable analyses, differences between cumulative incidences.[22] The following variables were investigated for their influence on cumulative incidence of grade II-IV acute GVHD and EFS: patient gender, patient age, diagnosis, risk stratification, HLA-disparity, stem cell source, CMV serology in the donor/recipient pair, using of TBI during the conditioning regimen and dose of ATLG (see also Supplementary file). Impact of chronic GVHD on EFS was also investigated. Multivariable analysis was performed using the Cox proportional hazard regression model.[20, 21] All variables resulting statistically significant in univariable analysis and the variables used for patients stratification were included in the multivariable model. Other details on statistical analysis are shown in the supplementary file.

Role of the funding source

Neovii Biotech, which provided ATLG (Grafalon ®) for the conduction of the study, had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

The first, second, and last authors of this manuscript designed the study; the first and last authors had full access to all the data in the study and wrote the first draft, and all the coauthors made the decision to submit the manuscript for publication, while no one who is not an author contributed to the writing of the manuscript.

RESULTS

From January 2008 to September 2012, 187 patients were screened for inclusion in the present study, at seven centres belonging to the AIEOP network. Seven patients were excluded from randomization, because they did not fulfil all the inclusion criteria. One hundred and eighty patients were enrolled and randomized; 89 were assigned to the high-ATLG group and 91 to the low-ATLG group (see Figure 1). Eight patients, five belonging to the high-ATLG group and three to the low-ATLG group, did not proceed to transplantation due to relapse before HSCT. The remaining 172 patients, 84 belonging to the ATLG 30 mg/kg group and 88 belonging to the 15 mg/kg group, were transplanted and included in this analysis.

Detailed patient and transplant characteristics are shown in Table 1. The two randomization groups were comparable for all demographic and transplant-related variables evaluated.

The median follow-up for the whole study population was 3·4 years (range, 1 day – 7·9 years). It was 4·2 years (range, 3·3 – 7·9) for patients alive and 0·7 years (range, 1 day – 4·2 years) for those who died. Considering only surviving patients, the median follow-up was 4·2 years (range, 3·3 – 7·3) for the 30 mg/kg arm and 4·1 years (range, 3·4 – 7·9) for the 15 mg/kg arm (P=0·461).

Two patients (one per randomization arm) did not engraft. The median time to neutrophil engraftment in the whole study population was 20 days (range, 9-43), with no difference in the 2 randomization arms (data not shown). The median time to platelet engraftment was 24 days (range, 11-96). It was 26 days (range, 11-96) in the high-ATLG group and 22 days (range, 9-93) in the low-ATLG group (P = 0·0107).

Figure 2 and Table S1 summarize the cumulative incidence of acute and chronic GVHD. In detail, 40 patients (24%) developed grade I acute GVHD, 44 (25%) grade II, six (3%) grade III and six (3%) grade IV, while 76 children (44%) did not present any grade of acute GVHD. No difference was observed between the two randomization arms. The overall 100-day cumulative incidence of grade II-IV acute GVHD was 33% (95% CI, 26-40), while that of grade III-IV acute GVHD was 7% (95% CI, 4-12). The cumulative incidence of grade II-IV and of grade III-IV acute GVHD was comparable between the two randomization arms (see also Figure 2, panel A and B, and Table S1 for further details). Analysis of the variables influencing occurrence of grade II-IV acute GVHD is shown in Table S2.

One hundred and fifty-six patients surviving more than 100 days after HSCT were evaluated for chronic GVHD occurrence. Chronic GVHD vas absent in 124 patients (79%), clinically limited in 20 (13%) and clinically extensive in 12 (8%). The overall cumulative incidence of chronic GVHD (limited + extensive) was 21% (95% CI, 15-28), with no difference was observed between the two randomization arms (see also Figure 2, panel C, and Table S1).

The overall cumulative incidence of extensive chronic GVHD alone was 8% (95% CI, 4-14), being 6% (95% CI, 2-14) for the high-ATLG patients and 10% for the low-ATLG group (hazard ratio [HR] 0·57 [95% CI 0·17-1·91], P=0·364, see also Figure 2, panel D).

Twenty-tree patients (13%) died in remission due to transplant-related causes, 15 in the high-ATLG group and eight in the low-ATLG group. The overall cumulative incidence of NRM was

14% (95% CI, 9-21), being 19% for patients who received ATLG at a total dose of 30 mg/kg and 9% for those given 15 mg/kg (HR 2·14 [95% CI 0·91-5·05], P = 0·09) (Figure 3C). Table S3 reports in detail the different causes of death in the two arms.

Table S4 shows the cumulative incidence of viral infections (HCMV, EBV, and Adenovirus both reactivation and disease) and of invasive aspergillosis in the whole study population and in the two randomization arms. Notably, children receiving the higher ATLG dose had a greater cumulative incidence of viral reactivations as compared to those who received the lower dosage. The difference was statistically significant for EBV reactivation (31/84, 37% vs. 20/88, 23%; P=0·038) and for Adenovirus reactivation (10/84, 12% vs. 1/88, 1%; P=0·004). Also the incidence of invasive aspergillosis was slightly higher in the high-ATLG group (5/84, 7% vs. 2/88, 2%), but this difference was not statistically significant (P=0·257).

Twenty-nine patients (17%) relapsed at a median of 6.6 months after HSCT (range, 1·9-21·8), the cumulative incidence of relapse being 15% (95% CI, 12-24). Seventeen relapses occurred in the high-ATLG group and 12 in the low-ATLG group. Figure 3D shows the cumulative incidence of relapse of the study population. The cumulative incidence of relapse was 20% (95% CI, 13-31) for the high-ATLG group and 14% (95% CI, 8-23) for the low-ATLG group (HR 1·69 [95% CI 0·81-3·54], P=0·254).

One-hundred and twenty-three patients (72%) were alive at time of the last follow-up: 120 were in continuous complete remission after HSCT, while three were alive after a post-transplant leukaemia relapse. At time of writing, no child has developed secondary malignancy.

Figures 3A and 3B show the results of OS and EFS analysis. The 5-year OS probability was 70% (95% CI, 62-77) for the whole study population. It was 62% (95% CI, 50-73) for the high-ATLG patients and 78% (95% CI, 69-87) for the low-ATLG patients (HR 1·80 [95% CI 1·01-3·20], P = 0·045)..

Overall, the 5-year EFS was 69% (95% CI, 62-76); it was 61% (95% CI, 50-72) and 77% (95% CI, 68-85) in patients who received ATLG at the dose of 30 mg/kg or of 15 mg/kg, respectively (HR 1·87 [95% CI 1·07-3·28], P = 0·028). Analysis of other variables potentially influencing EFS is shown in Table S5.

Table S6 and Figure S1 summarize the results of survival analysis for specific subgroups of patients, including acute leukaemia patients and subsets of patients stratified according to disease risk category, degree of donor/recipient HLA compatibility, and source of stem cells. Children with SR disease had a better EFS probability as compared with HR patients (Figure S1A), as well as those transplanted using an HLA-identical or one allelic disparate donor had a better outcome than those transplanted from a donor showing a greater disparity with the recipient, although the difference in univariate analysis is not statistically significant (Figure S1B). Noteworthy, in all subgroup analyses, we observed an advantage in terms of EFS for patients who received ATLG at the dose of 15 mg/kg as compared to those who received the 30 mg/kg dose. This advantage was statistically significant (P<0·05) for all acute leukaemia patients, for those affected by ALL and for those receiving a transplant from a one-antigen or two-allele mismatched donor. The advantage was not statistically significant in the remaining comparisons, possibly due to the insufficient number of patients enrolled.

Chronic GVHD-free + relapse-free survival and extensive chronic GVHD-free + relapse-free survival were also evaluated (see Table S1 and Figure S2 for details). In both analyses, children who received low-ATLG had a slightly better outcome, even though in both cases the difference was not statistically significant.

Table 2 summarizes the results of multivariable analysis of grade II-IV acute GVHD, EFS, NRM and relapse. The use of a donor with either two allelic disparities or with one antigenic disparity was associated with an increased risk of grade II-IV acute GVHD, while a negative/negative

donor/recipient CMV serology correlated with a lower risk of developing this complication (see Table 2 for details). In multivariable analysis of EFS, the following variables were associated with worse outcome: ATLG dose of 30 mg/kg (relative risk 1·90; P = 0·026), HLA disparity > one allele (relative risk 2·08; P = 0·01) and HR disease (relative risk 2·46; P = 0·0015). Only HR disease group resulted significantly associated with a higher risk of relapse (relative risk = 3·52; P = 0·0012), while the ATLG dose of 30 mg/kg was associated with a higher risk of NRM (relative risk = 1·80; P = 0·047).

DISCUSSION

Although *in vivo* serotherapy has been largely used in the last two decades with the aim of reducing the incidence and severity of GVHD, few randomized trials have been conducted [4-9] and none of these in paediatric patients. Moreover, while all these controlled trials compared either rabbit-antihuman T-cell line (Jurkat) globulin (Grafalon®, Neovii Biotech) versus no ATLG [6, 8, 9] or rabbit-antihuman thymocyte globulin (Thymoglobulin®, Genzyme) versus no ATLG,[4, 5, 7] no randomized study has addressed the issue of drug dose. Thus, the optimum ATLG therapeutic window and the timing of treatment have not yet been defined. Herein, we report the results of the first randomized clinical trial designed to establish the better dose of rabbit ATLG able to prevent acute GVHD, while maintaining the capacity of effectively controlling/eradicating infections and leukemia re-growth in children undergoing transplantation with either BM- or PBSC-derived grafts from a UD.

Despite the heterogeneity of disease transplanted and of conditioning regimens employed, our results show that, in an era in which high-resolution molecular typing for class I and class II HLA loci has significantly improved the clinical outcome of UD-HSCT by decreasing the risk of

immunological complications[14, 23] lowering the ATLG dose to 15 mg/kg did not affect the time to engraftment, and more importantly, the incidence of acute or chronic GVHD, while was associated with an improved probability of EFS, mainly due to a reduced risk of NRM. In particular, children given high-ATLG had a higher incidence of infection-related deaths (seven vs three in the low-ATLG group); moreover, they also experienced an increased risk of adenovirus and EBV infections. Although we do not have data on virus-specific immune reconstitution in the two randomization arms, several studies showed that ATLG has a half-life of 7–14 days, which means that patients are exposed to ATLG both before and after HSCT[11, 24] and there is substantial evidence that higher doses of ATG are associated with slower recovery of pathogen-specific immunity and higher incidence of fatal infections.[4, 9, 10, 12] Adenovirus infections are much more of a problem in paediatric patients (20-26%) undergoing HSCT than in adults (9%), possibly because of the reservoir of adenovirus in the general paediatric population.[25, 26] Higher doses of ATLG depleting antiviral T cells transferred with the graft, because of the long half-life of the polyclonal antibodies, may have a detrimental effect on recovery of pathogen-specific T-cell immunity, which was demonstrated to be crucial for clearing the viral infection and preventing dissemination of adenovirus infection.[25] Previous reports have also documented that both T-cell depletion and high rabbit ATLG serum levels on day seven after HSCT are associated with increased risk of EBV infection and post-transplantation lymphoproliferative disorder.[27, 28] Two patients given high-dose ATLG in our study developed PTLD and one died for this complication. It is important to note that a recent study showed that detection of multiple double-stranded DNA viruses has a dose-dependent association with increased mortality after HSCT.[29]

The use of low-dose ATLG was associated with statistically better EFS in the subgroups of children with acute leukaemia or with ALL, which represents the more frequent indication for an

allograft in haematological malignancies of childhood. In particular, the 78% probability of EFS at five years observed in the 50 children with ALL given low-dose ATLG is comparable with that recently reported by Peters et al.[23] in 306 children transplanted from an UD using a standardized transplantation and GVHD prophylaxis protocol (71% at 4 years).

Remarkably, the advantage of using low-dose ATLG was evident also in patients given PBSC, a stem cell source that, in comparison to BM, was shown to be associated with more chronic GVHD in a prospective, randomized trial conducted in UD-HSCT recipients,[30] probably reflecting the protective effect of intensive GVHD prophylaxis with cyclosporine, methotrexate, and rabbit ATLG.

In our cohort, the most frequent cause of treatment failure after transplantation was disease recurrence, which occurred in the first or more rarely in the second year after transplantation. While the dose of ATLG employed did not statistically influence the probability of relapse, this was mainly affected by the disease risk category, confirming that the stratification criterion chosen had a high predictive value on final outcome.

In this study, the rate of the composite end-point of survival free from chronic GVHD and from relapse was comparable between children given either high- or low-dose ATLG, suggesting that a lower dose of serotherapy does not affect the quality of life of surviving patients.

Different types of ATLG preparations have been tested as part of conditioning regimens to achieve *in vivo* T-cell depletion/modulation and, thus, to prevent GVHD. The ATLG preparation used in our study is a polyclonal antihuman T-lymphocyte immune globulin derived from rabbits after immunization with the Jurkat human T-cell line. This product preferentially targets T cells; however, since other antigens like CD19 or CD138 are also targeted by ATLG,[31] we cannot exclude a direct antitumor effect of the drug in B-cell ALL and to a lesser extent in myeloid leukaemias.

Recent data on pharmacokinetics of ATLG in the paediatric population have shown that older children have a disproportionately higher exposure than younger children, because they have a lower clearance of the drug per kg.[10, 11] In addition, a recipient low lymphocyte count at time of ATLG infusion was shown to translate into high exposure of donor T cells to the drug.[10, 11] These observations provide a rationale for envisaging for the future more sophisticated strategies on ATLG dose and timing of administration based on pharmacokinetics parameters. In summary, our data obtained in a randomized clinical trial with a long follow-up indicate that, in children with haematological malignancies transplanted from an UD selected through high-resolution HLA typing, the use of low-dose rabbit ATLG results into better OS and EFS. Low-dose ATLG can spare life-threatening viral infections, without significantly increasing the incidence of acute and chronic GVHD and without adversely affecting other outcomes, like engraftment or relapse.

Research in context:

Evidence before this study

This study was designed in 2007 with the aim of identifying the recommended dose of rabbit anti-T-lymphocytes globulin (ATLG) for prevention of acute graft-versus-host disease (GVHD) in children with haematological malignancies given allogeneic haematopoietic stem cell transplantation (HSCT) from an unrelated donor. At that time, a previously published randomized trial conducted in adult HSCT recipients had shown that, in comparison to patients given placebo, those treated with high-dose ATLG had a lower incidence and severity of GvHD, at the price, however, of a greater risk of infectious-related mortality. A second randomised trial of ATLG in adults given allogeneic HSCT from an unrelated donor was published in 2009, with a follow-up report in 2011. This second study confirmed that patients given ATLG (60 mg/kg) benefited from lower incidence/severity of acute GVHD, but also found a decrease in incidence of chronic GVHD in comparison to those receiving the placebo. A third randomized trial published in 2016 in adults given unrelated donor HSCT and comparing rabbit ATLG versus placebo provided support to previous findings, since ATLG prophylaxis of GVHD was shown to decrease the number of patients needing immunosuppressive treatment at 1 year after transplantation.

However, none of the three studies focused on paediatric patients or addressed the issue of the dose of ATLG to be employed for preventing GVHD occurrence, while preserving recovery of pathogen-specific immunity.

We updated this information by searching MEDLINE for articles published from Jan 1, 2008, to Jan 1, 2017, using the algorithm “hematopoietic stem cell transplantation” OR “bone marrow transplantation” OR “stem cell transplantation” OR “peripheral blood stem cell transplantation” AND “antithymocyte globulin” OR “anti-T-lymphocyte globulin” OR “GvHD prophylaxis in

children", but identified no additional prospective randomised trials of ATLG done in children receiving unrelated donor transplantation

Added value of this study

This is the first randomized clinical trial comparing two different dosages of ATLG in unrelated donor HSCT recipients ever conducted. Moreover, none of the abovementioned studies have focused on paediatric patients, while our own prospective randomized trial included only children. We have a long follow-up (median value for surviving patients being 4.2 years, range 3.3-7.3), this rendering our data reliable and robust enough to draw definitive conclusions.

Implications of all the available evidence

The results of our study provide a clear and clinically useful message, namely that children with haematological malignancies given an unrelated donor HSCT should receive low-dose (15 mg/kg) instead of high-dose (30 mg/kg) ATLG for avoiding the risk of increasing non-relapse mortality, and thus, impairing the probability of event-free survival. The advantage of using low-dose ATLG is confirmed in the subset of children with acute lymphoblastic leukaemia, which represents the more frequent indication to HSCT. Noteworthy, decreasing the ATLG dose to 15 mg/kg did not affect the time to engraftment, and more importantly, the incidence of acute or chronic GVHD.

Authors' contribution

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Table 1. Patient and transplant characteristics.

	Randomization arm		
	15 mg/kg	**30 mg/kg**	**Total**
Number of patients	88 (51%)	84 (49%)	172 (100%)
Gender:			
Male	54 (61%)	54 (64%)	108 (63%)
Female	34 (39%)	30 (36%)	64 (37%)
Age at diagnosis (years)	7·5 (1-18)	7·0 (1-18)	7·0 (1-18)
Diagnosis:			
ALL	50 (57%)	44 (52%)	94 (55%)
AML	21 (24%)	21 (25%)	42 (24%)
CML	1 (1%)	2 (2%)	3 (2%)
MDS	12 (14%)	13 (16%)	25 (14%)
NHL	4 (4%)	4 (5%)	8 (5%)

Age at HSCT (years)	9·0 (1-19)	9·0 (1-19)	9·0 (1-19)
Disease risk category:			
Standard risk	57 (65%)	50 (60%)	107 (62%)
High risk	31 (35%)	34 (40%)	65 (38%)
Year of transplantation			
2008	12 (14%)	8 (9%)	20 (12%)
2009	13 (15%)	18 (21%)	31 (18%)
2010	16 (18%)	15 (18%)	31 (18%)
2011	26 (30%)	24 (29%)	50 (29%)
2012	21 (23%)	19 (23%)	40 (23%)
Centre of transplantation			
Pavia	20 (23%)	22 (26%)	42 (24%)
Rome	22 (25%)	17 (20%)	39 (23%)
Monza	10 (12%)	13 (16%)	23 (13%)
Bologna	11 (12%)	9 (11%)	20 (12%)
Turin	11 (12%)	7 (8%)	18 (10.5%)

Pisa	8	(9%)	10	(12%)	18	(10.5%)
Genoa	6	(7%)	6	(7%)	12	(7%)
Donor – recipient HLA disparity						
Identical or 1 allele disparity	64	(73%)	61	(73%)	125	(73%)
1 Antigen or > 1 allele disparity	24	(27%)	23	(27%)	47	(27%)
Stem cell source						
Bone marrow	72	(82%)	70	(83%)	142	(79%)
Peripheral blood	16	(18%)	14	(17%)	30	(21%)
Conditioning regimen						
Busulfan-based	30	(34%)	38	(45%)	68	(40%)
Busulfan + Cyclophosphamide + Melphalan	18		25		43	
Busulfan + Thiotepa + Cyclophosphamide	7		8		15	
Busulfan + Cyclophosphamide	5		5		10	
Treosulfan- based	13	(15%)	13	(15%)	26	(15%)
Thiotepa + Treosulfan + Fludarabine	7		9		16	
Treosulfan + Fludarabine	6		4		10	

TBI-based	45	(51%)	33	(39%)	78	(45%)
TBI + Thiotepa + Cyclophosphamide	29		20		49	
TBI + Thiotepa + Fludarabine	11		7		18	
TBI + VP16	5		6		11	
Cell dose infused						
Bone marrow (TNC x 10^8/kg)	4	(2-7)	5	(2-9)	4	(2-9)
Peripheral blood (CD34+ cell x 10^6/kg)	7	(3-8)	6	(3-8)	6	(3-9)

Table 2. Multivariable analysis on grade II-IV acute GVHD, EFS, NRM and relapse risk

	Relative risk	(95% CI)	P
GRADE II-IV ACUTE GVHD			
Disease risk category			
HR vs. SR	0·59	(0·32-1·08)	0·087
HLA compatibility			
> 1 allele mismatch vs. Identical or 1 allele mismatch	2·22	(1·29-3·85)	**0·0043**
Stem cell source			
Peripheral blood vs. bone marrow	0·76	(0·36-1·61)	0·470
Donor -> recipient CMV serology			
Negative -> positive vs. Negative -> negative	11·52	(1·57-84·47)	**0·016**
Positive -> negative vs. Negative -> negative	7·42	(0·98-56·69)	0·053
Positive -> positive vs. Negative -> negative	10·34	(1·43-74·78)	**0·021**
Randomization arm			
ATG dose: 30 mg/kg vs. 15 mg/kg	0·76	(0·42-1·38)	0·370
EVENT-FREE SURVIVAL			
Disease risk category			
HR vs. SR	2·46	(1·41-4·27)	**0·0015**
HLA compatibility			
> 1 allele mismatch vs. Identical or 1 allele mismatch	2·08	(1·2-3·7)	**0·0111**
Stem cell source			
Peripheral blood vs. bone marrow	0·92	(0·41-2·07)	0·885
Randomization arm			
ATG dose: 30 mg/kg vs. 15 mg/kg	1·90	(1·07-3·3)	**0·0256**
NON-RELAPSE MORTALITY			
Disease risk category			
HR vs. SR	1·56	(0·68-3·54)	0·187
HLA compatibility			
> 1 allele mismatch vs. Identical or 1 allele mismatch	2·00	(0·87-4·59)	0·107
Stem cell source			

Peripheral blood vs. bone marrow	0·25	(0·03-1·88)	0·473
Randomization arm			
ATG dose: 30 mg/kg vs. 15 mg/kg	1·80	(1·07-3·0)	**0·047**
RELAPSE			
Disease risk category			
HR vs. SR	3·52	(1·65-7·51)	**0·0012**
HLA compatibility			
> 1 allele mismatch vs. Identical or 1 allele mismatch	2·12	(0·94-4·58)	0·0551
Stem cell source			
Peripheral blood vs. bone marrow	1·95	(0·81-4·67)	0·081
Randomization arm			
ATG dose: 30 mg/kg vs. 15 mg/kg	1·80	(0·85-3·81)	0·126

Figure legends

Figure 1. Trial profile of the whole population enrolled into the randomized trial.

Figure 2. Cumulative incidence of grade II-IV acute GVHD (A), grade III-IV acute GVHD (B), chronic GVHD (C) and extensive chronic GVHD (D) in the two randomization arms.

Figure 3. Five-year probability of overall survival (SURV) (A), event-free survival (EFS) (B), cumulative incidence of non-relapse mortality (NRM) (C) and relapse (REL) (D) according to randomization arm.

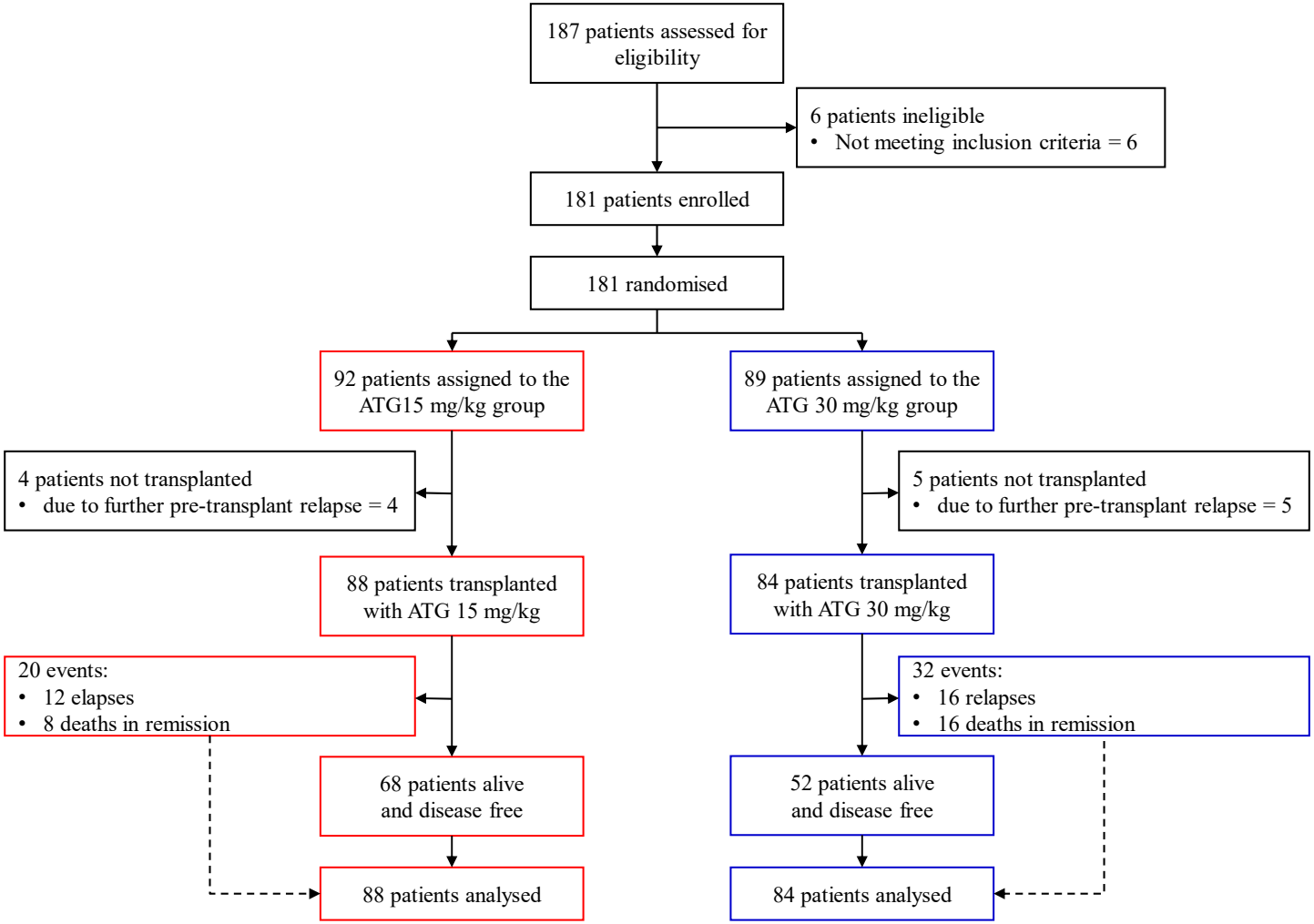
187 patients assessed for eligibility
6 patients ineligible
• Not meeting inclusion criteria = 6
181 patients enrolled
181 randomised
92 patients assigned to the ATG15 mg/kg group
89 patients assigned to the ATG 30 mg/kg group
4 patients not transplanted
• due to further pre-transplant relapse = 4
5 patients not transplanted
• due to further pre-transplant relapse = 5
88 patients transplanted with ATG 15 mg/kg
84 patients transplanted with ATG 30 mg/kg
20 events:
• 12 elapses
• 8 deaths in remission
32 events:
• 16 relapses
• 16 deaths in remission
68 patients alive and disease free
52 patients alive and disease free
88 patients analysed
84 patients analysed

Figure 1

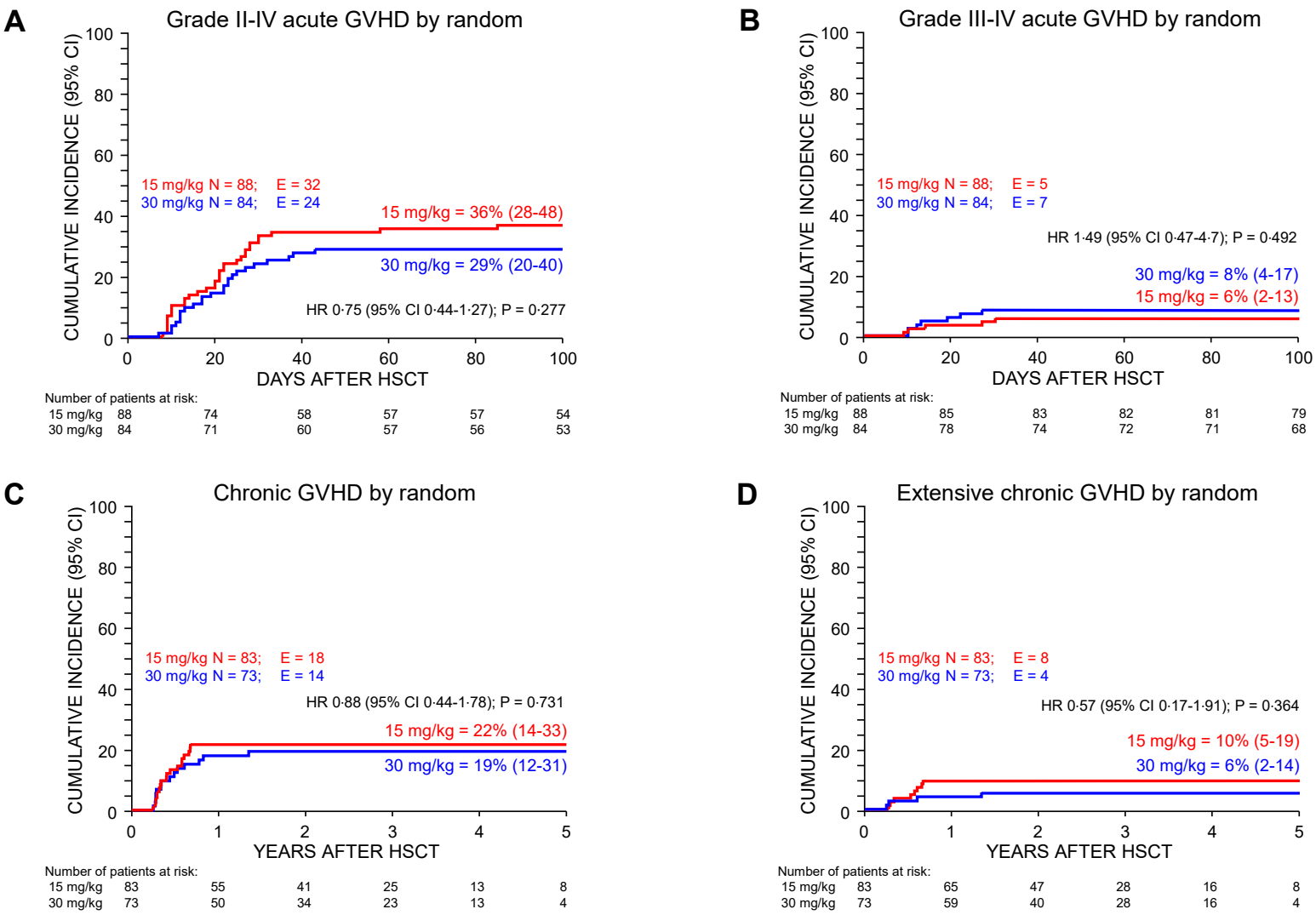
A
Grade II-IV acute GVHD by random
CUMULATIVE INCIDENCE (95% CI)
DAYS AFTER HSCT
15 mg/kg N = 88; E = 32
30 mg/kg N = 84; E = 24
15 mg/kg = 36% (28-48)
30 mg/kg = 29% (20-40)
HR 0·75 (95% CI 0·44-1·27); P = 0·277
Number of patients at risk:
15 mg/kg 88 74 58 57 57 54
30 mg/kg 84 71 60 57 56 53
B
Grade III-IV acute GVHD by random
CUMULATIVE INCIDENCE (95% CI)
DAYS AFTER HSCT
15 mg/kg N = 88; E = 5
30 mg/kg N = 84; E = 7
HR 1·49 (95% CI 0·47-4·7); P = 0·492
30 mg/kg = 8% (4-17)
15 mg/kg = 6% (2-13)
Number of patients at risk:
15 mg/kg 88 85 83 82 81 79
30 mg/kg 84 78 74 72 71 68
C
Chronic GVHD by random
CUMULATIVE INCIDENCE (95% CI)
YEARS AFTER HSCT
15 mg/kg N = 83; E = 18
30 mg/kg N = 73; E = 14
HR 0·88 (95% CI 0·44-1·78); P = 0·731
15 mg/kg = 22% (14-33)
30 mg/kg = 19% (12-31)
Number of patients at risk:
15 mg/kg 83 55 41 25 13 8
30 mg/kg 73 50 34 23 13 4
D
Extensive chronic GVHD by random
CUMULATIVE INCIDENCE (95% CI)
YEARS AFTER HSCT
15 mg/kg N = 83; E = 8
30 mg/kg N = 73; E = 4
HR 0·57 (95% CI 0·17-1·91); P = 0·364
15 mg/kg = 10% (5-19)
30 mg/kg = 6% (2-14)
Number of patients at risk:
15 mg/kg 83 65 47 28 16 8
30 mg/kg 73 59 40 28 16 4

Figure 2

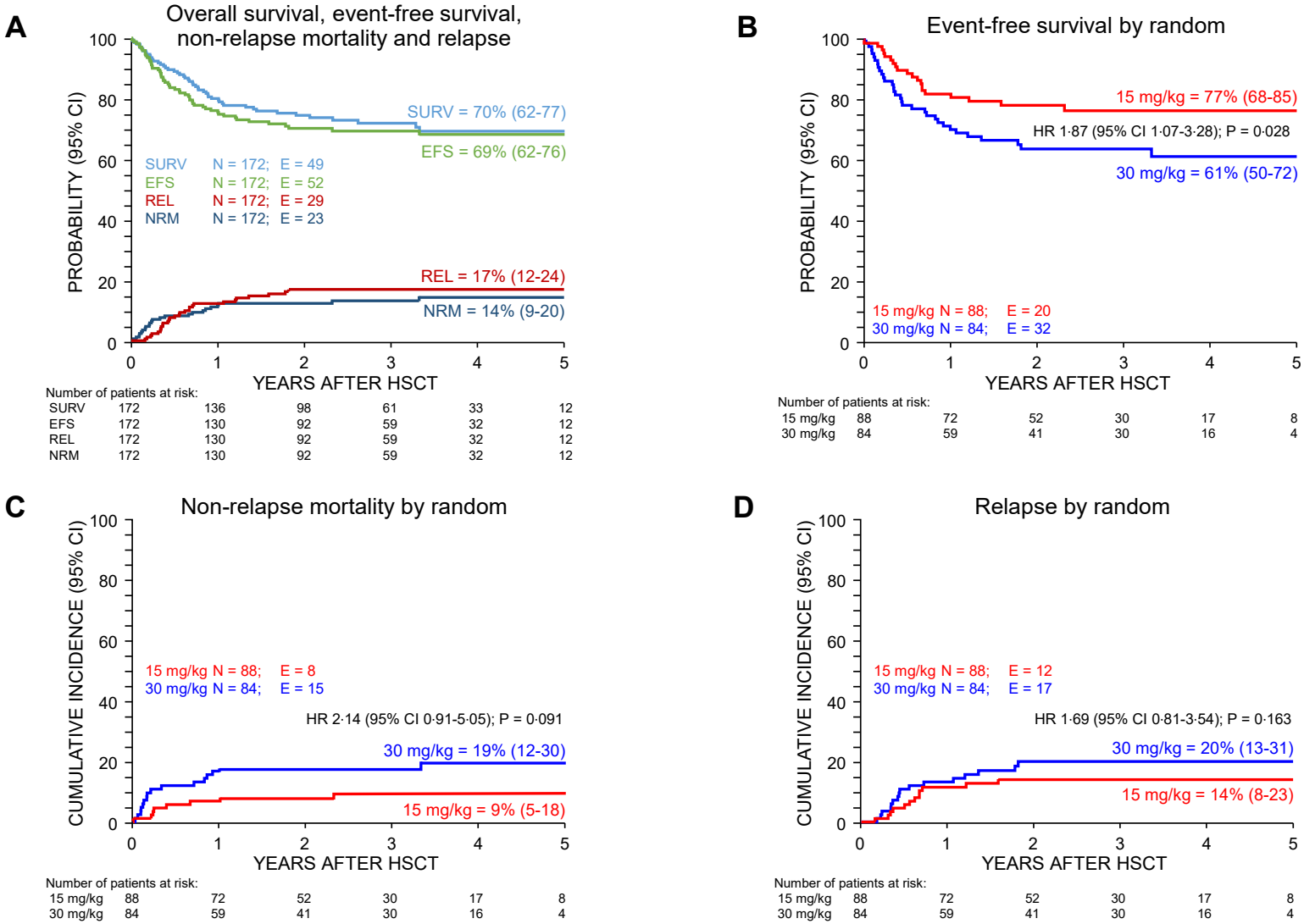
A
Overall survival, event-free survival, non-relapse mortality and relapse
PROBABILITY (95% CI)
YEARS AFTER HSCT
SURV = 70% (62-77)
EFS = 69% (62-76)
REL = 17% (12-24)
NRM = 14% (9-20)
SURV N = 172; E = 49
EFS N = 172; E = 52
REL N = 172; E = 29
NRM N = 172; E = 23
Number of patients at risk:
SURV 172 136 98 61 33 12
EFS 172 130 92 59 32 12
REL 172 130 92 59 32 12
NRM 172 130 92 59 32 12
B
Event-free survival by random
PROBABILITY (95% CI)
YEARS AFTER HSCT
15 mg/kg = 77% (68-85)
HR 1·87 (95% CI 1·07-3·28); P = 0·028
30 mg/kg = 61% (50-72)
15 mg/kg N = 88; E = 20
30 mg/kg N = 84; E = 32
Number of patients at risk:
15 mg/kg 88 72 52 30 17 8
30 mg/kg 84 59 41 30 16 4
C
Non-relapse mortality by random
CUMULATIVE INCIDENCE (95% CI)
YEARS AFTER HSCT
15 mg/kg N = 88; E = 8
30 mg/kg N = 84; E = 15
HR 2·14 (95% CI 0·91-5·05); P = 0·091
30 mg/kg = 19% (12-30)
15 mg/kg = 9% (5-18)
Number of patients at risk:
15 mg/kg 88 72 52 30 17 8
30 mg/kg 84 59 41 30 16 4
D
Relapse by random
CUMULATIVE INCIDENCE (95% CI)
YEARS AFTER HSCT
15 mg/kg N = 88; E = 12
30 mg/kg N = 84; E = 17
HR 1·69 (95% CI 0·81-3·54); P = 0·163
30 mg/kg = 20% (13-31)
15 mg/kg = 14% (8-23)
Number of patients at risk:
15 mg/kg 88 72 52 30 17 8
30 mg/kg 84 59 41 30 16 4

Figure 3

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