Individualized rabbit anti-thymocyte globulin dosing in adult haploidentical hematopoietic cell transplantation with high-risk hematologic malignancy: Exposure-response analysis and population pharmacokinetics simulations

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Abstract

Hematopoietic cell transplantation (HCT) for hematologic malignancies with non-remission disease and/or prior post-transplant relapse have poor relapse-free survival. We previously demonstrated the efficacy of haploidentical reduced-intensity HCT regimen with glucocorticoid-based graft-versus-host disease (GVHD) prophylaxis. We recently showed a possible association between rabbit antithymocyte globulin (rATG) exposure and acute GVHD (aGVHD) risk, leading to hypothesize that optimization of rATG exposure may further improve this regimen. We retrospectively examined the exposure-response association of rATG and key clinical outcomes post haploidentical HCT. We subsequently developed an individualized rATG dosing that optimizes rATG exposure using a previously developed population pharmacokinetic model.

Of the 103 patients analyzed, the median age was 47 years (range: 17–70) and majority had a non-remission disease prior to HCT (88%). rATG concentration on day 0 of HCT (C_{day_0}) was the strongest predictor of Grade 2-4 aGVHD through day +100. Patients with C_{day_0} ≥20 μg/mL had an approximately 3-fold lower risk of Grade 2-4 aGVHD (hazard ratio [HR]: 0.32, 95%confidence interval [CI]: 0.16, 0.62) and Grade 3-4 aGVHD (HR: 0.33, 95%CI: 0.16, 0.68) as well as an approximately 2-fold lower risk of overall mortality (HR: 0.47, 95%CI: 0.28, 0.77) and relapse (HR: 0.50, 95%CI: 0.26, 0.94).

In conclusion, this reduced-intensity haploidentical HCT regimen with exposure-optimized rATG may provide a promising option to patients undergoing high-risk HCT for hematologic malignancy. The developed rATG dosing warrant prospective validation.

Introduction

The risk of acute graft-versus-host disease (aGVHD) is historically higher in patients undergoing hematopoietic cell transplantation (HCT) from a human leucocyte antigen (HLA) haploidentical donor than from an HLA-matched donor[1-3]. Intensified GVHD prophylaxis regimens are commonly utilized to overcome this higher degree of immunological barrier. However, such regimens are double-edged swords and potentially impair beneficial graft-versus-leukemia (GVL) effects by donor T cells. The advent of post-transplant cyclophosphamide regimen expanded the safe utilization of haploidentical HCT[4], but relapse remains the leading cause of death in patients post HCT[5]. Such risk is particularly higher in patients with active disease prior to HCT[5].

Rabbit anti-thymocyte globulin (rATG), a rabbit-derived polyclonal antibody against human T cells, has been employed as an immunosuppressive agent for aGVHD prophylaxis in haploidentical HCT[6-9]. We have investigated a haploidentical HCT protocol using glucocorticoid and low-dose rATG as GVHD prophylaxis for hematological malignancy with a very high-risk disease profile (e.g., active disease, prior HCT failures)[10-12]. This regimen utilizes glucocorticoid to preferentially provide augmented GVL effects by donor T cells without increased risk of severe GVHD[13]. rATG doses are administered immediately prior to stem cell infusion as a secondary immunomodifier, and its exposure may have critical impacts on donor T cells to determine the GVL-GVHD balance.

rATG has large between-subject pharmacokinetic variabilities that lead to disparities in its efficacy-toxicity balances when one-size-fits-all dosing regimens are used (i.e., a uniform "mg/kg" dosing)[14,15]. Admiraal et al. described a narrow therapeutic window of rATG in HCT; namely, a higher exposure prior to day 0 of HCT decreases the risks of aGVHD and graft failure, while a higher exposure post day 0 delays T-cell immune reconstitution[14,15]. By leveraging the knowledge of population pharmacokinetics (PK), the same group derived individualized rATG dosing regimen and successfully improved timely immune reconstitution without compromising other key outcomes of HCT[16].

The present study was built upon our recent work on pharmacokinetic-pharmacodynamics of rATG in our reduced-intensity haploidentical HCT regimen. First, in a small preliminary cohort with 24 adult patients, we discovered a protective effect of higher day 0 total rATG concentration (C_{day_0}) against aGVHD[17]. Subsequently, we developed a novel population PK model to describe serum total rATG concentration in this population, measured by enzyme-linked immunosorbent assay (ELISA)[18]. This model identified that patient ideal body weight (IBW) was the only influential covariate that affects C_{day_0}. In the present study with a larger cohort, we aimed to further delineate the exposure-response associations of rATG, and thereby, to propose a new individualized dosing regimen to optimize rATG exposure through model-informed precision dosing approach.

Methods

Study settings

This retrospective study included the same patient population as the recent population PK study except for exclusion of 2 non-haploidentical HCT patients[18]. The present study included all patients, who underwent reduced-intensity HCT from haploidentical HCT donors in Hyogo Medical University Hospital in Hyogo, Japan between June 2014 and December 2019. This regimen was preferentially used in patients had active disease prior to HCT and/or a prior failure of HCT. All patients received T cell-repleted peripheral blood stem cells as the graft source, and rATG was administered as part of the conditioning regimen. Haploidentical HCT in this study included transplantation from a related donor with 1–3 mismatch out of 6 loci in *HLA* gene (i.e., HLA-A, HLA-B, and HLA-DR) in the GVH direction. All patients or their families provided written informed consent to participate in the study. The study protocol was approved by the Institutional Review Board of Hyogo Medical University.

Conditioning and GVHD prophylaxis regimen

The standard reduced-intensity conditioning regimen in this cohort consisted of fludarabine (30 mg/m^2/day, days -9 to -4), high-dose cytarabine (2.0 g/m^2/day, days -9 to -6), melphalan (70 mg/m^2/day, days -3 to -2), and total body irradiation (3 Gy). rATG was administered at a dose of 1.25 mg/kg/ day for 2 days (days -2 to -1) (thymoglobulin®, Sanofi Gen- zyme, the USA). Melphalan was replaced by busulfan (3.2 mg/kg/day, days -3 to -2) in those who had previous exposure to melphalan. High-dose cytarabine was omitted in patients with a low pre-HCT disease burden

and/or with a poor general condition. The standard initial GVHD prophylaxis included tacrolimus (target trough: 10–12 ng/mL) and methylprednisolone (1.0 mg/kg/day).

Pharmacokinetic analysis

Patient blood samples were collected on day 0 and once a week thereafter at weeks 1–5 after HCT, and serum rATG concentrations were quantified using the Thymoglobulin Assay Kit-IBL® (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan), an ELISA-based assay that measured total ATG in specimens[19]. Within-day and between-day precision were 3.5% and 3.2%, respectively. Properly diluted patient serum samples (100- to 3000-fold) were analyzed to obtain the measured concentration within the linear range of the assay. Subsequently, no samples were outside of the linear range of the assay.

Study outcomes

The primary outcome was the cumulative incidence of Grade 2–4 aGVHD through day +100. Grading and staging of aGVHD were based on the standard definitions[20]. Secondary outcomes were day +100 Grade 3–4 aGVHD, 2-year overall survival (OS), 2-year non-relapse mortality (NRM), 2-year relapse (including disease persistence and progression after HCT), 2-year relapse-free survival (RFS), and days to neutrophil engraftment. NRM was defined as death from any cause other than relapse. Relapse was treated as a competing risk for aGVHD and NRM. Death was treated as a competing risk for aGVHD and relapse.

Exposure-response model development

The effects of optimal rATG dose on the risk of day +100 Grade 2–4 aGVHD was analyzed in the following six steps. The below steps 1 to 4 treated the outcomes as dichotomous variables for the sake of better interpretability, while the steps 5 to 6 as time-to-event variables. The steps 1 to 4 describe the probabilities of an event over the index period without accounting for censoring events; and thus, the results for the outcomes up to day +100 are statistically correct (no censoring) while those up to day +730 should be interpreted as approximation (censored 10 of 103 patients). Of note, the difference in PK assays precluded evaluation of previously identified thresholds by Admiraal et al.[14,15], where only the active fraction of ATG ("arbitrary unit/mL") was measured by quantitative flow cytometry, while total ATG (μg/mL) was measured using ELISA in the present study[19,21].

(1) We evaluated the following three exposure variables of interest based on the previous reports: area-under-the-curve of rATG concentration-time from the start of first infusion to day 0 ($AUC_{pre\text{-}day\_0}$), C_{day_0}, and AUC post day 0. For the rATG exposure post HCT, we only evaluated AUC from day 0 to day +7 ($AUC_{day\_0\text{-}7}$) because nonnegligible fraction of aGVHD events occurred after this period (1 patient [2%] from day 0 through day +7, 7 [16%] through day +14, and 14 [33%] through day +21). We focused on the ATG exposure before the aGVHD onset to identify an actionable exposure target. These exposure variables in each individual were estimated by the previously developed, validated population PK model[18]. We analyzed the area under the receiver operating characteristics curves (ROC-AUCs) for these 3 exposure variables and compared their statistical superiority ($p<0.05$)[22]. We planned *a priori* to select C_{day_0} as the exposure variable of choice if there were no statistical differences. C_{day_0} has a clear logistical advantage over the other 2

exposure variables because it can be measured by a single sample, while the others require multiple sampling and estimation by a population PK model.

(2) Using the selected exposure variable, the optimal thresholds for clinical outcomes were identified according to the maximum Youden index (calculated as follows: sensitivity + specificity − 1).

(3) To further elucidate the exposure thresholds, we conducted classification tree analysis[23] to identify the best classification model to predict day +100 Grade 2–4 aGVHD. Specifically, this method served to explore multiple potential exposure thresholds (e.g., “U” or “inverted U” shape in the exposure-response relationship) or subgroup-specific exposure thresholds (e.g., higher exposure target for those with higher HLA mismatch). The developed trees were pruned to the smallest size within one standard error above the minimum cross-validated error to avoid overfitting.[23] The following predictor candidates were evaluated to determine the best predictive classifiers for each outcome: selected rATG exposure variable (above step #1), diagnosis (categorical: acute myeloid leukemia/myelodysplastic syndrome, acute lymphoblastic leukemia/lymphoblastic lymphoma vs. others), age (continuous), sex (categorical: male vs. female), number of previous HCT performance (continuous), complete remission prior to HCT (dichotomous: yes vs. no), number of HLA mismatch graft-versus-host direction (continuous), number of HLA-mismatch host-versus-graft direction (continuous), donor relation (categorical: first-degree vs. second- or higher degree relative), graft cell dose per patient body weight (total nucleated cell, $CD34^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$ [continuous for all]). Conditioning

regimens were tested as a dichotomous variable: the standard regimen (i.e., fludarabine, cytarabine, melphalan, rATG, and total body irradiation 3 Gy) vs. others.

(4) We further interrogated exposure-response relationships visually by local polynomial regression analysis by dichotomizing the outcomes (i.e., assuming no censoring events as approximation) and also by cumulative incidence analysis with quartile exposure subgroups.

(5) We performed univariate analysis to test the significance of a rATG exposure risk groups (identified in the above steps #2 and #3) on all clinical outcomes. Similarly, univariate analysis was performed to evaluate the association of the same clinical factors (i.e., the above step #3) with clinical outcomes as described. Time-to-event test was used for Grade 2–4 and 3–4 aGVHD, OS, NRM, relapse, and RFS (Cox proportional hazard for OS and RFS, Fine-Grey test for the others to account for competing risks). Linear regression testing was used for days to neutrophil engraftment. A p value <0.05 was considered statistically significant in all statistical analyses.

(6) Using significant exposure risk group-outcome pairs (identified in the above step #5), we performed multivariable analysis to identify the final models after adjusting for significant clinical covariates. Finally, the effects of exposure-based risk groups were visualized using cumulative incidence function plots.

Dose simulations

We performed dose-exposure simulations using the previously developed population PK model to derive individualized dosing regimens. Monte Carlo simulation was conducted to assess the probability to attain target exposure.

The simulation scenarios included an IBW range of 40–80 kg and a rATG dose range of 1.0–2.0 mg/kg of IBW/dose. The Devine formula was used to calculate IBW[24]. In all simulation scenarios, rATG was administered at the assigned dose over 6 hours daily on days −2 and −1 of HCT, and rATG PK was simulated 1000 times. In this simulation analysis, we assessed rATG dosing at each IBW that attains the target exposure at 80% of probability.

Software information

The following software was used in the analysis: NONMEM® version 7.5 (ICON Development Solutions, Ellicott City, MD, USA), Perl-speaks-NONMEM (Uppsala University, Uppsala, Sweden), and R 4.2.0. (R Core Team, Vienna, Australia). We used R packages *GGally* (correlation matrix), *pROC* (ROC), *rpart* (classification tree), *survival* (Fine-Grey test), *stats* (linear regression), and *cmprsk* (cumulative incidence plot).

Results

Study settings

Table 1 shows the characteristics of the present study cohort with 103 patients. The median follow-up of survivors was 1126 days (range, 132–2404 days). The most

common diagnosis was acute myeloid leukemia (52%). Majority of the patients were either not in remission prior to HCT (88%), and approximately half (49%) had a history of HCT failure prior to the index HCT procedure. A total of 44 patients (43%) had both non-remission pre-HCT status and a history of previous HCT failure. Nearly all (96%) received HCT from HLA 2–3 antigen-mismatched donors in the graft-versus-host direction. The combination of fludarabine, high-dose cytarabine, melphalan, rATG, and total body irradiation was the most commonly used conditioning regimen (79%). In all patients, tacrolimus and methylprednisolone were used as post-HCT pharmacological GVHD prophylaxis. The absolute lymphocyte count (ALC) before the first rATG infusion was negligible in all patients (median: 7 cells/μL, range: 0–58). The dose of rATG was 2.5 and 3.0 mg/kg by total body weight in 89%, and 11% of the patients, respectively. Most of the patients received rATG equally split on days −2 and −1 (76% with 2.5 mg/kg and 8% with 3 mg/kg), and the others on days −3, −2, and −1 (13% with 2.5 mg/kg and 3% with 3 mg/kg).

Study outcomes in the overall cohort

The primary and secondary outcomes of all patients are shown in **Table 2**. The cumulative incidence of day +100 Grade 2–4 aGVHD was 41.0% (95%confidence interval [CI]: 31.2, 50.1) including 42 patients (Grade 2, 3, and 4: 11, 27, and 4 patients, respectively). Of these 42, 8 (19%), 10 (24%), and 36 (86%) patients developed Grade ≥3 skin GVHD, Grade ≥1 liver GVHD, and Grade ≥1 gut GVHD, respectively. The median time to the onset of Grade 2–4 aGVHD through day +100 was 30 days (range, 4–96 days). Neutrophil engraftment was achieved in 101 patients (98%) at the median of 10 days (range, 8–14 days). In this cohort with high

2-year mortality (68%), relapse was the most common cause of death (24 patients [32%]). The other deaths were attributed to infection, GVHD, thrombotic microangiopathy or veno-occlusive disease, and other causes in 22 (29%), 5 (7%), 5 (7%), and 19 (25%) patients, respectively. The cumulative incidence of chronic GVHD was unable to calculate due to the low number of moderate/severe chronic GVHD events (n = 7, 7%).

Exposure-response model development

(1) The three tested exposure variables were highly correlated (**Supplemental Figure 1**), and there was no statistically significant superiority between these variables in predictability of day +100 Grade 2–4 aGVHD based on ROC-AUC ($p \geq 0.05$) (**Supplemental Figure 2**). Thus, we selected C_{day_0} as the exposure variable of choice in subsequent analyses as planned a priori because of its logistical advantage.

(2) The optimal C_{day_0} threshold for predicting day +100 Grade 2–4 aGVHD was ≥20 μg/mL according to the maximum Youden index (**Supplemental Figure 2**). The same C_{day_0} threshold was also identified for day +100 Grade 3–4 aGVHD and 2-year OS, whereas higher C_{day_0} thresholds were associated with 2-year NRM, relapse, and RFS (range: 26–31 μg/mL) (**Supplemental Figure 2**).

(3) The classification tree model also revealed the same, single threshold for the entire cohort (i.e., C_{day_0} ≥20 μg/mL) as the best predictor of day +100 Grade 2–4 aGVHD. There was no second C_{day_0} threshold or subgroup-specific C_{day_0}

thresholds. No other outcomes, except for day +100 Grade 2−4 aGVHD, retained any classifiers after accounting for cross-validated errors.

(4) Visual examination of the exposure-response associations corroborated the C_{day_0} ≥20 μg/mL as a predictor of day +100 Grade 2–4 and 3–4 aGVHD (**Figure 1**), and this threshold apparently dichotomized the probabilities of these outcomes into the high and low probability groups. Although the median probabilities of these two outcomes were apparently increasing at the highest end of C_{day_0}, the wide 95%CI range (grey zones in **Figure 1**) indicated a higher degree of uncertainty in these ranges. In fact, monodirectional inverse associations were demonstrated between C_{day_0} quartile group and day +100 Grade 2–4 or 3–4 aGVHD (**Supplemental Figure 3**). Although a clear trend between C_{day_0} and 2-year OS, relapse, or RFS was difficult to discern in the smooth regression (**Figure 1**), the median point (C_{day_0} ≥22.5 μg/mL) dichotomized the cohort into high vs. low risks of OS, relapse, and RFS in the quartile groups (**Supplemental Figure 3**). No apparent associations were observed for NRM or relapse.

(5) In univariate analysis, C_{day_0} risk group (<20 vs. ≥20 μg/mL) was significantly associated with day +100 Grade 2–4 and 3–4 aGVHD as well as 2-year OS and RFS (**Supplemental Table 1**). Several clinical factors were also significantly associated with outcomes: $CD3^+$ and $CD56^+$ cell doses for Grade 3–4 aGVHD, complete remission status and previous HCT for OS, none for NRM, patient age and past HCT for relapse, and complete remission status and past HCT for RFS.

(6) Finally, the cumulative incidences of outcomes are shown in **Figure 2**. After adjusting for significant clinical factors, patients with a C_{day_0} ≥20 μg/mL as compared to C_{day_0} <20 μg/mL had favorable risks of Grade 2–4 aGVHD (hazard ratio [HR]: 0.31, 95%CI: 0.16, 0.57) and Grade 3–4 aGVHD (HR: 0.33, 95%CI: 0.16, 0.68), OS (HR: 0.47, 95%CI: 0.28, 0.77), and RFS (HR: 0.62, 95%CI: 0.39, 0.99) (**Supplemental Table 2**).

Dose simulation

For the identified exposure variable, C_{day_0}, our previous report revealed that the only influential covariate was IBW because of its effect on the rATG volume of distribution.[18] Therefore, we examined the probability of target rATG attainment by rATG dosage based on IBW (i.e., mg/kg of IBW). The predicted distribution of C_{day_0} according to dose and IBW are shown in **Supplemental Figure 4**. The daily rATG dose that attained a target C_{day_0} of ≥20 μg/mL with 80% probability was 1.5 mg/kg of IBW per dose administered on days −2 and −1 of HCT (total 3.0 mg/kg of IBW). The distribution of the 20th percentile values are shown as a heatmap in **Supplemental Figure 5**, which confirms that 1.5 mg/kg of IBW being the optimal dosing across all range of IBW from 40–80 kg.

Discussion

The present study is a culmination of our work in PKPD of rATG in reduced-intensity haploidentical HCT in adult patients with particularly high-risk hematologic malignancy. This expanded exposure-response analysis further characterized the

beneficial, higher rATG C_{day_0} effects that we discovered in our previous study[17]. Furthermore, by leveraging our previous population PK model[18], we derived a novel rATG dosing regimen to individualize rATG dosing to improve clinical outcomes through optimization of rATG exposure. Specifically, a C_{day_0} of ≥20 μg/mL was associated with approximately a 3-fold risk reduction for both day +100 Grade 2–4 aGVHD and day +100 Grade 3–4 aGVHD. Importantly, no adverse exposure-response association was observed for relapse or NRM, implying absence of clinically significant interference of GVL effect or immune reconstitution. As a result, a C_{day_0} of ≥20 μg/mL resulted in approximately 2-fold risk reduction for both 2-year mortality and relapse in this high-risk HCT cohort with hematologic malignancy.

The initiatives led by Admiraal et al. successfully developed individualized rATG dosing regimen in non-haploidentical HCT settings by applying PKPD methodology over the past decade[14,15], leading to improved immune reconstitution in heterogenous pediatric HCT cohort as compared to one-size-fits-all dosing (75% vs. 51%, $p < 0.001$)[16]. The dose optimization scheme used in ours is similar to Admiraal's, but there are notable differences between these 2 bodies of PKPD work in rATG for HCT conditioning. First, our work was focused on a uniquely high-risk adult hematologic malignancy population, majority of which had an active disease and/or prior HCT failure and pursued this haploidentical HCT regimen as the last resort. Thus, the main clinical interest was to improve their dismal relapse-free survival. Second, likely reflecting the disparities in the studied HCT settings, our dosing regimen is a function of IBW alone, whereas the Admiraal's was of ALC and total body weight[14,15]. Lastly, Admiraal, et al. performed their PKPD study based on

active fraction of rATG measured by quantitative flow cytometry[21], while we used ELISA to measure total rATG including both active and inactive fractions. Subsequently, the findings generated by these 2 distinct bioassay methods may not be translatable to each other. Nonetheless, we internally validated our PK model, and the strong correlation between the optimal C_{day_0} threshold and clinical outcomes in our study support further investigations of our findings.

The present study has important clinical implications in the context of haploidentical HCT for high-risk hematologic malignancy. Among many successes that have enabled improved access and outcomes of HCT over the past decades, the advent of post-transplantation cyclophosphamide was revolutionary and opened the new horizon of safe haploidentical HCT. However, post-HCT relapse remains the main cause of HCT failure, and such risk is even higher in those who could not attain disease remission before HCT or relapsed after HCT. Over the past 2 decades, our group has explored the potential utility of reduced-intensity regimen in this very high-risk HCT population with the current combination of low-dose rATG and glucocorticoid. Glucocorticoid administration could modify T-cells distribution in the peripheral blood and promote GVL effect by migrating donor T cells into the bone marrow[13,25]. This regimen requires fine tuning of GVHD prophylaxis to optimize the GVL vs. GVHD balance. In fact, the patients with Grade 0–1 aGVHD had significantly better 2-year OS than those with Grade ≥2 aGVHD in our previous study (50% [95%CI: 22.9, 72.2] vs. 20.0% [95%CI: 3.1, 47.5], p = 0.031)[17]. The present study suggested that a higher exposure to rATG would independently improve aGVHD risk, OS, and relapse-free survival with comparable NRM. Although with a

limited sample size, OS in our optimal rATG exposure cohort (43% at 2 years [95%CI: 32, 57]) was apparently more favorable than the registry data at the Center for International Blood and Marrow Transplant Research in haploidentical HCT for AML without pre-HCT remission (28% at 3 years [95%CI: 25, 32])[5]. This encouraging data call for further investigation of our regimen in this high-risk HCT population.

The most intriguing clinical implication of the present study is the predicted superior outcomes by the new dosing regimen based on IBW. Given the limited number of trial candidates and the observed large outcome disparities by the C_{day_0} target attainment in the present study, a prospective validation of this new dosing method would better fit the scheme of a single-arm study with comparison to a historical cohort for rather than a conventional randomized-controlled study. The same approach was taken by Admiraal et al. to validate their rATG dosing regimen[16]. Correlative studies of interest include rATG PK (both active and total), immune reconstitution, and viral infection surveillance. Future studies should further delineate the target rATG exposures (e.g., higher end of threshold, subgroup-specific target). If a target exposure windrow is confirmed to be sufficiently narrow in relation to the between-subject PK variabilities, reactive dose optimization strategies (i.e., therapeutic drug monitoring) may be beneficial. For this purpose, an ELISA-based bioassay would likely be logistically more favorable than quantitative flowcytometry.

Limitations should be noted in the interpretation of the study findings. First, our population PK and exposure-response models have been tested only in the present study cohort at a single center. Because of the relatively small sample size, our

study may be limited in delineating either minor effects and/or rare events. It is also possible that other exposure measures (e.g., $AUC_{0\text{-infinity}}$, $AUC_{pre\text{-}day0}$, $AUC_{post\text{-}day0}$) may be more clinically influential in other settings. Second, we were unable to measure active rATG fraction, which by itself does not undermine the statistical associations observed in our study. However, comparative studies for active vs. total rATG would be of interest in future research (e.g., correlations to each other, superiority as a predictor of clinical outcomes). Moreover, we also did not have the data on T-cell reconstitution in our cohort. However, in our very high-risk HCT cohort, the main ATG toxicity is expected to be impaired GVL effects and protective immunity against pathogens. The former is represented by relapse in the present study. Although NRM partially represents the latter, compromised anti-viral immunity has the potential to confound the aGVHD risk, as GVHD prophylaxis is typically reduced in the presence of viral infections. Lastly, like any other model-based simulations, the predictions made in our study need proper external validations, which can be done as a single-arm prospective study as noted above.

The present study demonstrated yet another example of clinically impactful application of model-informed precision dosing, an increasingly utilized tool in the clinical field of HCT[26-28]. However, a substantive heterogeneity in HCT approaches in the field poses a hindrance because gained E-R knowledge is considered specific to the tested HCT setting (e.g., our rATG C_{day_0} target may not be applicable in different conditioning, GVHD prophylaxis, or disease cohort). An emerging novel approach to this conundrum is mechanistic mathematical PKPD modeling (i.e., quantitative systems pharmacology)[29,30]. This type of *in silico* HCT models can possibly identify

individualized HCT regimens based on robust simulations of various HCT scenarios and predicted outcomes.

In conclusion, we demonstrated that optimization of rATG exposure on C_0 may make our reduced-intensity haploidentical HCT regimen an attractive option in adult patients with high-risk hematological disease. Our serial work in PKPD of rATG enabled development of an individualized dosing regimen to optimize rATG exposure, which warrants future validation in a prospective study.

Authorship:

Contribution: M.T. and T.T. designed the study; T.T. designed the methodology and performed the computational analysis; M.T. wrote the original draft preparation; K.M. performed bioanalysis; M.J. reviewed computational analysis; M.T., K.K., H.T., K.I., and S.Y. acquired clinical sample; M.T. performed data curation; All authors reviewed and edited the draft.

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Table 1. Characteristics of study patients

Variables		n = 103
Demographic		
Male sex		67 (64%)
Age (years)		47 (17–70)
Weight (kg)		57.1 (37.8–84.8)
Ideal body weight (kg)		63.3 (41.0–77.9)
Absolute lymphocyte count, within 1 day before the first ATG dose (cells/μL)		7 (0–58)
HCT profiles		
Disease		
AML		54 (52%)
ALL/LBL		24 (23%)
MDS		5 (5%)
Malignant lymphoma		19 (18%)
Myelofibrosis		1 (1%)
Complete remission prior to HCT		12 (12%)
Prior HCT history		
None		53 (51%)
1		44 (43%)
≥2		6 (6%)
Donor		
First-degree relative		90 (87%)
Second-degree relative		3 (3%)
Third-degree relative		6 (6%)
Others		4 (4%)
HLA mismatch		
Graft-versus-host direction		
	1	4 (4%)
	2	45 (44%)
	3	54 (52%)
Host-versus-graft direction		
	1	7 (7%)
	2	37 (36%)
	3	59 (57%)
Conditioning regimen		
Flu/Ara-C/Mel/ATG/TBI (3 Gy)		81 (79%)

Flu/Ara-C/CY/ATG/TBI (8 Gy)	7 (7%)
Flu/Ara-C/BU/ATG/TBI (3 Gy)	3 (3%)
Flu/Mel/ATG/TBI (3 Gy)	9 (9%)
Flu/CY/ATG/TBI (3 Gy)	1 (1%)
Flu/BU/ATG/TBI (3 Gy)	2 (2%)
Stem cell dose*	
Total nucleated cell (x10^8/kg)	8.0 (1.8–19.6)
CD34+ (x10^6/kg)	5.4 (2.2–14.9)
CD3+ (x$10^{8/}$kg)	2.1 (0.4–5.7)
CD4+ (x10^8/kg)	1.3 (0.2–4.6)
CD56+ (x10^8/kg)	0.2 (0.04–1.2)

*Cell dose is calculated based on kg of recipient total body weight.

Abbreviations: IgG, immunoglobulin G; HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndromes; CR, complete remission or response; HLA, human leukocyte antigen; Ara-C, high-dose cytarabine; Flu, fludarabine; Mel, melphalan; rATG, rabbit anti-thymocyte globulin; TBI, total body irradiation; CY, cyclophosphamide; BU, busulfan; aGVHD, acute graft-versus-host disease; mPSL, methylprednisolone

Table 2. Clinical outcomes of all patients (n = 103)

Clinical outcomes	Event (%)	Cumulative incidence (95%CI)
Grade 2–4 aGVHD, day +100	42 (41%)	41.0% (31.2, 50.1)
Grade 3–4 aGVHD, day +100	31 (30%)	30.1% (21.5, 39.1)
Death, +2 year	66 (64%)	67.2% (56.7, 75.7)
Relapse or death, +2 year	75 (73%)	72.8% (63.0, 80.4)
NRM, +2 year	34 (33%)	33.0% (24.1, 42.2)
Relapse, +2 year	41 (40%)	40.0% (30.3, 49.2)
cGVHD moderate-severe, +2 year	7 (7%)	5.8% (2.4, 11.6)

Abbreviations: aGVHD, acute graft-versus-host disease; NRM, non-relapse mortality;

cGVHD, chronic graft-versus-host disease

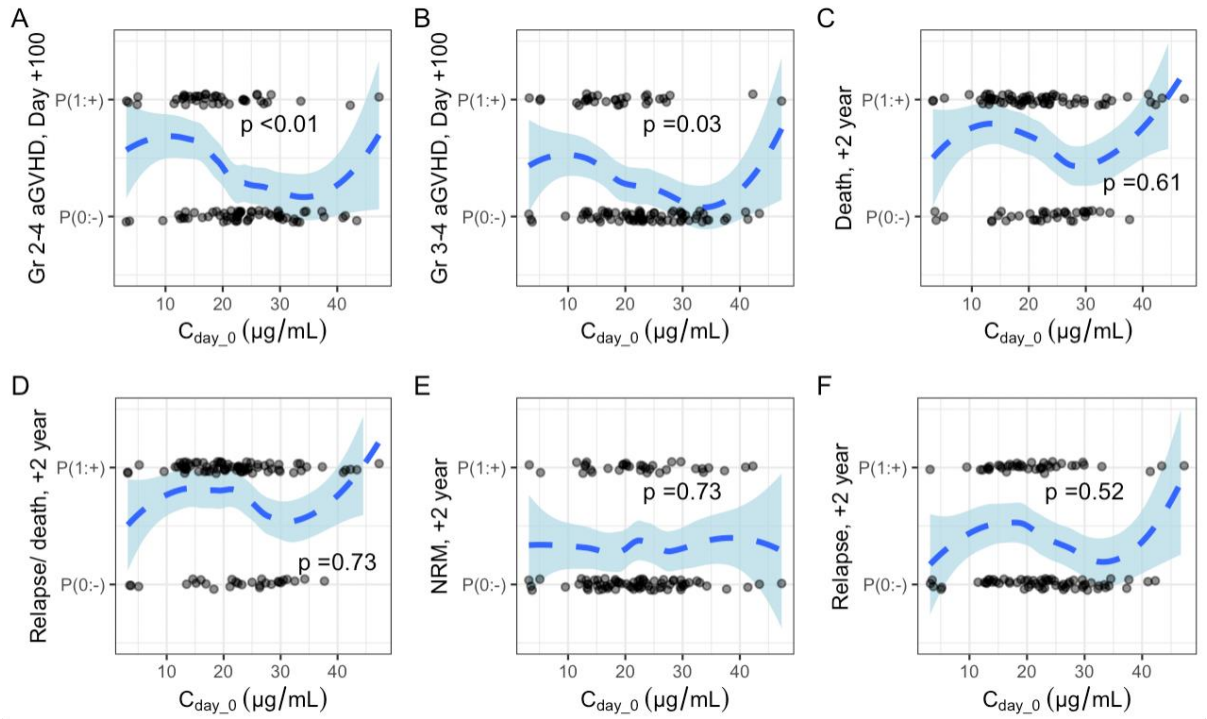


Figure 1. Exposure-response relations by local polynomial regression

Note: The dotted lines represent the typical trend by local polynomial regression (i.e., Loess smooth regression). The shaded area is 95% confidence interval for the typical value. Each dot represents a single patient with associated C_{day_0} and the probability of the event (0:-, event absent, 1:+, event present).

The median probabilities of aGVHD outcomes were apparently increasing at the highest end of C_{day_0}, the wide 95%CI range (grey zones) indicate a higher degree of uncertainty in these ranges.

Abbreviations: aGVHD = acute graft-versus-host disease, C_{day_0} = rATG concentration on day 0 of hematopoietic cell transplantation, NRM = non-relapse mortality, RFS = relapse-free survival

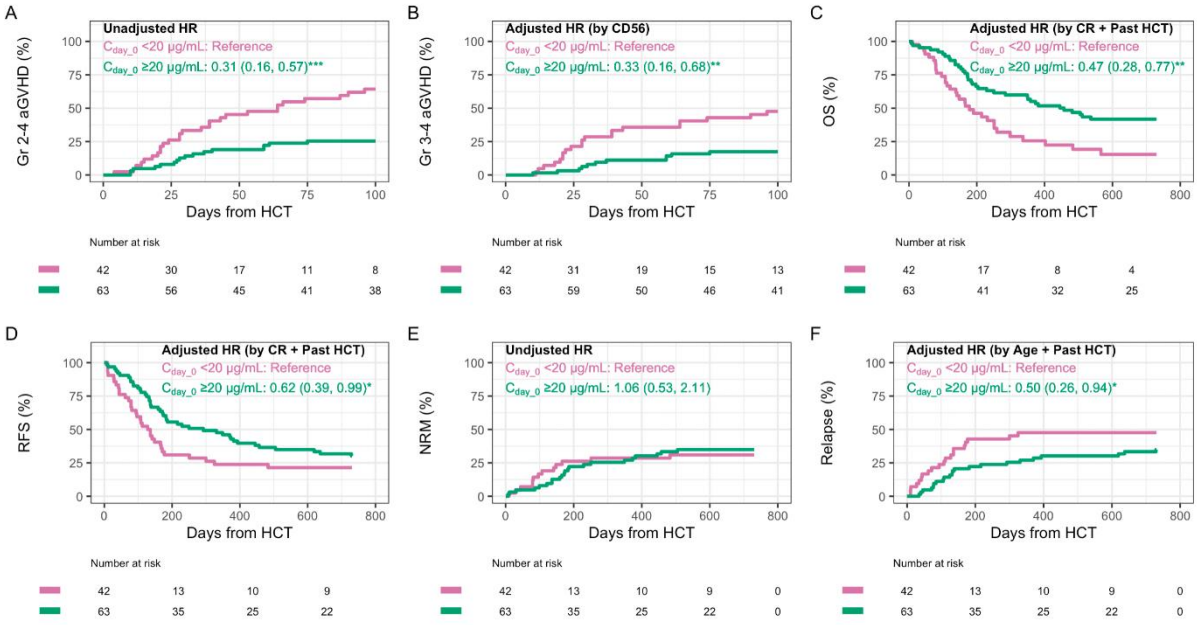


Figure 2. Cumulative incidence of clinical outcomes by Cday-0 risk groups

Note: Cumulative Incidences are depicted for (A) Grade 2–4 aGVHD, (B) Grade 3–4 aGVHD, (C) OS, (D) RFS, (E) NRM, and (F) relapse/progression. The study cohort is subgrouped based on the C_{day_0} threshold (< or ≥ 20 µg/mL). Hazard ratio are adjusted when significant clinical factors were identified for each outcome.

Cumulative incidence estimates:

A) Gr 2–4 aGVHD through Day +100 (C_{day_0} <20 µg/mL: 64.3% [95%CI: 47.4, 77.0]), C_{day_0} ≥20 µg/mL: 24.6% [95%CI: 14.6, 36.0],

B) Gr 3–4 aGVHD through Day +100 (C_{day_0} <20 µg/mL: 47.6% [95%CI: 31.7, 61.9], C_{day_0} ≥20 µg/mL: 18.0% [95%CI: 9.6, 28.7]),

C) 2-year OS (C_{day_0} <20 µg/mL: 15.4% [95%CI: 6.2, 35.0], C_{day_0} ≥20 µg/mL: 43.2% [95%CI: 31.6, 56.9]),

D) 2-year RFS (C_{day_0} <20 µg/mL: 21.4% [95%CI: 11.5, 37.8], C_{day_0} ≥20 µg/mL: 31.1% [95%CI: 21.0, 44.6]),

E) 2-year NRM (C_{day_0} <20 μg/mL: 31.0% [95%CI: 17.6, 45.4], C_{day_0} ≥20 μg/mL: 34.4% [95%CI: 22.7, 46.4]),

F) 2-year relapse (C_{day_0} <20 μg/mL: 47.6% [95%CI: 31.7, 61.9], C_{day_0} ≥20 μg/mL: 34.4% [95%CI: 22.7, 46.4])

Abbreviations: C_{day_0} = total rATG concentration on day 0 of HCT (μg/mL), HCT = hematopoietic cell transplantation, aGVHD = acute graft-versus-host disease, NRM = non-relapse mortality, OS = overall survival, RFS = relapse-free survival

Individualized rabbit anti-thymocyte globulin dosing in adult haploidentical hematopoietic cell transplantation with high-risk hematologic malignancy: Exposure-response analysis and population pharmacokinetics simulations

Masahiro Teramoto, Takuto Takahashi, Kana Matsumoto, Mutaz Jaber, Katsuji Kaida, Hiroya Tamaki, Kazuhiro Ikegame, Satoshi Yoshihara

Supplemental File

Supplemental Table 1. Univariate analysis on clinical outcomes by C_{day_0} ≥ 20 μg/mL and clinical factors

	C_{day_0} ≥20 μg/mL	Clinical factors			
Outcome	P value	Variables	N	Groups	P value
Gr 2-4 aGVHD, day +100	<0.001	.	.	.	.
Gr 3-4 aGVHD, day +100	<0.01	CD56 dose*	103	.	0.03
Overall survival, +2 year	<0.01	CR	91	Yes	0.02
			12	No	
		Past HCT	55	None	.
			44	Once	0.52
			6	Twice or more	0.046
Relapse-free-survival, +2 year	0.03	CR	91	Yes	<0.01
			12	No	
		Past HCT	55	None	.
			44	Once	0.64
			6	Twice or more	0.02
Non-relapse mortality, +2 year	0.92	.	.	.	
Relapse, +2 year	0.09	Age	103	.	0.04
		Past HCT	55	None	.
			44	Once	0.34
			6	Twice or more	0.04
Neutrophil engraftment	0.24	.	.	.	

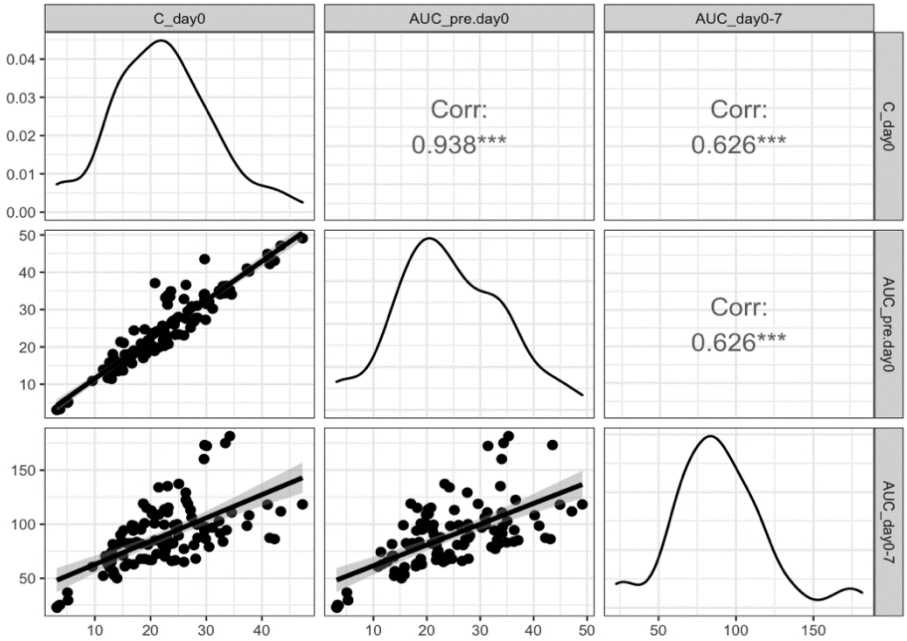
*CD56+ cell dose in the graft ($x10^8$/kg)

Abbreviations: C_{day_0}, total rabbit anti-thymocyte globulin serum concentration on day 0 of hematopoietic cell transplantation; aGVHD, acute graft-versus-host disease; OS, overall survival; CR, complete remission prior to HCT; NRM, non-relapse mortality; RFS, relapse-free survival; HCT, hematopoietic cell transplantation

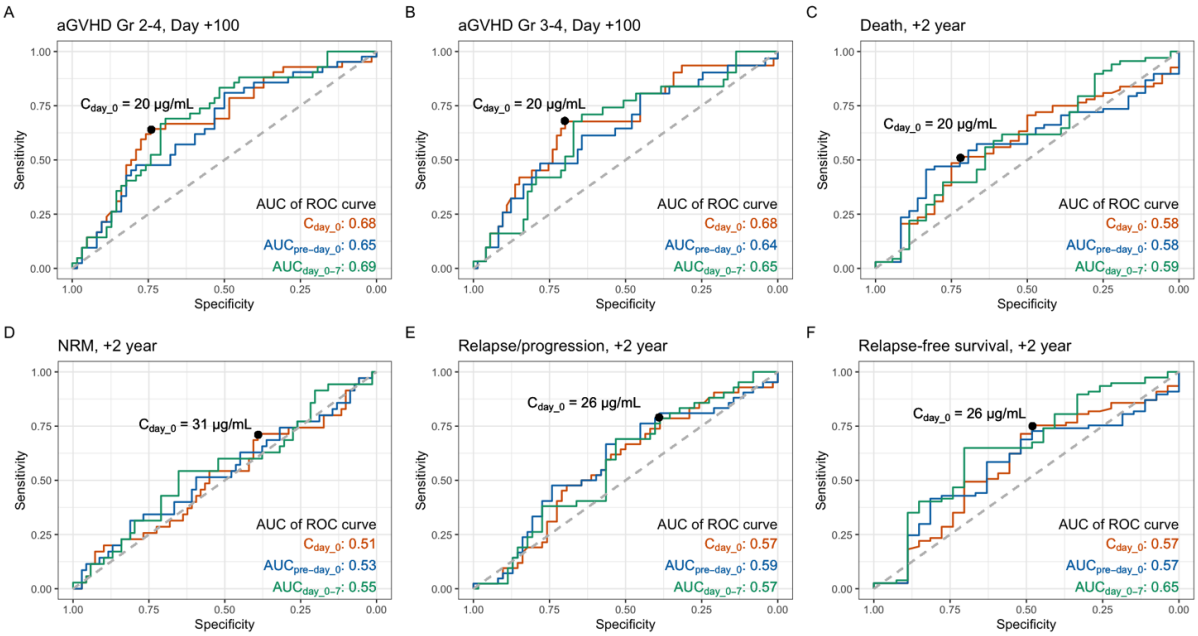
Supplemental Table 2. Multivariable analysis of clinical outcomes by rabbit ATG C_{day_0} and clinical factors

Outcome	Variables	N	Groups	HR (95%CI)	P value
Gr 2-4 aGVHD, day +100	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	0.31 (0.16, 0.57)	<0.001
Gr 3-4 aGVHD, day +100	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	0.33 (0.16, 0.68)	<0.01
	CD56 dose*	103	N/A (continuous)	4.62 (0.90, 23.9)	0.07
Overall survival, +2 year	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	0.47 (0.28, 0.77)	<0.01
	CR	91	Yes	Reference	.
		12	No	0.36 (0.13, 1.01)	0.05
	Past HCT	55	None	Reference	.
		44	Once	1.24 (0.75, 2.01)	0.4
		6	Twice or more	2.83 (1.16, 6.89)	0.02
Relapse-free-survival, +2 year	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	0.62 (0.39, 0.99)	0.047
	CR	91	Yes	Reference	.
		12	No	0.28 (0.10, 0.78)	0.01
	Past HCT	55	None	Reference	.
		44	Once	1.20 (0.74, 1.93)	0.46
		6	Twice or more	2.99 (1.23, 7.31)	0.02
Non-relapse mortality, +2 year	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	1.06 (0.53, 2.11)	0.88
Relapse, +2 year	rATG C_{day_0}	42	<20 µg/mL	Reference	.
		61	≥20 µg/mL	0.50 (0.26, 0.94)	0.03
	Age	103	.	0.97 (0.95, 0.996)	0.02
	Past HCT	55	None	Reference	.
		44	Once	0.89 (0.42, 1.90)	0.76
		6	Twice or more	2.44 (0.81, 7.31)	0.11

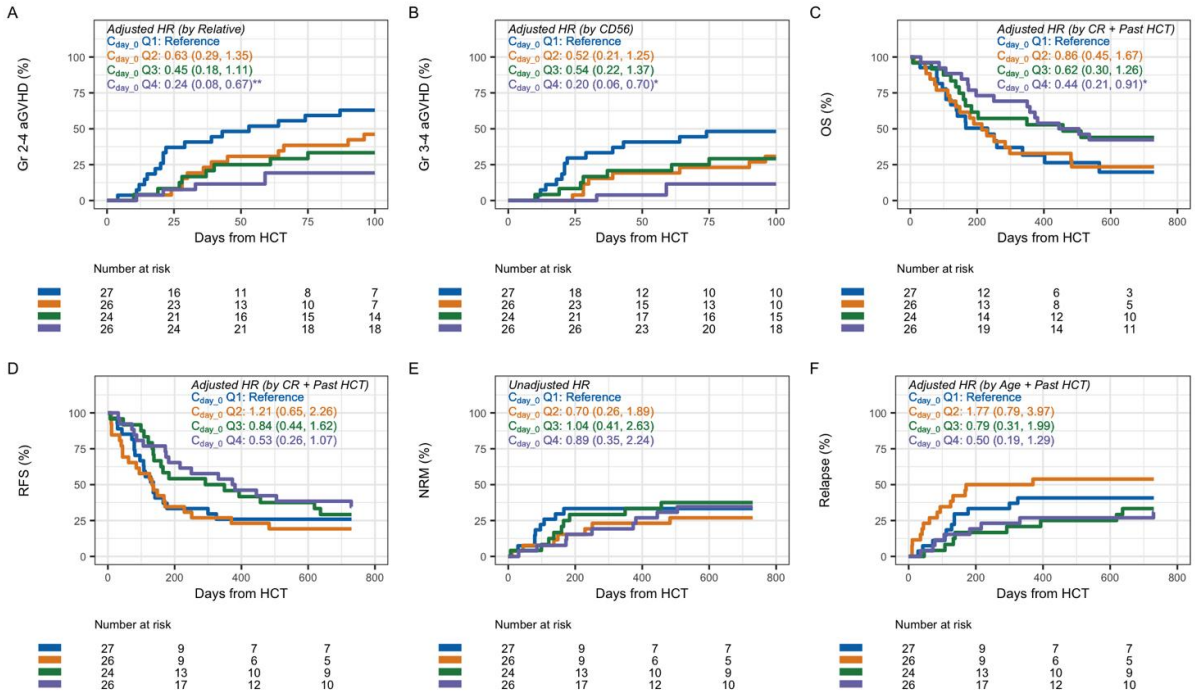
*CD56+ cell dose in the graft (x10^8/kg)
Abbreviations: C_{day_0} = concentration at day 0 of HCT, CR = complete remission prior to HCT, aGVHD = acute graft-versus-host disease, Gr = Grade, HCT = hematopoietic cell transplantation, Ref: reference group



Supplemental Figure 1. Correlation matrix of rATG exposure measures
Shown are correlation characteristics among C_{day_0}, $AUC_{pre\text{-}day\_0}$, and $AUC_{day\_0\text{-}7}$. The diagonal grids show the density plots. Scatter plots are shown in the left lower with linear regression, and correlation coefficients are shown in the right upper grids (*** indicates p <0.001 by Pearson test). All of the 3 variables show high correlation between each other. Abbreviations: C_{day_0}, total rabbit anti-thymocyte globulin concentration on day 0 of hematopoietic cell transplantation (μg/mL); AUC, area under the receiver operating characteristic curve (i.e., cumulative exposure) of rabbit anti-thymocyte globulin; $AUC_{pre\text{-}day\_0}$, AUC from the start of the first infusion until day 0 of hematopoietic cell transplantation (μg·hr/mL); $AUC_{day\_0–7}$, AUC from day 0 of hematopoietic cell transplantation until infinity



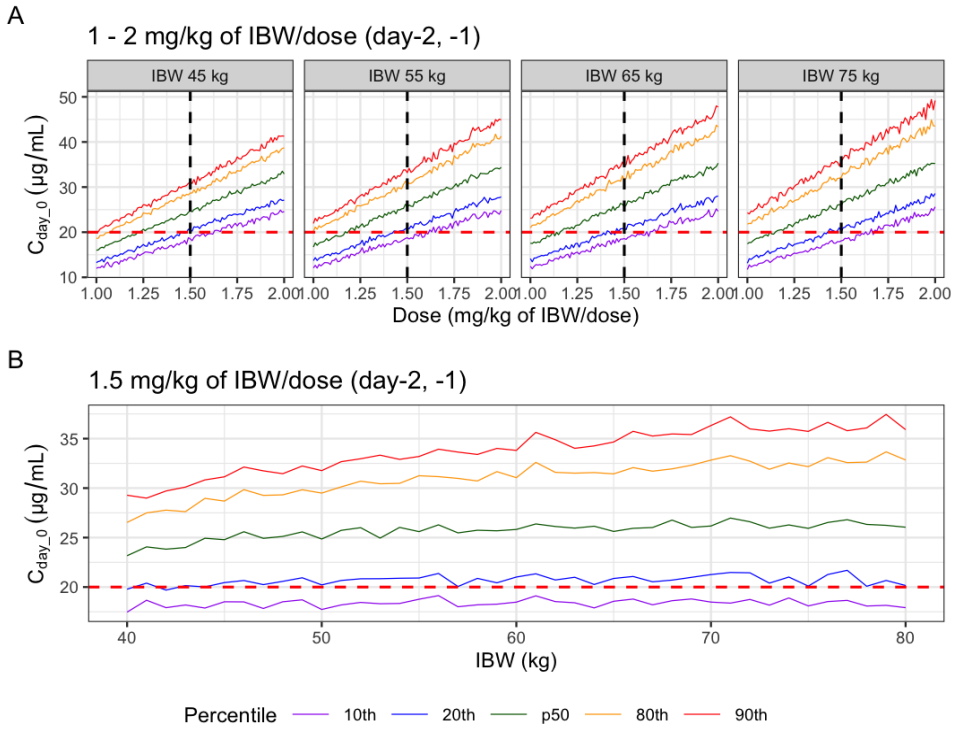
Supplemental Figure 2. Receiver operator characteristics of exposure variables
The best threshold is indicated by the black dot, calculated by the maximum Youden index. Abbreviations: aGVHD Gr 2–4, acute graft-versus-host disease Grade 2–4; C_{day_0}, rATG concentration on day 0 of hematopoietic cell transplantation; AUC_{day_0-7}, rATG area-under-the-curve from day 0 until day 7; $AUC_{pre-day\_0}$, rATG area-under-the-curve from the start of infusion until day 0; NRM, nonrelapse mortality



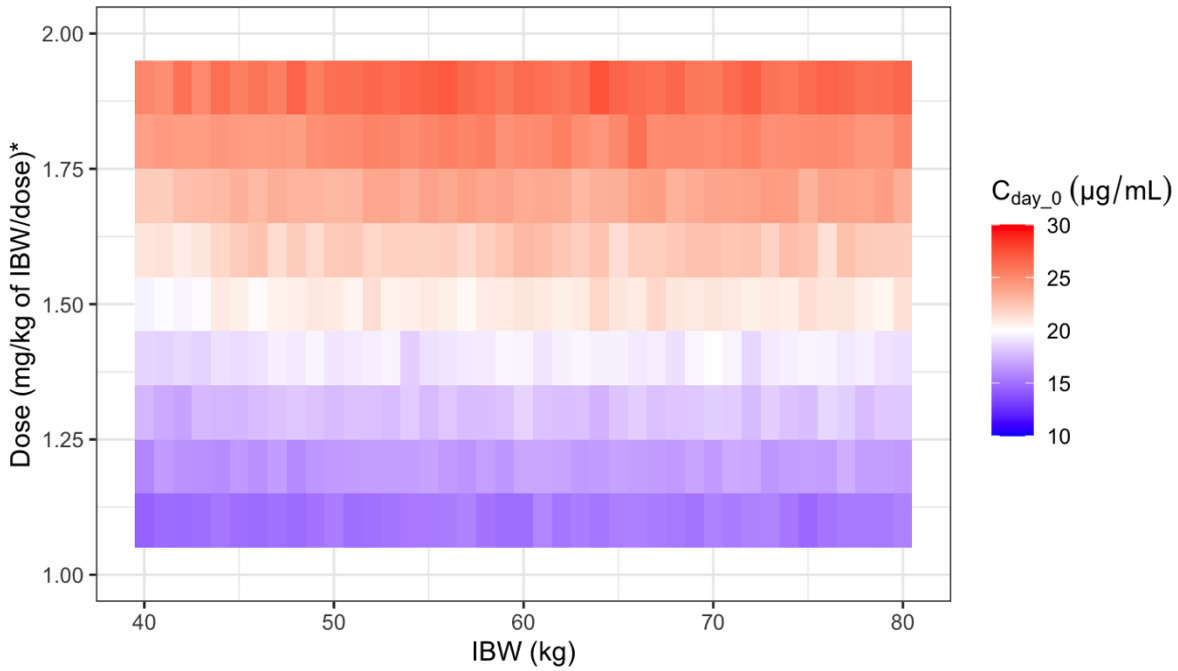
Supplemental Figure 3. Cumulative incidence of clinical outcomes in C_{day_0} quartile groups

Cumulative Incidences are depicted for (A) grade 2–4 aGVHD, (B) grade 3–4 aGVHD, (C) OS, (D) RFS, (E) NRM, and (F) relapse/progression. The study cohort is subgrouped based on the quartile C_0 groups (Q1: 3.2–16.0 µg/mL, Q2: 16.1–22.4 µg/mL, Q3: 22.5–27.5 µg/mL, Q4: 27.6–47.3 µg/mL). Hazard ratios are adjusted when significant clinical factors were identified for each outcome.

Abbreviations: C_0, total rabbit anti-thymocyte globulin concentration on day 0 of hematopoietic cell transplantation (µg/mL); HCT, hematopoietic cell transplantation; aGVHD, acute graft-versus-host disease; OS, overall survival; RFS, relapse-free survival; NRM, non-relapse mortality



Supplemental Figure 4. Simulated distribution of C_{day_0} by rATG dose and IBW
The distributions of C_{day_0} were simulated in 1000 virtual scenarios for each dose-IBW setting after two doses of rATG at the specified dosing (mg/kg of IBW per dose) on days −2 and −1 of HCT. The representative percentile values are shown in lines. (A) C_{day_0} by dosing for 4 IBW tiers (45, 55, 65, and 75 kg). The dosing of 1.5 mg/kg of IBW per dose attains 20 µg/mL by approximately 20th percentile of the population (i.e., at least 80% probability of target attainment). (B) The distribution of C_{day_0} when 1.5 mg/kg IBW per dose is given to a range of IBW. This plot confirms the attainment of 20 µg/mL over a continuous IBW range from 40–80 kg.
Abbreviations: C_{day_0}, total rabbit anti-thymocyte globulin concentration on day 0 of hematopoietic cell transplantation; HCT, hematopoietic cell transplantation; IBW, ideal body weight; rATG, rabbit anti-thymocyte globulin



Supplemental Figure 5. C_{day_0} values at the 20th percentile of the simulated distribution
The distributions of C_{day_0} are simulated in 1000 virtual patients in each dose-IBW setting after 2 doses of rATG at the specified dose (mg/kg of IBW/dose) on day –2 and –1 of HCT. The 20th percentile value in the 1000 simulated C_{day_0} values in each setting is shown, where these 20th percentile values represent the exposure levels attained at an 80% probability. The graph indicates that the target C_{day_0} concentration of 20 µg/mL is attained at 80% probability by 1.5 mg/kg of IBW dose across the IBW range.
*The y-axis represents the ATG dose in mg/kg of IBW per dose. The C_{day_0} was simulated after 2 ATG infusions at the same dose on days -2 and -1.
Abbreviations: C_{day_0}, total rabbit anti-thymocyte globulin concentration on day 0 of hematopoietic cell transplantation (µg/mL); HCT, hematopoietic cell transplantation; IBW, ideal body weight; rATG, rabbit anti-thymocyte globulin