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CD25-based graft-*versus*-host disease prophylaxis after haploidentical hematopoietic stem cell transplantation: comparison of two prophylactic strategies

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WS, LHX, XNC, and YC conceived and designed the study. QLL, AZ, HRC, HY,

LLL, YXY, and MRW collected and curated the data. QLL, YXY, and MRW performed the formal statistical analyses. QLL, AZ, and HRC conducted the investigation. QLL and XYS drafted the original manuscript. WS, LHX, XNC, and YC critically revised and edited the manuscript and supervised the study. All authors reviewed and approved the final version of the manuscript.

Running Head:

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Interested collaborators may direct data requests to the corresponding author.

Trial Registration:

This study was registered in the Chinese Clinical Trial Registry (ChiCTR2200060184).

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Abstract

Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is widely used for hematologic malignancies, yet balancing graft-versus-host disease (GVHD) prophylaxis and immune reconstitution remains challenging. In this prospective, non-randomized study, we compared a higher-dose anti-CD25 strategy that omitted methotrexate with a conventional lower-dose anti-CD25 regimen that included methotrexate. Lower cumulative anti-thymocyte globulin exposure was observed in the higher-dose group. From May 2022 to January 2025, 276 patients were enrolled (lower-dose group, $n=149$; higher-dose group, $n=127$). After a median follow-up of 28.4 months, the higher-dose strategy was associated with a lower 100-day cumulative incidence of grade III–IV acute GVHD (7.9% vs. 17.5%; adjusted subdistribution hazard ratio, 0.39; 95% confidence interval, 0.18–0.88; $p=0.022$) and improved graft-versus-host disease-free, relapse-free survival (GRFS) (adjusted hazard ratio, 0.59; 95% confidence interval, 0.43–0.83; $p=0.002$). Chronic GVHD, overall survival, disease-free survival, relapse, and non-relapse mortality did not differ significantly between groups. Exploratory immune monitoring showed transiently lower $CD4^+$ T-cell counts at day +30 in the higher-dose group, whereas longitudinal immune reconstitution trajectories and infectious complications were broadly comparable. These findings suggest that a higher-dose anti-CD25, methotrexate-free prophylactic approach may reduce acute GVHD and improve GRFS after haplo-HSCT, while its effects on long-term disease control and survival require confirmation in multicenter randomized trials. Trial registration: Chinese Clinical Trial Registry, ChiCTR2200060184.

Introduction

Haploidentical hematopoietic stem cell transplantation (haplo-HSCT) has evolved into a predominant approach for allogeneic hematopoietic stem cell transplantation in China,¹ yielding clinical outcomes comparable to those of HLA-matched cohorts.²⁻⁷ A critical challenge in this setting remains preserving graft-versus-leukemia (GVL) effects to prevent relapse while minimizing graft-versus-host disease (GVHD).⁸⁻¹⁰ Interleukin-2 receptor α -chain (CD25) has been identified as a critical therapeutic target.¹¹ CD25 blockade has been shown to selectively deplete activated T cells, thereby mitigating GVHD. In addition, it may play a role in modulating regulatory T-cell function within the immune microenvironment.¹²⁻¹⁶

Although optimizing immune reconstitution is critical for minimizing the risk of infection and preventing relapse,¹⁷⁻²⁰ the immunological consequences of intensified graft-versus-host disease (GVHD) prophylaxis remain poorly understood. Previous evidence suggests that a higher-dose anti-CD25 monoclonal antibody regimen is superior to a lower-dose strategy in preventing GVHD.^{21,22} Beyond GVHD control, the higher-dose strategy has also demonstrated statistically significant benefits in enhancing engraftment and mitigating treatment-induced mucosal damage, thereby lowering the incidence of oral mucositis (OM) and infections.²³⁻²⁵ However, it is unclear whether this intensified suppression of interleukin-2 signaling may compromise lymphocyte subset recovery.²⁶

To address this gap, we prospectively compared lower- and higher-dose anti-CD25

prophylactic strategies in patients undergoing haplo-HSCT. This study aimed to characterize differences in immune reconstitution between the two strategies and determine their associations with post-transplant clinical outcomes.

Methods

Study design and participants

We performed a prospective, single-center, non-randomized comparative cohort study of 276 patients with hematologic malignancies who received haplo-HSCT at Union Hospital (Wuhan, China) between May 2022 and January 2025. All participants provided written informed consent. The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval No. [2021] IEC (0723-01); Date of Approval: May 9, 2022).

Transplantation procedures

Stem cell harvesting and processing followed previously described methods.²¹ Conditioning regimens included myeloablative conditioning (MAC), reduced-intensity conditioning (RIC), and non-myeloablative regimens. GVHD prophylaxis comprised rabbit anti-thymocyte globulin (rATG), mycophenolate mofetil (MMF), and tacrolimus. Both prophylactic strategies were clinically available at our center during the study period. After the treating physician explained the composition and potential benefits and risks of the two complete prophylactic strategies, treatment assignment was primarily based on the patient's preference between these strategies rather than on selection of the

anti-CD25 dose in isolation. No prespecified patient-, donor-, disease-, or transplantation-related algorithm was used to determine treatment assignment. The lower-dose group received anti-CD25 monoclonal antibody 25 mg on days 0 and +4 with short-term methotrexate (MTX), whereas the higher-dose group received 50 mg on days 0 and +4 without MTX. No post-transplant cyclophosphamide was administered. The two prophylactic strategies are summarized in Figure 1. Full dosing details are provided in Supplementary Methods S1.

Endpoints and definitions

The primary endpoint was the cumulative incidence of acute GVHD (aGVHD). Secondary endpoints included chronic GVHD (cGVHD), cumulative incidence of relapse (CIR), non-relapse mortality (NRM), overall survival (OS), disease-free survival (DFS), and GVHD-free relapse-free survival (GRFS). GVHD diagnosis and grading,^{27,28} and survival/risk definitions^{21,29,30} followed standard consensus guidelines.

An exploratory endpoint was immune reconstitution, assessed in an exploratory subcohort (n = 177) with sufficient longitudinal immune-monitoring data. Detailed eligibility criteria for the immune-reconstitution cohort are provided in Supplementary Methods S3.3 and Figure S3. Recovery thresholds were: CD3⁺ >955/ μ L, CD3⁺CD4⁺ >550/ μ L, CD3⁺CD8⁺ >320/ μ L, CD19⁺ >90/ μ L, and natural killer (NK) cells >150/ μ L. Supportive care followed routine institutional guidelines (Supplementary Methods S2).

Statistical analysis

Categorical and continuous variables were compared using χ^2 /Fisher's exact test

and the Mann–Whitney U test. Kaplan–Meier analysis with the log-rank test and cumulative incidence functions with Gray’s test were used to evaluate survival and competing-risk outcomes, respectively. Potential temporal bias was assessed by comparing treatment allocation across transplantation years using Fisher's exact test. In addition, transplantation year was incorporated into sensitivity analyses of the primary study outcomes. Inverse probability of treatment weighting (IPTW) based on propensity scores estimated from a multivariable logistic regression model was applied. The propensity score model included all prespecified baseline characteristics. Covariate balance was assessed using Love plots and absolute standardized mean differences (SMD; threshold <0.1); covariates retaining SMD >0.1 after weighting were included as adjusters for doubly robust estimation. Weighted Cox and Fine–Gray models with robust variance estimators were applied. Extended IPTW diagnostics are provided in Supplementary Methods S3.1. Median follow-up duration was estimated using the reverse Kaplan–Meier method. A two-sided $p < 0.05$ was considered significant. Analyses used SPSS 26.0 and R 4.5.1.

Results

Patient characteristics

The study cohort comprised 276 patients (156 males and 120 females) with a median age of 35 years (range, 14–59 years). The patients were divided into the higher-dose group ($n=127$, 46.0%) and the lower-dose group ($n=149$, 54.0%). A summary of the primary clinical characteristics of the two groups is presented in Table 1.

Detailed baseline characteristics before and after IPTW adjustment are shown in

Supplementary Table S1. As shown in Supplementary Table S1 and visualized in the Love plot (Figure S1), IPTW adjustment successfully balanced the majority of baseline covariates. However, five variables (diagnosis, human leukocyte antigen [HLA] matching, donor/recipient cytomegalovirus [CMV] status, donor/recipient Epstein–Barr virus [EBV] status, and conditioning regimen) showed residual imbalances ($0.1 < \text{SMD} < 0.2$) and were thus included as adjustors in the subsequent multivariable models.

Both treatment strategies were used concurrently throughout the study period, and no significant association was observed between transplantation year and treatment assignment (Table S5, $p = 0.996$). Furthermore, additional adjustment for transplantation year in the doubly robust IPTW models did not materially alter the treatment-effect estimates for the major clinical endpoints examined (Table S6). These results support the robustness of the primary findings to potential calendar-time bias.

Clinical Outcomes

The cumulative-incidence curves showed significantly lower incidences of aGVHD in the higher-dose group across all severity grades (Figure 2A–C). For Grade I–IV aGVHD, the 100-day cumulative incidence was 27.6% in the higher-dose group, compared to 40.3% in the lower-dose group ($p = 0.026$) with an adjusted subdistribution hazard ratio (aSHR) of 0.72 (95% CI, 0.44–1.16, $p = 0.175$; Table 2). The higher-dose group also showed a significant reduction in the incidence of Grade II–IV aGVHD (15.0% vs. 29.5%, $p = 0.004$; aSHR 0.45, 95% CI, 0.25–0.84, $p = 0.011$). Most notably, the risk of Grade III–IV aGVHD was markedly lower in the higher-dose group (7.9% vs. 17.5%, $p = 0.017$). This finding was confirmed using the multivariable model (aSHR

0.39, 95% CI, 0.18–0.88, $p=0.022$). In contrast, no significant differences in the cumulative incidence of cGVHD at any severity level were observed between the two groups (all $p>0.05$; Figure 2D–F). Detailed cumulative incidence rates for both aGVHD and cGVHD at multiple time points are provided in Table S2.

The data cutoff date was September 6, 2025. The median follow-up duration was 28.4 months (95% CI, 24.8–35.5 months). By this time, 63 deaths (22.8%) had been recorded: 32 (25.2%) in the higher-dose group and 31 (20.8%) in the lower-dose group. The median time from transplantation to death was 5.0 months (95% CI, 3.6–6.4) in the higher-dose group and 4.5 months (95% CI, 3.2–5.8) in the lower-dose group.

Relapse and GVHD were the most frequent adjudicated primary causes of death (Table S3). Acute GVHD-related death occurred in 4/149 patients (2.7%) in the lower-dose group and 8/127 (6.3%) in the higher-dose group. The unweighted cumulative incidence comparison did not reach statistical significance (Gray's test, $p=0.143$). Although acute GVHD-related deaths were more frequent in the higher-dose group (8/127 vs. 4/149), the number of events was small and the confidence intervals around the weighted effect estimates were wide and included the null value. The IPTW-weighted Fine–Gray model yielded an sHR of 3.35 (95% CI, 0.96–11.72; $P=0.058$), and the cause-specific Cox model yielded an HR of 3.27 (95% CI, 0.94–11.43; $P=0.063$). Overall GVHD-related and relapse-related mortality were also numerically higher in the higher-dose group, although none of the weighted comparisons reached statistical significance (Table S10).

Median overall survival was not reached in either cohort. The Kaplan–Meier estimates of OS, DFS, and GRFS are provided in Table S2. The higher-dose group demonstrated significantly better GRFS than the lower-dose group, with 1- and 2-year estimates of 68.9% and 59.1% versus 56.2% and 50.3%, respectively (Figure 3C). The adjusted hazard ratio (aHR) for GRFS was 0.59 (95% CI, 0.43–0.83, $p=0.002$; Table 3). The distribution of first GRFS-defining events is summarized in Table S9. Grade III–IV aGVHD was the first event in 16 patients (10.7%) in the lower-dose group and 8 (6.3%) in the higher-dose group, while moderate-to-severe cGVHD was the first event in 24 (16.1%) and 19 (15.0%), respectively.

However, OS and DFS were comparable across the two groups (Figure 3A–B), with aHRs of 1.26 ($p=0.427$) and 1.04 ($p=0.884$), respectively.

Similarly, competing-risk analyses revealed no significant differences in the CIR or NRM between the cohorts (Figure 3D–E). Although the higher-dose group showed higher CIR and lower NRM numerically (Table S2), these differences were not statistically significant (aSHR 1.07, $p=0.857$ for CIR; aSHR 0.82, $p=0.632$ for NRM; Table 3).

After IPTW adjustment, the incidence, frequency, and duration of CMV and EBV viraemia did not differ significantly between the two groups. Bloodstream infection occurred in 19 patients (12.8%) in the lower-dose group and 4 (3.1%) in the higher-dose group ($p=0.014$). Infectious complications in the full cohort are summarized in Table S8.

Immune Reconstitution

In an exploratory analysis, immune reconstitution was evaluated in a subcohort of 177 patients with longitudinal data available. Baseline comparisons between patients included and excluded from the immune-reconstitution cohort are shown in Supplementary Table S7. Treatment allocation and transplantation year were similar between the two populations. All included patients received peripheral blood stem-cell grafts. Differences were observed in HCT-CI category, ECOG performance status, disease status, disease-risk category, HLA disparity, donor/recipient CMV serostatus, and stem-cell source.

This subcohort comprises 89 (50.3%) patients in the lower-dose group and 88 (49.7%) in the higher-dose group. Baseline characteristics for this subcohort were comparable between the two groups (Table S4).

At day +30 post-transplantation, the absolute CD4⁺ T-cell count was markedly lower in the higher-dose group compared to the lower-dose group (median 70.4/ μ L vs. 279.2/ μ L). This difference remained statistically significant in the IPTW-adjusted analysis ($p=0.036$; Table 4). However, this effect was transient, with CD4⁺ counts becoming comparable between the groups from day +60 through day +540 (all $p>0.05$). No statistically significant differences were observed in absolute counts for other lymphocyte subsets at any scheduled time point (all $p>0.05$).

When death before recovery was treated as a competing event, the cumulative incidence of sustained recovery was similar between groups for all five lymphocyte subsets (all $p>0.05$; Figure 4). In longitudinal mixed-effects models, lymphocyte subset counts changed significantly over time (all p for time <0.05), and no significant group-

by-time interactions were observed (all p for interaction >0.05 ; Figure S2).

Discussion

In this prospective, non-randomized cohort study, the higher-dose anti-CD25, methotrexate-free strategy was associated with a lower incidence of both grade II–IV and grade III–IV aGVHD and with improved GRFS. By contrast, relapse, NRM, OS, and DFS did not differ significantly between groups. Immune monitoring identified a transient reduction in day +30 CD4⁺ T-cell counts in the higher-dose group, with no substantial between-group differences in subsequent immune reconstitution or infectious outcomes.

The observed reduction in aGVHD is biologically plausible given the central role of interleukin-2 signaling in early donor T-cell activation after transplantation.^{12,26,31,32} However, these findings should be interpreted in the context of the prophylactic regimen as a whole rather than as evidence of an isolated effect of anti-CD25 dose escalation. The protocol-defined strategies differed with respect to both anti-CD25 dose and MTX administration. By contrast, cumulative rATG dosing was individualized in clinical practice and was not assigned according to treatment group, although overall rATG exposure was lower in the higher-dose group. This observed co-treatment imbalance was included in the propensity-score model and was well balanced after IPTW. Even so, the independent contributions of anti-CD25 dose, MTX omission, and differences in actual rATG exposure cannot be fully disentangled in this non-randomized study. Nonetheless, the lower incidence of aGVHD in the higher-dose group despite MTX omission and lower observed rATG exposure is consistent with the possibility that more

intensive IL-2 receptor blockade contributed to improved GVHD control.^{13,29,33}

Beyond GVHD control, MTX omission may also have implications for hematopoietic recovery and mucosal toxicity. In a previous institutional analysis of an earlier, partially overlapping cohort, the MTX-free regimen was associated with faster neutrophil and platelet engraftment and a lower incidence of severe oral mucositis than the MTX-containing regimen.²¹ These observations are consistent with the antiproliferative and cytotoxic effects of MTX on rapidly dividing hematopoietic and mucosal cells^{34,35} and suggest that MTX omission may reduce selected treatment-related toxicities.

The immune-reconstitution findings provide additional context for interpreting the biological effects of the higher-dose anti-CD25, MTX-free strategy. Although CD4⁺ T-cell counts were lower at day +30 in the higher-dose group, this difference was not sustained at subsequent assessments. Consistent with the cross-sectional findings, longitudinal mixed-effects models showed progressive recovery across all evaluated lymphocyte subsets, with no significant treatment-by-time interactions, suggesting that the overall trajectory of immune reconstitution was not substantially altered by treatment strategy.²⁰ Similarly, competing-risk analyses of sustained recovery, with death treated as a competing event, showed no significant between-group differences in the recovery of CD3⁺, CD4⁺, CD8⁺, CD19⁺, or NK cells. Taken together, these findings suggest that more intensive IL-2 receptor blockade may transiently influence early lymphocyte kinetics during the period of greatest alloreactive risk^{14,22,36,37} without producing a persistent divergence in subsequent immune recovery.

The transient differences in early immune kinetics were not accompanied by a

detectable increase in infectious complications. After IPTW adjustment, the incidence, frequency, and duration of CMV and EBV viraemia were similar between groups, whereas bacterial bloodstream infections occurred less frequently in the higher-dose group.³⁸ These clinical findings suggest that the transient reduction in early CD4⁺ T-cell counts was not associated with an increased overall burden of infection.

Several limitations warrant consideration. First, although IPTW improved the balance of measured covariates, treatment allocation was non-randomized and was primarily determined by patient preference following physician counseling. In addition, anti-CD25 was administered as a fixed dose, and body-weight-normalized anti-CD25 exposure was not a prespecified treatment variable or analysis in the present study. Therefore, whether interindividual variation in anti-CD25 exposure expressed as mg/kg influences clinical outcomes remains to be determined. Residual confounding from unmeasured factors, including potential confounding by indication, therefore cannot be excluded. Second, the immune-reconstitution analyses were conducted in a prespecified exploratory subset of the overall cohort. Although treatment allocation and transplantation year were similar between patients included and not included in these analyses, differences in several disease- and transplantation-related characteristics may limit the generalizability of the immune findings and introduce selection bias. Third, early post-transplant events, particularly aGVHD and its subsequent treatment with systemic corticosteroids, may have acted as time-dependent determinants of immune recovery. aGVHD occurring before the day +30 immune assessment was numerically more frequent in the lower-dose group than in the higher-dose group (6/89 vs. 3/88). This imbalance would

be expected, if anything, to reduce CD4⁺ T-cell counts in the lower-dose group and would therefore tend to attenuate, rather than explain, the lower day +30 CD4⁺ T-cell counts observed in the higher-dose group. Nevertheless, residual time-dependent confounding related to GVHD occurrence, GVHD treatment, and systemic corticosteroid exposure cannot be excluded. Moreover, because detailed functional immune assessments were unavailable, the independent effects of the prophylactic strategy, GVHD-related events, and their treatment on immune reconstitution could not be fully disentangled.

In conclusion, the higher-dose anti-CD25, MTX-free strategy was associated with a lower incidence of aGVHD and improved GRFS, without significant differences in relapse, NRM, OS, or DFS and with broadly similar long-term immune-reconstitution and infectious outcomes. Given the non-randomized design and the concurrent differences in prophylactic-regimen components, these findings warrant confirmation in multicenter randomized trials of haploidentical transplantation.

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Tables:

Table 1. Summary of selected baseline patient and transplant characteristics

Characteristics	Before IPTW			After IPTW		
	Lower-dose n=149	Higher-dose n=127	SMD	Lower-dose n=282.30	Higher-dose n=272.46	SMD
Patient age, median (IQR)	34.0 (26.0,45.0)	35.0 (27.5,46.5)	0.096	36.00 (26.0,46.21)	34.00 (27.0,44.0)	0.076
Patient gender, n (%)			0.006			0.006
Male	84 (56.4)	72 (56.7)		156.6 (55.5)	150.4 (55.2)	
Female	65 (43.6)	55 (43.3)		125.7 (44.5)	122.1 (44.8)	
HCT-CI, n (%)			0.406			0.047
0	44 (29.5)	23 (18.1)		62.6 (22.2)	64.4 (23.6)	
1–2	71 (47.7)	53 (41.7)		118.0 (41.8)	115.6 (42.4)	
>2	34 (22.8)	51 (40.2)		101.0 (36.0)	92.5 (33.9)	
Diagnosis, n (%)			0.379			0.133
ALL	47 (31.5)	32 (25.2)		78.4 (27.8)	74.7 (27.4)	
AML	70 (47.0)	73 (57.5)		147.2 (52.1)	134.6 (49.4)	
MDS	12 (8.1)	16 (12.6)		30.5 (10.8)	26.6 (9.8)	
LBL	20 (13.4)	6 (4.7)		26.2 (9.3)	36.5 (13.4)	
Disease status before HSCT, n (%)			0.169			0.026
CR, MRD-	113 (75.8)	102 (80.3)		228.3 (80.9)	217.7 (79.9)	
CR, MRD+	25 (16.8)	14 (11.0)		36.2 (12.8)	37.2 (13.7)	
NR	11 (7.4)	11 (8.7)		17.8 (6.3)	17.5 (6.4)	
Gender match, n (%)			0.134			0.055
Female to male	25 (16.8)	28 (22.0)		40.3 (14.3)	44.3 (16.3)	
Others	124 (83.2)	99 (78.0)		242.0 (85.7)	228.1 (83.7)	
Conditioning regimen, n (%)			0.041			0.132
Myeloablative, n (%)	122 (81.9)	102 (80.3)		216.8 (76.8)	223.6 (82.1)	
Reduced-intensity, n (%)	26 (17.4)	24 (18.9)		64.3 (22.8)	47.6 (17.5)	
Non-myeloablative, n (%)	1 (0.7)	1 (0.8)		1.3 (0.4)	1.3 (0.5)	
Stem cell source, n (%)			0.251			0.040
Peripheral blood stem cell	144 (96.6)	115 (90.6)		267.8 (94.9)	255.9 (93.9)	
Peripheral blood stem cell + Bone marrow	5 (3.4)	12 (9.4)		14.5 (5.1)	16.5 (6.1)	
Cumulative rATG dose category, n (%)			0.652			0.016
≤6 mg/kg	56 (37.6)	87 (68.5)		146.9 (52.0)	139.6 (51.2)	
> 6mg /kg	93 (62.4)	40 (31.5)		135.4 (48.0)	132.8 (48.8)	

Detailed patient characteristics before and after IPTW are provided in Supplementary Table S1.

The median (range) cumulative rATG dose was 7.5 (6–10) mg/kg in the lower-dose group and 6.0 (5–8) mg/kg in the higher-dose group. For propensity-score estimation and covariate-balance assessment, cumulative rATG exposure was categorized as ≤ 6 versus >6 mg/kg.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CR, complete remission; HCT-CI, Hematopoietic Cell Transplantation–Comorbidity Index; HSCT, hematopoietic stem cell transplantation; IPTW, inverse probability of treatment weighting; IQR, interquartile range; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndrome(s); MRD[−], measurable residual disease negative; MRD⁺, measurable residual disease positive; NR, no remission; SMD, standardized mean difference.

Table 2. Subdistribution hazard ratio (SHR) for higher dose compared with lower dose for acute and chronic GVHD

Outcome	Unweighted			Weighted Univariable			Weighted Multivariable		
	SHR	95% CI	<i>P</i> value	SHR	95% CI	<i>P</i> value	SHR	95% CI	<i>P</i> value
Grade I–IV aGVHD	0.61	0.41–0.90	0.014	0.77	0.45–1.31	0.331	0.72	0.44–1.16	0.175
Grade II–IV aGVHD	0.49	0.27–0.87	0.015	0.45	0.24–0.84	0.013	0.45	0.25–0.84	0.011
Grade III–IV aGVHD	0.20	0.06–0.60	0.004	0.39	0.18–0.86	0.019	0.39	0.18–0.88	0.022
≥Mild cGVHD	0.76	0.50–1.15	0.191	0.66	0.40–1.09	0.101	0.66	0.41–1.08	0.097
≥Moderate cGVHD	0.81	0.46–1.43	0.462	0.64	0.33–1.26	0.196	0.66	0.35–1.24	0.194
≥Severe cGVHD	0.89	0.50–1.56	0.677	0.65	0.33–1.29	0.222	0.69	0.36–1.31	0.254

The doubly robust model was adjusted for the treatment group and covariates that remained unbalanced after IPTW (SMD > 0.1), including Diagnosis, HLA, Donor/recipient CMV, Donor/recipient EBV, and Conditioning regimen. *P* values in weighted models were calculated using robust standard errors.

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CI, confidence interval; IPTW, inverse probability of treatment weighting; SHR, subdistribution hazard ratio; SMD, standardized mean difference.

Table 3. Treatment effects on survival and competing-risk outcomes

Outcome	Unweighted			Weighted Univariable			Weighted Multivariable		
	HR/SHR	95% CI	<i>P</i> value	HR/SHR	95% CI	<i>P</i> value	HR/SHR	95% CI	<i>P</i> value
OS	1.23	0.75–2.01	0.421	1.15	0.65–2.03	0.634	1.26	0.71–2.23	0.427
DFS	1.07	0.69–1.66	0.771	0.93	0.55–1.57	0.788	1.04	0.63–1.71	0.884
GRFS	0.65	0.48–0.87	0.004	0.57	0.39–0.83	0.004	0.59	0.43–0.83	0.002
CIR	1.32	0.73–2.37	0.358	1.01	0.51–2.03	0.969	1.07	0.53–2.12	0.857
NRM	0.77	0.39–1.50	0.438	0.81	0.38–1.71	0.578	0.82	0.37–1.82	0.632

The doubly robust models were adjusted for treatment group and residual covariates with standardized mean differences >0.1 after IPTW, including Diagnosis, HLA, donor/recipient CMV serostatus, donor/recipient EBV serostatus, and conditioning regimen. Robust standard errors were used for all IPTW-weighted analyses. The lower-dose group was the reference group.

Abbreviations: CI, confidence interval; CIR, cumulative incidence of relapse; DFS, disease-free survival; GRFS, graft-versus-host disease-free, relapse-free survival; HR, hazard ratio; IPTW, inverse probability of treatment weighting; NRM, non-relapse mortality; OS, overall survival; SHR, subdistribution hazard ratio; SMD: Standardized mean difference.

Table 4. Immune reconstitution of lymphocyte subsets at different time points (Days Post-Transplantation)

Lymphocyte Subset	Lower-dose	Higher-dose	P value	
	n=89	n=88	Unweighted	IPTW-Weighted
CD3 ⁺ cell, median (IQR), cells/μL				
Day 30	1243.9 (466.7–3169.4)	1050.8 (268.1–1914.1)	0.331	0.320
Day 60	1130.5 (667.0–2334.9)	981.4 (436.4–1608.3)	0.130	0.132
Day 90	1298.6 (531.3–2684.8)	824.1 (410.2–1180.7)	0.096	0.117
Day 180	820.5 (610.5–1496.9)	1075.6 (411.3–2093.9)	0.690	0.713
Day 270	1285.0 (888.5–2406.7)	1248.0 (885.4–1966.4)	0.146	0.141
Day 360	1504.4 (917.9–2829.8)	1275.7 (753.3–1783.0)	0.291	0.320
Day 540	1129.7 (791.2–1824.5)	1544.3 (1107.4–1917.0)	0.414	0.392
CD4 ⁺ cell, median (IQR), cells/μL				
Day 30	279.2 (87.8–908.8)	70.4 (50.7–203.2)	0.035	0.036
Day 60	156.4 (70.4–437.1)	114.2 (51.3–217.9)	0.305	0.370
Day 90	153.0 (80.4–446.4)	142.0 (71.1–255.3)	0.687	0.622
Day 180	163.3 (94.4–275.2)	192.3 (113.9–389.0)	0.659	0.671
Day 270	275.0 (171.3–403.6)	217.3 (146.2–459.4)	0.780	0.757
Day 360	362.3 (230.1–586.7)	256.5 (180.4–354.9)	0.095	0.109
Day 540	340.4 (200.7–410.1)	319.7 (261.6–487.2)	0.820	0.831
CD8 ⁺ cell, median (IQR), cells/μL				
Day 30	752.7 (199.9–1967.4)	653.1 (201.1–1506.4)	0.740	0.731
Day 60	893.7 (463.8–1489.9)	695.0 (305.2–1183.0)	0.255	0.263
Day 90	846.4 (309.1–1853.2)	596.4 (329.9–918.0)	0.177	0.213
Day 180	609.9 (409.0–1190.3)	785.1 (303.5–1499.3)	0.649	0.649
Day 270	896.0 (614.7–1891.9)	953.2 (639.1–1681.6)	0.994	0.994
Day 360	1026.4 (582.0–2349.1)	928.5 (531.4–1441.9)	0.393	0.393
Day 540	734.7 (501.0–1336.3)	1092.1 (797.8–1400.1)	0.494	0.489
CD19 ⁺ cell, median (IQR), cells/μL				
Day 30	13.6 (6.3–36.0)	35.9 (17.3–56.0)	0.111	0.113
Day 60	25.8 (8.6–102.2)	50.8 (17.8–128.6)	0.117	0.134
Day 90	41.9 (14.3–118.7)	77.7 (32.1–142.6)	0.419	0.392
Day 180	85.2 (25.7–152.6)	99.8 (39.9–202.8)	0.533	0.563
Day 270	98.6 (51.5–216.8)	101.9 (54.6–257.5)	0.862	0.826
Day 360	193.6 (55.0–344.5)	212.0 (99.6–355.5)	0.393	0.389
Day 540	318.1 (108.9–447.1)	265.1 (198.5–602.6)	0.459	0.459
NK cell, median (IQR), cells/μL				
Day 30	815.4 (308.1–2528.1)	368.1 (160.0–436.4)	0.062	0.073
Day 60	322.4 (158.1–1456.5)	291.7 (154.4–543.6)	0.287	0.287
Day 90	358.3 (122.9–1166.6)	306.7 (195.1–540.0)	0.803	0.852
Day 180	165.8 (116.6–408.4)	291.2 (175.8–744.9)	0.245	0.214
Day 270	222.2 (159.6–460.2)	259.0 (156.8–639.5)	0.689	0.661
Day 360	220.4 (139.5–725.7)	314.0 (200.5–753.1)	0.420	0.420
Day 540	140.6 (127.0–558.6)	254.7 (200.2–574.6)	0.531	0.530

Abbreviations: IPTW, inverse probability of treatment weighting; IQR, interquartile range; NK, natural killer.

Figure Legends:

Figure 1. A schematic diagram of the proposed GVHD prophylaxis regimen.

Abbreviations: Anti-CD25 mAb, anti-CD25 monoclonal antibody; ATG, anti-thymocyte globulin; BM, bone marrow; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; MTX, methotrexate; PBSC, peripheral blood stem cell.

Figure 2. Unweighted cumulative incidence of acute and chronic graft-versus-host disease. (A) Grade I–IV aGVHD by day +100. (B) Grade II–IV aGVHD by day +100.

(C) Grade III–IV aGVHD by day +100. (D) Mild or more severe cGVHD. (E) Moderate or more severe cGVHD. (F) Severe cGVHD. The displayed cumulative-incidence curves, numbers at risk, and *p* values are based on the unweighted cohort. Between-group *p* values were calculated using Gray's test. Relapse and death without prior graft-versus-host disease were treated as competing events.

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; haplo-HSCT, haploidentical hematopoietic stem cell transplantation.

Figure 3. Unweighted survival and competing-risk outcomes. (A) Probability of OS. (B) Probability of DFS. (C) Probability of GRFS. (D) CI of relapse. (E) CI of NRM.

The displayed curves, numbers at risk, and *P* values are based on the unweighted cohort. Between-group *p* values for overall survival, disease-free survival, and graft-versus-host disease-free, relapse-free survival were calculated using the log-rank test. *p* values for relapse and non-relapse mortality were calculated using Gray's test. Relapse and non-relapse mortality were treated as competing events for each other.

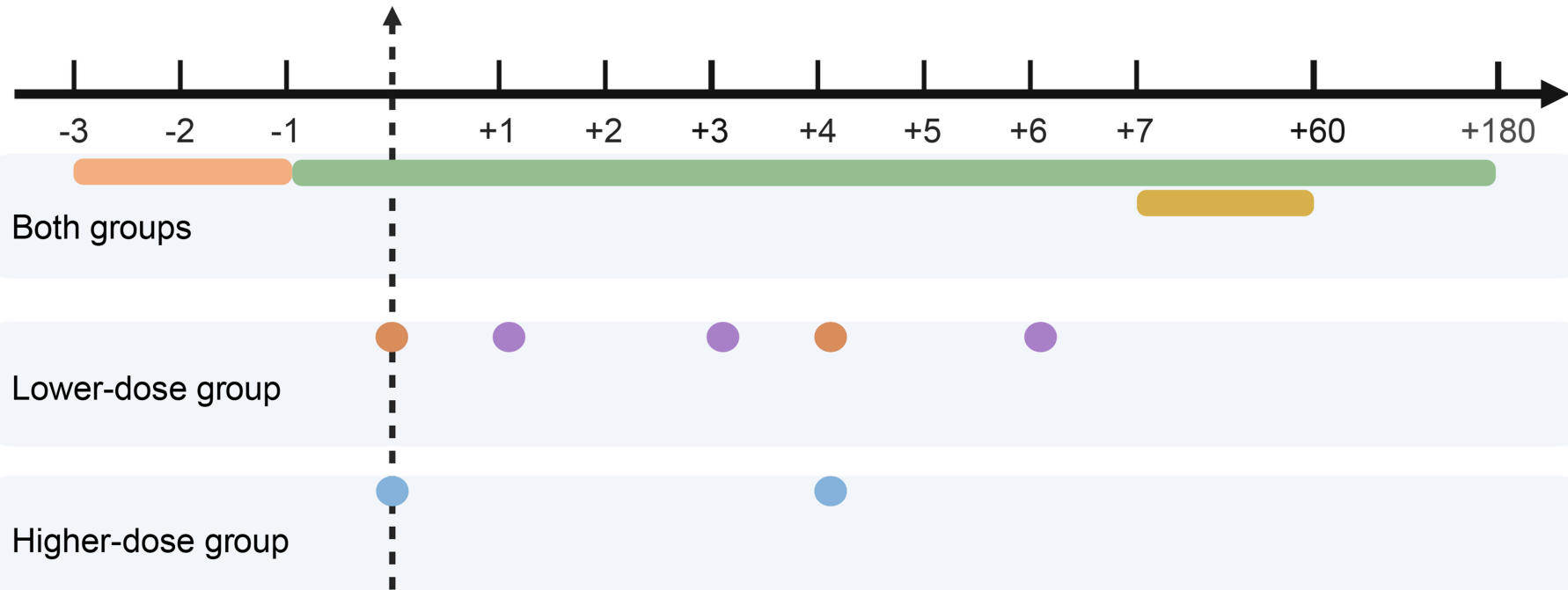
Abbreviations: DFS, disease-free survival; GRFS, graft-versus-host disease-free and

relapse-free survival; haplo-HSCT, haploidentical hematopoietic stem cell transplantation; NRM, non-relapse mortality; OS, overall survival.

Figure 4. Unweighted cumulative incidence of sustained lymphocyte-subset recovery after haplo-HSCT. (A) CD3⁺ Cell. (B) CD4⁺ Cell. (C) CD8⁺ Cell. (D) CD19⁺ Cell. (E) NK Cell. Sustained recovery was defined as the first of three consecutive measurements above the predefined threshold. Death before sustained recovery was treated as a competing event. The displayed cumulative-incidence curves, numbers at risk, subdistribution hazard ratios, and *p* values are based on the unweighted immune-reconstitution cohort. Subdistribution hazard ratios and *p* values were estimated using univariable Fine–Gray models, with the lower-dose group as the reference.

Abbreviations: haplo-HSCT, haploidentical hematopoietic stem cell transplantation; NK, natural killer; SHR, subdistribution hazard ratio.

PBSC ± BM graft infusion



Green bar: Tacrolimus (0.03 mg/kg), twice daily, -1d~+180d

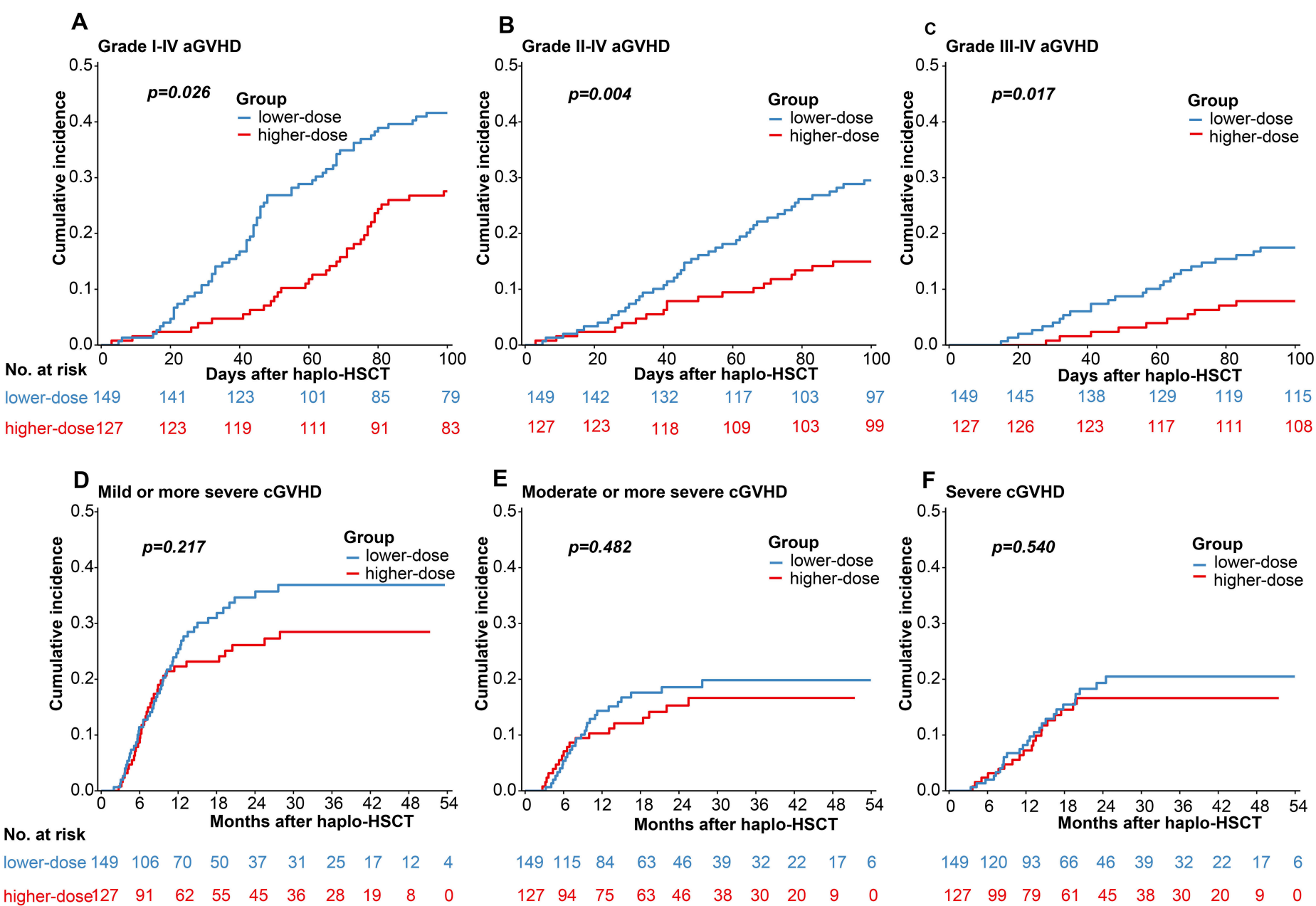
Yellow bar: MMF (5 mg/kg), twice daily, +7~+60d

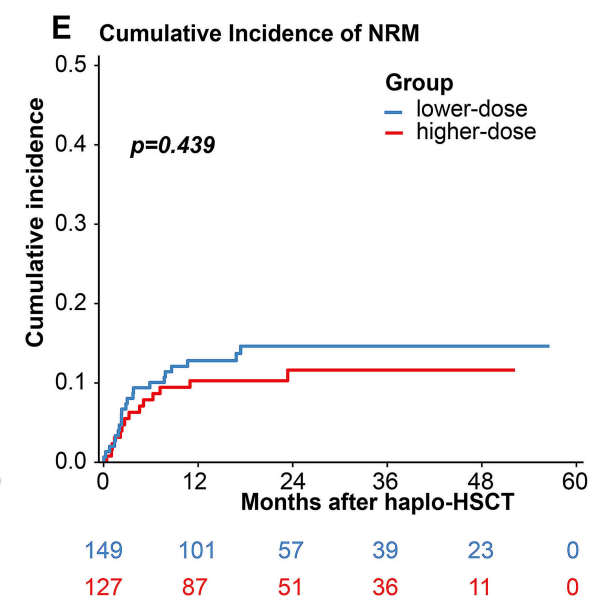
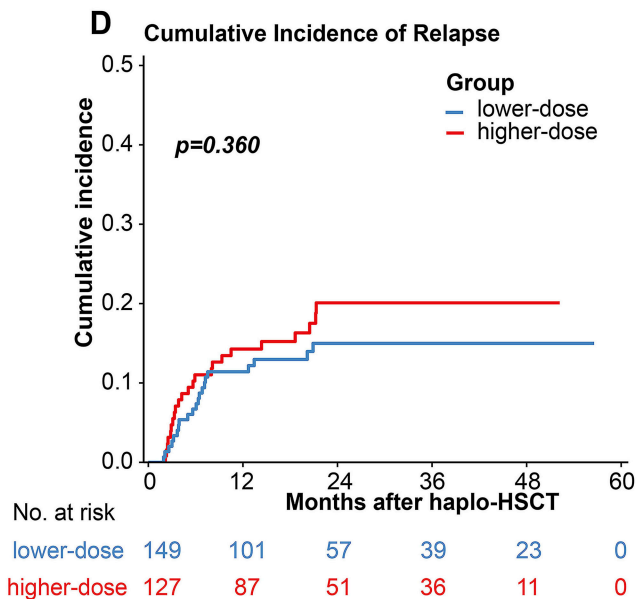
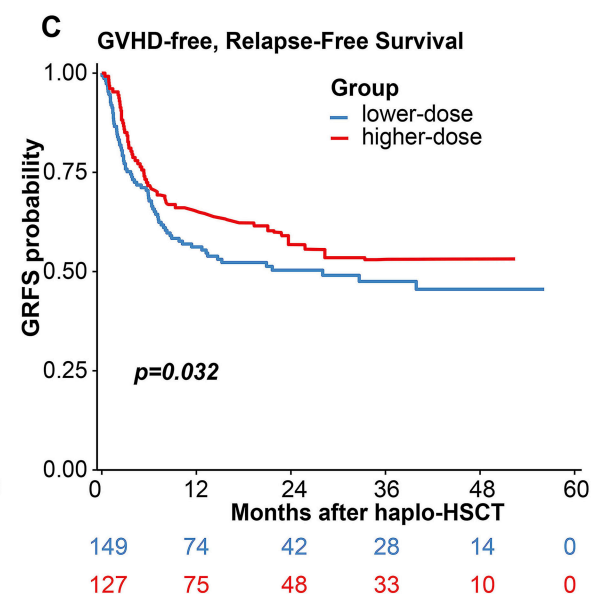
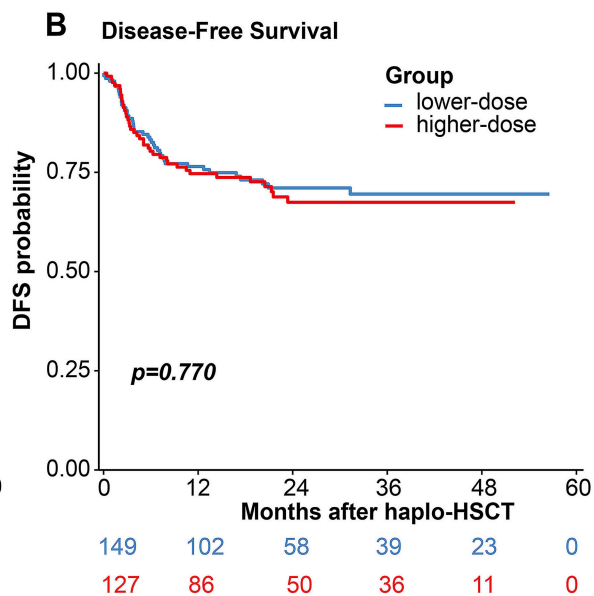
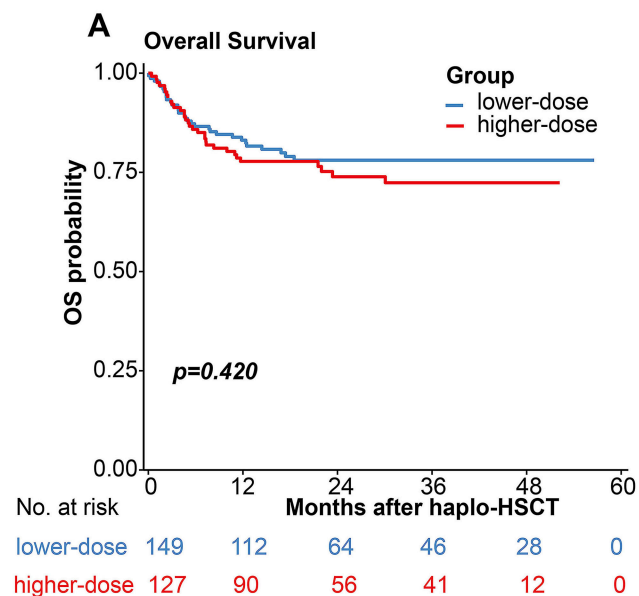
Orange bar: ATG: 5-10 mg/kg, days -3 ~ -1d

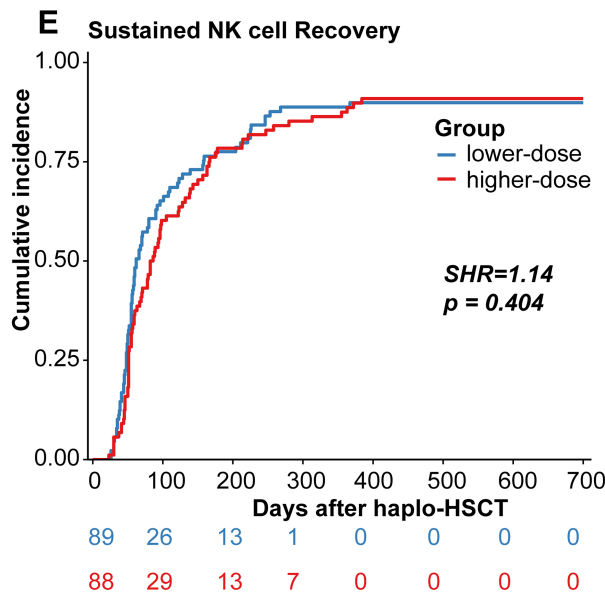
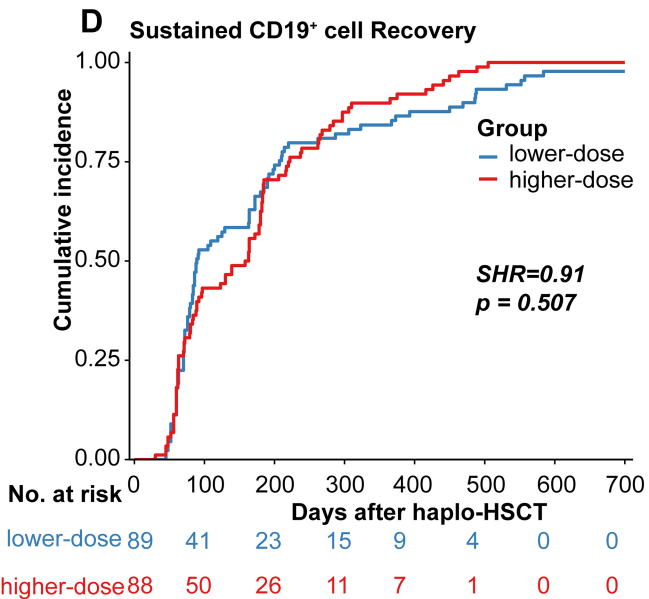
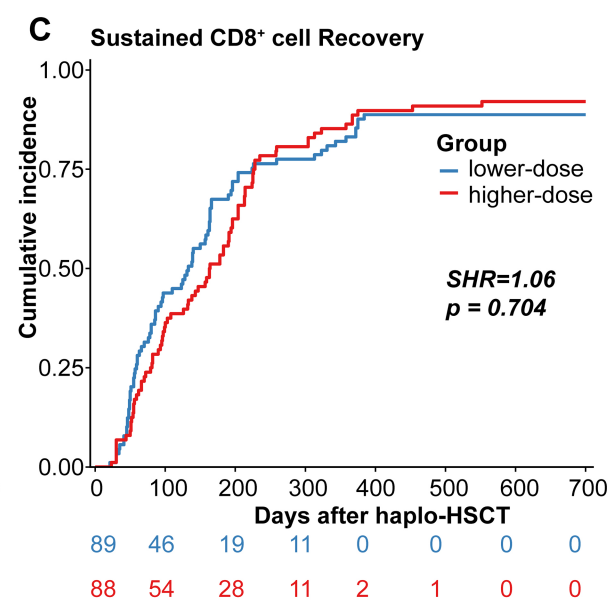
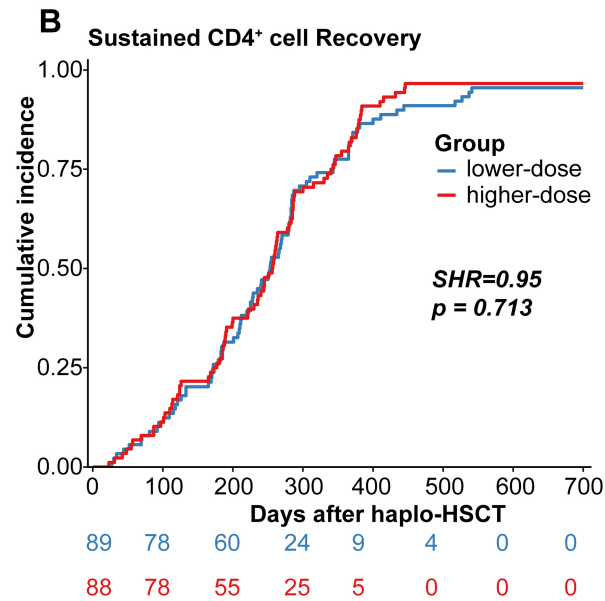
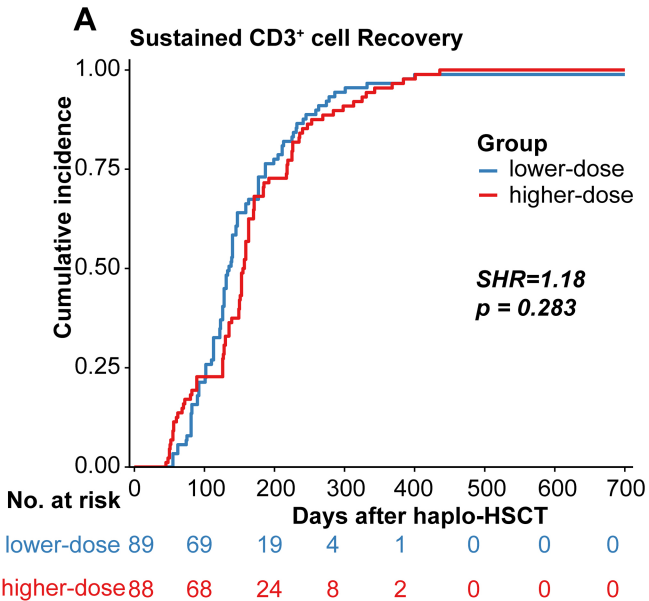
Orange dot: Anti-CD25 mAb: 25 mg IV on days 0 and +4

Blue dot: Anti-CD25 mAb: 50 mg IV on days 0 and +4

Purple dot: MTX: 15 mg/m² on day 1; 10 mg/m² on days 3 and +6







Supplementary Methods

The following sections provide extended methodological details corresponding to abbreviated descriptions in the main text.

S1. Transplantation Procedures

S1.1 Stem cell harvesting and processing

Stem cell harvesting and processing were performed consistently with our previous studies.¹ Briefly, peripheral blood stem cells were mobilized with granulocyte colony-stimulating factor (G-CSF) and collected by apheresis. All grafts were processed and infused fresh without ex vivo T-cell depletion.

S1.2 Conditioning regimens

Conditioning intensity was determined by disease status and patient fitness. MAC was administered to 224 patients (n=224, 81%), RIC to 50 patients (n=50, 18%), and non-myeloablative conditioning to 2 patients (n=2, <1%). Specific regimen composition followed institutional protocols as previously described.¹

S1.3 GVHD prophylaxis

All patients received rabbit anti-human thymocyte globulin (rATG; Thymoglobuline®, Sanofi, Lyon, France), tacrolimus, and mycophenolate mofetil (MMF) as the backbone of GVHD prophylaxis. rATG was administered intravenously on days -3 to -1. The cumulative dose was individualized by the attending transplant physician according to the patient's overall clinical condition, disease status, and anticipated transplant-related risk. Cumulative rATG doses ranged from 5 to 10 mg/kg. The median cumulative dose was 7.5 mg/kg (range, 6–10 mg/kg) in the lower-dose group and 6.0 mg/kg (range, 5–8 mg/kg) in the higher-dose group. Tacrolimus was administered at 0.03 mg/kg twice daily from day -1 through day +180, and MMF was administered at 5 mg/kg twice daily from day +7 through day +60. The anti-CD25 monoclonal antibody used in this study was Jennip® (recombinant humanized anti-CD25 monoclonal antibody injection; Sunshine Guojian Pharmaceutical [Shanghai] Co., Ltd., Shanghai, China). Jennip® is a recombinant humanized IgG1/κ monoclonal

antibody that targets the α -chain of the interleukin-2 receptor (CD25, IL-2R α) and is approved by the National Medical Products Administration (NMPA) of China (Approval No. S20110001). After physician counseling regarding the available anti-CD25/MTX prophylaxis strategies, treatment allocation was primarily determined by patient preference. Patients were assigned to one of two anti-CD25 monoclonal antibody strategies:

Lower-dose group: Anti-CD25 monoclonal antibody 25 mg intravenously on days 0 and +4, combined with short-term MTX (15 mg/m² on day +1; 10 mg/m² on days +3 and +6).

Higher-dose group: Anti-CD25 monoclonal antibody 50 mg intravenously on days 0 and +4, without MTX.

No post-transplant cyclophosphamide (PTCy) was administered to any patient.

S2. Supportive Care

Supportive care followed routine institutional guidelines consistent with our previous studies.¹⁻³ Protocols included:

Antimicrobial prophylaxis: Antibacterial prophylaxis was administered during the neutropenic period per institutional protocol.

Antifungal prophylaxis: Systemic antifungal agents were given to all patients from the start of conditioning through engraftment.

Antiviral prophylaxis and surveillance: All patients received acyclovir for herpes simplex/varicella-zoster prophylaxis. CMV and EBV were monitored weekly by quantitative PCR from engraftment; preemptive therapy was initiated per institutional thresholds.

Pneumocystis jirovecii prophylaxis: Trimethoprim-sulfamethoxazole (or pentamidine in cases of intolerance) was administered from engraftment through at least 6 months post-transplant.

Supportive transfusions: Packed red blood cells and platelets were transfused

to maintain hemoglobin ≥ 60 g/L and platelet count $\geq 10 \times 10^9/L$ ($\geq 20 \times 10^9/L$ in febrile patients).

S3. Statistical Analysis — Extended Details

S3.1 IPTW weight diagnostics

Propensity scores were estimated using a multivariable logistic regression model with treatment group as the dependent variable. The model included the following prespecified baseline characteristics: patient age, donor age, patient gender, HCT-CI category, ECOG performance status, Karnofsky Performance Status (KPS), diagnosis, disease status, disease risk, donor–recipient relationship, donor–recipient gender match, HLA disparity, ABO compatibility, pre-transplant CMV serostatus, pre-transplant EBV serostatus, conditioning regimen, stem cell source, cumulative rATG dose category (≤ 6 vs. > 6 mg/kg), infused nucleated cell dose, and infused CD34⁺ cell dose.

Standard unstabilized IPTW weights were applied without truncation or trimming, as extreme weights were not observed (maximum weight < 10). Covariate balance was assessed visually using Love plots and quantitatively using absolute standardized mean differences, with an absolute SMD < 0.1 indicating adequate balance. Covariates with residual SMD > 0.1 after weighting were retained as adjusters in weighted regression models, yielding doubly robust estimates.

S3.2 Competing-risk and cause-specific mortality analyses

Competing event pairs were defined as: (1) relapse versus non-relapse mortality for CIR and NRM; and (2) relapse and death without GVHD versus GVHD for GVHD-related outcomes. The Fine–Gray sub-distribution hazard model was applied for competing-risk endpoints. Robust (sandwich) variance estimators were used in all weighted models.

Additional post hoc cause-specific mortality analyses were performed according to the adjudicated primary cause of death. Separate endpoints were defined for acute GVHD-related mortality, overall GVHD-related mortality (acute or chronic

GVHD), and relapse-related mortality. For each endpoint, death from any other cause was treated as a competing event, while patients alive at the last follow-up were censored. Unweighted cumulative incidence functions were compared using Gray's test. IPTW-weighted Fine–Gray models with robust variance estimators were used to estimate subdistribution hazard ratios. IPTW-weighted cause-specific Cox models with robust variance estimators were performed as complementary analyses. Given the limited number of endpoint-specific deaths, the weighted outcome models included treatment group as the only regression term after application of the prespecified IPTW weights.

S3.3 Immune reconstitution analysis

Immune reconstitution was evaluated as a prespecified exploratory analysis using prospectively collected immune-monitoring data. Longitudinal immune monitoring was performed as part of routine post-transplant follow-up. Because evaluation of immune reconstitution required serial measurements across predefined assessment windows, only patients with sufficient longitudinal immune-monitoring data were included in the immune-reconstitution cohort ($n = 177$). The patient-selection process is summarized in Figure S3, and baseline characteristics of included and non-included patients are compared in Table S7.

Assessments were grouped into predefined windows centered on days +30, +60, +90, +180, +270, +360, and +540. Recovery was defined as the first of three consecutive measurements exceeding the predefined lower-limit-of-normal (LLN) thresholds: $CD3^+ > 955/\mu\text{L}$, $CD3^+CD4^+ > 550/\mu\text{L}$, $CD3^+CD8^+ > 320/\mu\text{L}$, $CD19^+ > 90/\mu\text{L}$, and NK cells $> 150/\mu\text{L}$. Time to immune recovery was evaluated using the Fine–Gray subdistribution hazard model, with death before recovery treated as a competing event.

Longitudinal immune-cell trajectories were additionally evaluated using linear mixed-effects models fitted to log-transformed cell counts [$\log(\text{count} + 1)$]. The models included fixed effects for treatment group, predefined categorical time window, treatment-by-time interaction, age, sex, and cumulative rATG dose category, with a

patient-specific random intercept.

S3.4 Software

Analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY) and R 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Key R packages included *survival*, *cmprsk*, *WeightIt*, and *cobalt* (for IPTW and balance diagnostics).

Supplementary Tables

Table S1. Baseline patient and transplant characteristics

Characteristics	Before IPTW			After IPTW		
	Lower-dose n=149	Higher-dose n=127	SMD	Lower-dose n=282.30	Higher-dose n=272.46	SMD
Patient age, median (IQR)	34.0 (26.0,45.0)	35.0 (27.5,46.5)	0.096	36.00 (26.0,46.21)	34.00 (27.0,44.0)	0.076
Donor age, median (IQR)	33.0 (22.0,46.0)	30.0 (20.0,43.0)	0.126	31.0 (19.40,44.57)	32.00 (19.00,47.02)	0.078
Patient gender, n (%)			0.006			0.006
Male	84 (56.4)	72 (56.7)		156.6 (55.5)	150.4 (55.2)	
Female	65 (43.6)	55 (43.3)		125.7 (44.5)	122.1 (44.8)	
HCT-CI, n (%)			0.406			0.047
0	44 (29.5)	23 (18.1)		62.6 (22.2)	64.4 (23.6)	
1–2	71 (47.7)	53 (41.7)		118.0 (41.8)	115.6 (42.4)	
>2	34 (22.8)	51 (40.2)		101.0 (36.0)	92.5 (33.9)	
ECOG, n (%)			0.191			0.039
0–1	143 (96.0)	116 (91.3)		261.9 (92.8)	255.5 (93.8)	
>1	6 (4.0)	11 (8.7)		20.4 (7.2)	17.0 (6.2)	
KPS, n (%)			0.136			0.042
≥ 90	122 (81.9)	97 (76.4)		227.2 (80.5)	223.7 (82.1)	
< 90	27 (18.1)	30 (23.6)		55.1 (19.5)	48.7 (17.9)	
Diagnosis, n (%)			0.379			0.133
ALL	47 (31.5)	32 (25.2)		78.4 (27.8)	74.7 (27.4)	
AML	70 (47.0)	73 (57.5)		147.2 (52.1)	134.6 (49.4)	
MDS	12 (8.1)	16 (12.6)		30.5 (10.8)	26.6 (9.8)	
LBL	20 (13.4)	6 (4.7)		26.2 (9.3)	36.5 (13.4)	
Disease status before HSCT, n (%)			0.169			0.026
CR, MRD-	113 (75.8)	102 (80.3)		228.3 (80.9)	217.7 (79.9)	
CR, MRD+	25 (16.8)	14 (11.0)		36.2 (12.8)	37.2 (13.7)	
NR	11 (7.4)	11 (8.7)		17.8 (6.3)	17.5 (6.4)	
Risk stratification, n (%)			0.113			0.071
Low	32 (21.5)	29 (22.8)		51.0 (18.1)	49.8 (18.3)	
Intermediate	35 (23.5)	24 (18.9)		56.1 (19.9)	46.7 (17.1)	
High	82 (55.0)	74 (58.3)		175.2 (62.1)	176.0 (64.6)	
Donor/recipient relationship, n (%)			0.195			0.073
Parent donor	49 (32.9)	31 (24.4)		88.2 (31.3)	88.7 (32.6)	
Sibling donor	36 (24.2)	35 (27.6)		60.9 (21.6)	63.3 (23.2)	
Cousin donor	4 (2.7)	5 (3.9)		5.1 (1.8)	6.1 (2.2)	
Child donor	60 (40.3)	56 (44.1)		128.1 (45.4)	114.4 (42.0)	
Gender match, n (%)			0.134			0.055
Female to male	25 (16.8)	28 (22.0)		40.3 (14.3)	44.3 (16.3)	

Characteristics	Before IPTW			After IPTW		
	Lower-dose	Higher-dose	SMD	Lower-dose	Higher-dose	SMD
	n=149	n=127		n=282.30	n=272.46	
Others	124 (83.2)	99 (78.0)		242.0 (85.7)	228.1 (83.7)	
HLA, n (%)			0.179			0.144
5/10M	94 (63.1)	78 (61.4)		181.2 (64.2)	170.2 (62.5)	
6/10M	24 (16.1)	21 (16.5)		42.2 (15.0)	38.2 (14.0)	
7/10M	20 (13.4)	15 (11.8)		32.0 (11.3)	42.9 (15.7)	
8/10M	7 (4.7)	11 (8.7)		22.4 (7.9)	18.6 (6.8)	
9/10M	4 (2.7)	2 (1.6)		4.5 (1.6)	2.5 (0.9)	
ABO match, n (%)			0.075			0.023
Match	86 (57.7)	78 (61.4)		176.9 (62.7)	167.7 (61.5)	
Mismatch	63 (42.3)	49 (38.6)		105.4 (37.3)	104.8 (38.5)	
Donor/recipient CMV serostatus, n (%)			0.120			0.173
Negative/negative	60 (40.3)	47 (37.0)		111.9 (39.6)	89.8 (33.0)	
Negative/positive	58 (38.9)	52 (40.9)		114.9 (40.7)	112.4 (41.3)	
Positive/negative	6 (4.0)	8 (6.3)		11.7 (4.1)	12.9 (4.8)	
Positive/positive	25 (16.8)	20 (15.7)		43.8 (15.5)	57.3 (21.0)	
Donor/recipient EBV serostatus, n (%)			0.114			0.113
Negative/negative	134 (89.9)	110 (86.6)		251.5 (89.1)	244.8 (89.8)	
Negative/positive	3 (2.0)	3 (2.4)		10.5 (3.7)	5.3 (2.0)	
Positive/negative	6 (4.0)	8 (6.3)		8.9 (3.2)	10.8 (4.0)	
Positive/positive	6 (4.0)	6 (4.7)		11.4 (4.0)	11.6 (4.2)	
Conditioning regimen, n (%)			0.041			0.132
Myeloablative	122 (81.9)	102 (80.3)		216.8 (76.8)	223.6 (82.1)	
Reduced-intensity	26 (17.4)	24 (18.9)		64.3 (22.8)	47.6 (17.5)	
Non-myeloablative	1 (0.7)	1 (0.8)		1.3 (0.4)	1.3 (0.5)	
Stem cell source, n (%)			0.251			0.040
Peripheral blood stem cell	144 (96.6)	115 (90.6)		267.8 (94.9)	255.9 (93.9)	
Peripheral blood stem cell + Bone marrow	5 (3.4)	12 (9.4)		14.5 (5.1)	16.5 (6.1)	
Cumulative rATG dose category, n (%)			0.652			0.016
≤6 mg/kg	56 (37.6)	87 (68.5)		146.9 (52.0)	139.6 (51.2)	
>6 mg/kg	93 (62.4)	40 (31.5)		135.4 (48.0)	132.8 (48.8)	
Median nucleated cells, ×10 ⁸ /kg (range)	11.12 (3.76,36.22)	10.52 (2.64,26.45)	0.293	10.53 (3.76,36.22)	10.56 (2.64,26.45)	0.051
Median CD34 ⁺ cells, ×10 ⁶ /kg (range)	5.73 (2.35,20.00)	6.14 (1.63,17.64)	0.002	5.73 (2.35,20.00)	6.26 (1.63,17.64)	0.075

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin;

CMV, cytomegalovirus; CR, complete remission; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group score; HCT-CI, Hematopoietic Cell Transplantation–Comorbidity Index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; IPTW, inverse probability of treatment weighting; IQR, interquartile range; KPS, Karnofsky performance status; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndrome(s); MRD[−], measurable residual disease negative; MRD⁺, measurable residual disease positive; NR, no remission; SMD, standardized mean differences.

Table S2. Clinical outcomes after haplo-HSCT

Characteristic	Lower-dose n=149	Higher-dose n=127	<i>P</i> value
Grade I–IV aGVHD at 100 days (% [95% CI])	40.27 (32.36–48.18)	27.56 (19.75–35.37)	0.026
Grade II–IV aGVHD at 100 days (% [95% CI])	29.53 (22.17–36.89)	14.96 (8.73–21.19)	0.004
Grade III–IV aGVHD at 100 days (% [95% CI])	17.45 (11.33–23.57)	7.87 (3.17–12.58)	0.017
≥Mild cGVHD at 1 year (% [95% CI])	25.40 (18.28–32.52)	22.29 (14.96–29.62)	0.217
at 2 years (% [95% CI])	34.65 (26.56–42.74)	26.12 (18.23–34.00)	
at 3 years (% [95% CI])	36.90 (28.51–45.30)	28.49 (20.19–36.78)	
≥Moderate cGVHD at 1 year (% [95% CI])	14.34 (8.63–20.06)	10.29 (4.96–15.62)	0.482
at 2 years (% [95% CI])	18.58 (12.03–25.12)	15.29 (8.67–21.92)	
at 3 years (% [95% CI])	19.84 (12.94–26.73)	16.66 (9.61–23.71)	
≥Severe cGVHD at 1 year (% [95% CI])	8.22 (3.74–12.71)	7.25 (2.66–11.83)	0.540
at 2 years (% [95% CI])	12.34 (8.49–19.39)	10.63 (4.71–15.65)	
at 3 years (% [95% CI])	15.59 (8.38–22.76)	12.65 (5.72–19.64)	
NRM at 1 year (% [95% CI])	12.81 (7.41–18.21)	10.28 (4.96–15.60)	0.439
at 2 years (% [95% CI])	14.64 (8.78–20.50)	11.62 (5.76–17.48)	
at 3 years (% [95% CI])	14.64 (8.78–20.50)	11.62 (5.76–17.48)	
CIR at 1 year (% [95% CI])	11.41 (6.28–16.53)	14.27 (8.13–20.41)	0.360
at 2 years (% [95% CI])	14.99 (8.96–21.02)	20.92 (12.49–27.69)	
at 3 years (% [95% CI])	14.99 (8.96–21.02)	20.92 (12.49–27.69)	
OS at 1 year (% [95% CI])	83.1 (77.3–89.4)	77.8 (70.8–85.4)	0.420
at 2 years (% [95% CI])	78.1 (71.4–85.3)	73.9 (66.2–82.5)	
at 3 years (% [95% CI])	78.1 (71.4–85.3)	72.4 (64.4–81.4)	
DFS at 1 year (% [95% CI])	76.5 (69.9–83.6)	74.7 (67.4–82.7)	0.770
at 2 years (% [95% CI])	71.1 (63.9–79.1)	67.5 (59.2–76.9)	
at 3 years (% [95% CI])	69.5 (62.0–78.0)	67.5 (59.2–76.9)	
GRFS at 1 year (% [95% CI])	56.2 (48.7–64.8)	68.9 (59.6–75.3)	0.032
at 2 years (% [95% CI])	50.3 (42.7–59.3)	59.1 (51.7–67.9)	
at 3 years (% [95% CI])	47.5 (39.6–57.1)	53.6 (46.8–61.2)	

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CI, confidence interval; CIR, cumulative incidence of relapse; DFS, disease-free survival; GRFS, graft-versus-host disease-free, relapse-free survival; haplo-HSCT, haploidentical hematopoietic stem cell transplantation; NRM, non-relapse mortality; OS, overall survival.

Table S3. Primary causes of death in the study cohort

Cause of death	All patients n=276	Lower-dose n=149	Higher-dose n=127
Relapse, n (%)	23 (8.3)	8 (5.4)	15 (11.8)
GVHD, n (%)	22 (8.0)	9 (6.0)	13 (10.2)
Acute GVHD, n (%)	12 (4.3)	4 (2.7)	8 (6.3)
Chronic GVHD, n (%)	10 (3.6)	5 (3.4)	5 (3.9)
Infection, n (%)	8 (2.9)	5 (3.4)	3 (2.4)
Graft failure, n (%)	5 (1.8)	4 (2.7)	1 (0.8)
Organ failure, n (%)	3 (1.1)	3 (2.0)	0 (0.0)
Treatment-related complications, n (%)	1 (0.4)	1 (0.7)	0 (0.0)
Secondary malignancy, n (%)	1 (0.4)	1 (0.7)	0 (0.0)

Percentages were calculated using the total number of patients in each treatment group as the denominator. Causes of death represent the primary causes of death determined by review of the clinical records.

Abbreviations: GVHD, graft-versus-host disease.

Table S4. Baseline patient and transplant characteristics in the immune-reconstitution cohort (n=177)

Characteristics	Before IPTW			After IPTW		
	Lower-dose n=89	Higher-dose n=88	SMD	Lower-dose n=177.39	Higher-dose n=170.78	SMD
Patient age, median (IQR)	34 (26,47)	35 (28,49)	0.044	36 (27,48)	34.8 (28,45.6)	0.052
Donor age, median (IQR)	34 (20,48)	31 (20,45)	0.113	33 (19,46)	30 (18.7,45)	0.013
Patient gender, n (%)			0.010			0.026
Male	50 (56.2)	49 (55.7)		92.9 (52.4)	87.2 (51.1)	
Female	39 (43.8)	39 (44.3)		84.4 (47.6)	83.5 (48.9)	
HCT-CI, n (%)			0.284			0.031
0	22 (24.7)	14 (15.9)		35.1 (19.8)	31.7 (18.5)	
1–2	41 (46.1)	38 (43.2)		74.9 (42.2)	73.1 (42.8)	
>2	26 (29.2)	36 (40.9)		67.5 (38.0)	66.0 (38.6)	
ECOG, n (%)			0.162			0.037
0–1	83 (93.3)	78 (88.6)		159.4 (89.8)	155.3 (90.9)	
>1	6 (6.7)	10 (11.4)		18.0 (10.2)	15.5 (9.1)	
KPS, n (%)			0.094			0.034
≥90	75 (84.3)	71 (80.7)		145.2 (81.9)	142.0 (83.1)	
< 90	14 (15.7)	17 (19.3)		32.2 (18.1)	28.8 (16.9)	
Diagnosis, n (%)			0.422			0.084
ALL	31 (34.8)	28 (31.8)		54.6 (29.9)	57.4 (33.6)	
AML	39 (43.8)	48 (54.5)		95.0 (52.0)	83.0 (48.7)	
MDS	8 (9.0)	10 (11.4)		20.4 (11.2)	19.3 (11.3)	
LBL	11 (12.4)	2 (2.3)		12.6 (6.9)	10.8 (6.4)	
Disease status before HSCT, n (%)			0.194			0.069
CR, MRD-	73 (82.0)	72 (81.8)		147.5 (83.2)	139.2 (81.5)	
CR, MRD ⁺	14 (15.7)	11 (12.5)		25.3 (14.3)	25.2 (14.8)	
NR	2 (2.2)	5 (5.7)		4.6 (2.6)	6.4 (3.7)	
Risk stratification, n (%)			0.433			0.062
Low	34 (38.2)	18 (20.5)		48.6 (27.4)	43.8 (25.7)	
Intermediate	11 (12.4)	20 (22.7)		27.3 (15.4)	29.8 (17.5)	
High	44 (49.4)	50 (56.8)		101.5 (57.2)	97.1 (56.8)	
Donor/recipient relationship, n (%)			0.184			0.048
Parent donor	29 (32.6)	23 (26.1)		48.7 (27.4)	46.6 (27.3)	
Sibling donor	17 (19.1)	22 (25.0)		36.5 (20.6)	38.3 (22.4)	
Cousin donor	4 (4.5)	3 (3.4)		5.5 (3.1)	4.7 (2.8)	
Child donor	39 (43.8)	40 (45.5)		86.7 (48.9)	81.2 (47.5)	
Gender match, n (%)			0.210			0.074
Female to male	13 (14.6)	20 (22.7)		26.1 (14.7)	29.8 (17.4)	
Others	76 (85.4)	68 (77.3)		151.3 (85.3)	141.0 (82.6)	
HLA, n (%)			0.148			0.034

Characteristics	Before IPTW		SMD	After IPTW		SMD
	Lower-dose n=89	Higher-dose n=88		Lower-dose n=177.39	Higher-dose n=170.78	
5/10M	51 (57.3)	48 (54.5)		99.8 (56.3)	95.6 (56.0)	
6/10M	16 (18.0)	17 (19.3)		33.2 (18.7)	34.0 (19.9)	
7/10M	14 (15.7)	12 (13.6)		24.3 (13.7)	22.1 (12.9)	
8/10M	5 (5.6)	8 (9.1)		15.3 (8.6)	14.5 (8.5)	
9/10M	3 (3.4)	3 (3.4)		4.8 (2.7)	4.6 (2.7)	
ABO match, n (%)			0.084			0.001
Match	53 (59.6)	56 (63.6)		108.7 (61.3)	104.5 (61.2)	
Mismatch	36 (40.4)	32 (36.4)		68.7 (38.7)	66.3 (38.8)	
Donor/recipient CMV serostatus, n (%)			0.120			0.065
Negative/negative	35 (39.3)	31 (35.2)		68.9 (38.8)	61.6 (36.0)	
Negative/positive	27 (30.3)	34 (38.6)		59.0 (33.3)	60.8 (35.6)	
Positive/negative	6 (6.7)	8 (9.1)		12.1 (6.8)	12.7 (7.4)	
Positive/positive	21 (23.6)	15 (17.0)		37.4 (21.1)	35.8 (21.0)	
Donor/recipient EBV serostatus, n (%)			0.214			0.062
Negative/negative	79 (88.8)	80 (90.9)		158.4 (89.3)	155.5 (91.0)	
Negative/positive	2 (2.2)	3 (3.4)		5.6 (3.1)	4.5 (2.7)	
Positive/negative	4 (4.5)	1 (1.1)		4.9 (2.8)	3.5 (2.1)	
Positive/positive	4 (4.5)	4 (4.5)		8.5 (4.8)	7.2 (4.2)	
Conditioning regimen, n (%)			0.006			0.066
Myeloablative	71 (79.8)	70 (79.5)		133.9 (75.5)	133.7 (78.3)	
Reduced-intensity	18 (20.2)	18 (20.5)		43.4 (24.5)	37.1 (21.7)	
Non-myeloablative	0(0.0)	0(0.0)		0(0.0)	0(0.0)	
Stem cell source, n (%)			—			—
Peripheral blood stem cell	89 (100.0)	88 (100.0)		177.4 (100.0)	170.8 (100.0)	
Peripheral blood stem cell + Bone marrow	0(0.0)	0(0.0)		0(0.0)	0(0.0)	
Cumulative rATG dose category, n (%)			0.383			0.049
≤6 mg/kg	41 (46.1)	57 (64.8)		99.9 (56.3)	100.3 (58.7)	
>6 mg/kg	48 (53.9)	31 (35.2)		77.5 (43.7)	70.5 (41.3)	
Median nucleated cells, ×10 ⁸ /kg (range)	11.68 (4.38,36.22)	10.22 (2.96,26.45)	0.279	10.66 (4.38,36.22)	10.78 (2.96,26.45)	0.011
Median CD34 ⁺ cells, ×10 ⁶ /kg (range)	5.50 (3.00,18.12)	6.15 (1.63,17.64)	0.040	5.63 (3.00,18.12)	6.11 (1.63,17.64)	0.006

SMD was not estimable for stem-cell source because all patients in the immune-reconstitution cohort received peripheral blood stem-cell grafts.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CR, complete remission; EBV, Epstein–Barr virus; ECOG, Eastern Cooperative Oncology Group score; HCT–CI, Hematopoietic Cell Transplantation–Comorbidity Index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; IPTW, inverse probability of treatment weighting; IQR, interquartile range; KPS, Karnofsky performance status; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndrome(s); MRD[−], measurable residual disease negative; MRD⁺, measurable residual disease positive; NR, no remission; SMD, standardized mean differences.

Table S5. Distribution of treatment allocation according to transplantation year

Transplantation year	Lower-dose	Higher-dose	<i>P</i> value
2022, n (%)	44 (53.7)	38 (46.3)	0.996
2023, n (%)	48 (53.3)	42 (46.7)	
2024, n (%)	53 (54.6)	44 (45.4)	
2025, n (%)	4 (57.1)	3 (42.9)	

Table S6. Sensitivity analysis additionally adjusted for transplantation year in the doubly robust IPTW models

Outcome	Primary model	Sensitivity model
Grade I–IV aGVHD, SHR (95% CI), P	0.72 (0.44–1.16), P=0.175	0.75 (0.46–1.17), P=0.178
Grade II–IV aGVHD, SHR (95% CI), P	0.45 (0.25–0.84), P=0.011	0.47 (0.25–0.86), P=0.014
Grade III–IV aGVHD, SHR (95% CI), P	0.39 (0.18–0.88), P=0.022	0.37 (0.16–0.85), P=0.019
GRFS, HR (95% CI), P	0.59 (0.43–0.83), P=0.002	0.59 (0.42–0.82), P=0.002
OS, HR (95% CI), P	1.26 (0.71–2.23), P=0.427	1.24 (0.70–2.18), P=0.463
DFS, HR (95% CI), P	1.04 (0.63–1.71), P=0.884	1.01 (0.61–1.68), P=0.968
CIR, SHR (95% CI), P	1.07 (0.53–2.12), P=0.857	1.12 (0.58–2.17), P=0.739
NRM, SHR (95% CI), P	0.82 (0.37–1.82), P=0.632	0.74 (0.34–1.63), P=0.461

Primary model: doubly robust IPTW model. Sensitivity model: primary model additionally adjusted for transplantation year.

Abbreviations: aGVHD, acute graft-versus-host disease; CI, confidence interval; CIR, cumulative incidence of relapse; DFS, disease-free survival; GRFS, graft-versus-host disease-free, relapse-free survival; HR, hazard ratio; IPTW, inverse probability of treatment weighting; NRM, non-relapse mortality; OS, overall survival; SHR, subdistribution hazard ratio.

Table S7. Baseline characteristics of patients included and not included in the immune-reconstitution analysis

Characteristic	Included n=177	Not included n=99	P value
Patient age, median (IQR)	35.0 (27.0, 45.0)	35.0 (26.5, 46.0)	0.860
Treatment group, n (%)			
Lower-dose	89 (50.3)	60 (60.6)	0.127
Higher-dose	88 (49.7)	39 (39.4)	
Donor age, median (IQR)	30.0 (20.0, 44.0)	34.0 (21.0, 47.0)	0.292
Patient gender, n (%)			0.891
Male	99 (55.9)	57 (57.6)	
Female	78 (44.1)	42 (42.4)	
HCT-CI, n (%)			0.049
0	36 (20.3)	31 (31.3)	
1–2	79 (44.6)	45 (45.5)	
>2	62 (35.0)	23 (23.2)	
ECOG, n (%)			0.016
0–1	161 (91.0)	98 (99.0)	
>1	16 (9.0)	1 (1.0)	
KPS, n (%)			0.117
≥ 90	146 (82.5)	73 (73.7)	
< 90	31 (17.5)	26 (26.3)	
Diagnosis, n (%)			0.080
ALL	59 (33.3)	20 (20.2)	
AML	87 (49.2)	56 (56.6)	
MDS	18 (10.2)	10 (10.1)	
LBL	13 (7.3)	13 (13.1)	
Disease status before HSCT, n (%)			0.004
CR, MRD-	145 (81.9)	70 (70.7)	
CR, MRD+	25 (14.1)	14 (14.1)	
NR	7 (4.0)	15 (15.2)	
Risk stratification, n (%)			<0.001
Low	52 (29.4)	9 (9.1)	
Intermediate	31 (17.5)	28 (28.3)	
High	94 (53.1)	62 (62.6)	
Donor/recipient relationship, n (%)			0.259
Parent donor	52 (29.4)	28 (28.3)	
Sibling donor	39 (22.0)	32 (32.3)	
Cousin donor	7 (4.0)	2 (2.0)	
Child donor	79 (44.6)	37 (37.4)	
Gender match, n (%)			0.876
Female to male	33 (18.6)	20 (20.2)	
Others	144 (81.4)	79 (79.8)	
HLA, n (%)			0.037
5/10M	99 (55.9)	73 (73.7)	

Characteristic	Included n=177	Not included n=99	P value
6/10M	33 (18.6)	12 (12.1)	
7/10M	26 (14.7)	9 (9.1)	
8/10M	13 (7.3)	5 (5.1)	
9/10M	6 (3.4)	0 (0.0)	
ABO match, n (%)			0.395
Match	109 (61.6)	55 (55.6)	
Mismatch	68 (38.4)	44 (44.4)	
Donor/recipient CMV serostatus, n (%)			<0.001
Negative/negative	66 (37.3)	41 (41.4)	
Negative/positive	61 (34.5)	49 (49.5)	
Positive/negative	14 (7.9)	0 (0.0)	
Positive/positive	36 (20.3)	9 (9.1)	
Donor/recipient EBV serostatus, n (%)			0.125
Negative/negative	159 (89.8)	85 (85.9)	
Negative/positive	5 (2.8)	1 (1.0)	
Positive/negative	5 (2.8)	9 (9.1)	
Positive/positive	8 (4.5)	4 (4.0)	
Conditioning regimen, n (%)			0.069
Myeloablative	141 (79.7)	83 (83.8)	
Reduced-intensity	36 (20.3)	14 (14.1)	
Non-myeloablative	0 (0.0)	2 (2.0)	
Stem cell source, n (%)			<0.001
Peripheral blood stem cell	177 (100.0)	82 (82.8)	
Peripheral blood stem cell + Bone marrow	0 (0.0)	17 (17.2)	
Cumulative rATG dose category, n (%)			0.146
≤6 mg/kg	98 (55.4)	45 (45.5)	
>6 mg/kg	79 (44.6)	54 (54.5)	
Median nucleated cells, ×10 ⁸ /kg (range)	11.03 (2.96–36.22)	10.56 (2.64–29.95)	0.815
Median CD34+ cells, ×10 ⁶ /kg (range)	5.98 (1.63–18.12)	5.74 (2.35–20.00)	0.177
Transplantation year, n (%)			0.332
2022	54 (30.5)	28 (28.3)	
2023	63 (35.6)	27 (27.3)	
2024	56 (31.6)	41 (41.4)	
2025	4 (2.3)	3 (3.0)	

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CR, complete remission; EBV, Epstein–Barr virus; ECOG, Eastern Cooperative Oncology Group; HCT-CI, Hematopoietic Cell Transplantation–Comorbidity Index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; KPS, Karnofsky Performance Status; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndrome; MRD, measurable residual disease; NR, no remission.

Table S8. Infectious complications after haplo-HSCT

Infectious complications	Lower-dose n=149	Higher-dose n=127	<i>P</i> value	IPTW-adjusted <i>P</i> value
Pneumonia, n (%)	42 (28.2)	21 (16.5)	0.022	0.458
Bloodstream infection, n (%)	19 (12.8)	4 (3.1)	0.004	0.014
Viraemia				
CMV viraemia, n (%)	111 (74.5)	90 (70.9)	0.501	0.662
CMV viraemia episodes, median (range)	1 (1–6)	1 (1–4)	0.641	0.558
Longest CMV viraemia duration, days, median (range)	30 (3–201)	34 (5–123)	0.048	0.423
EBV viraemia, n (%)	45 (30.2)	49 (38.6)	0.162	0.863
EBV viraemia episodes, median (range)	1 (1–4)	1 (1–6)	0.920	0.944
Longest EBV viraemia duration, days, median (range)	16 (2–109)	10 (3–156)	0.675	0.761

The number and longest duration of viraemia episodes were calculated among patients who developed the corresponding viraemia.

Abbreviations: CMV, cytomegalovirus; EBV, Epstein–Barr virus; haplo-HSCT, haploidentical hematopoietic stem cell transplantation; IPTW, inverse probability of treatment weighting.

Table S9. First GRFS-defining event

First GRFS-defining event	Lower-dose n=149	Higher-dose n=127
Grade III–IV aGVHD, n (%)	16 (10.7)	8 (6.3)
Moderate-to-severe cGVHD, n (%)	24 (16.1)	19 (15.0)
Relapse, n (%)	20 (13.4)	20 (15.7)
Death without a prior GRFS-defining event, n (%)	11 (7.4)	4 (3.1)
Terminal event leading to death, n		
Infection	3	3
Graft failure	4	1
Organ failure	2	0
Treatment-related complications	1	0
Secondary malignancy	1	0
No GRFS event, n (%)	78 (52.3)	76 (59.8)

Percentages were calculated using the total number of patients in each treatment group as the denominator. For patients experiencing multiple GRFS-defining events, only the first event was included in this analysis. The listed terminal events refer only to patients whose first GRFS-defining event was death. These categories describe the immediate clinical event leading to death and are distinct from the adjudicated primary causes of death reported in Table S3. Terminal-event categories are mutually exclusive and sum to the corresponding total number of deaths without a prior GRFS-defining event.

Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; GRFS, graft-versus-host disease-free, relapse-free survival.

Table S10. Cause-specific mortality analyses

Outcome	Events, lower-dose/higher-dose	Gray's test P	IPTW-weighted Fine–Gray model	IPTW-weighted cause-specific Cox model
Acute GVHD-related mortality	4/8	0.143	sHR 3.35 (95% CI, 0.96–11.72); P=0.058	csHR 3.27 (95% CI, 0.94–11.43); P=0.063
Overall GVHD-related mortality	9/13	0.200	sHR 1.95 (95% CI, 0.77–4.91); P=0.158	csHR 1.90 (95% CI, 0.75–4.81); P=0.173
Relapse-related mortality	8/15	0.054	sHR 1.81 (95% CI, 0.67–4.90); P=0.240	csHR 1.77 (95% CI, 0.65–4.79); P=0.263

Event counts are presented for the lower-dose and higher-dose groups, respectively. Gray's test compared the unweighted cumulative incidence functions. Subdistribution hazard ratios and cause-specific hazard ratios compare the higher-dose group with the lower-dose group and were estimated using the prespecified inverse probability of treatment weights with robust variance estimators. Deaths from causes other than the event of interest were treated as competing events in the cumulative incidence and Fine–Gray analyses. Causes of death were classified according to the adjudicated primary cause.

Abbreviations: CI, confidence interval; csHR, cause-specific hazard ratio; GVHD, graft-versus-host disease; IPTW, inverse probability of treatment weighting; sHR, subdistribution hazard ratio.

Supplementary Figures

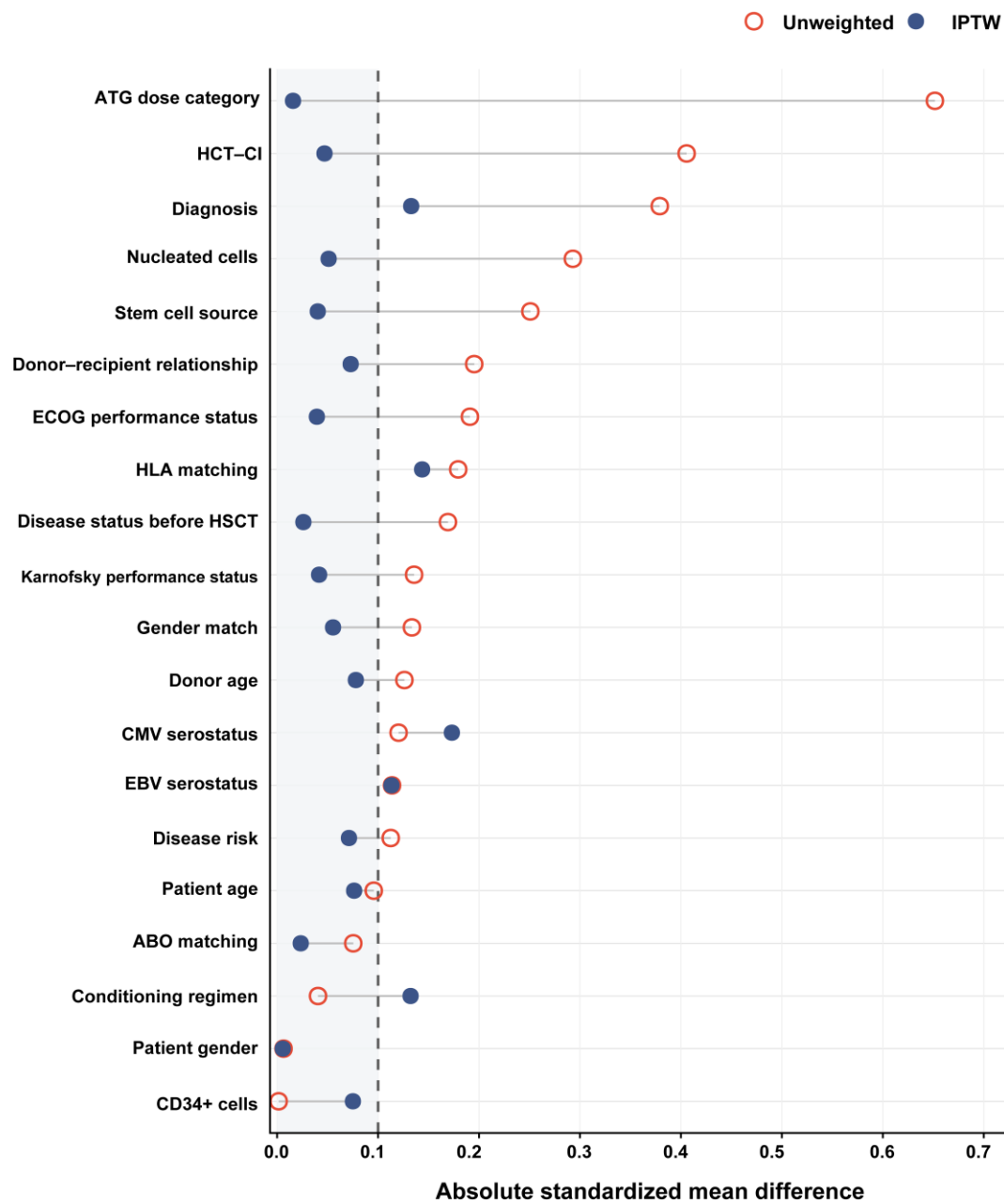


Figure S1. Assessment of covariate balance before and after IPTW.

Abbreviations: ABO, ABO blood group system; ATG, anti-thymocyte globulin; CD, cluster of differentiation; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; HCT-CI, Hematopoietic Cell Transplantation-Comorbidity Index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; IPTW, inverse probability of treatment weighting.

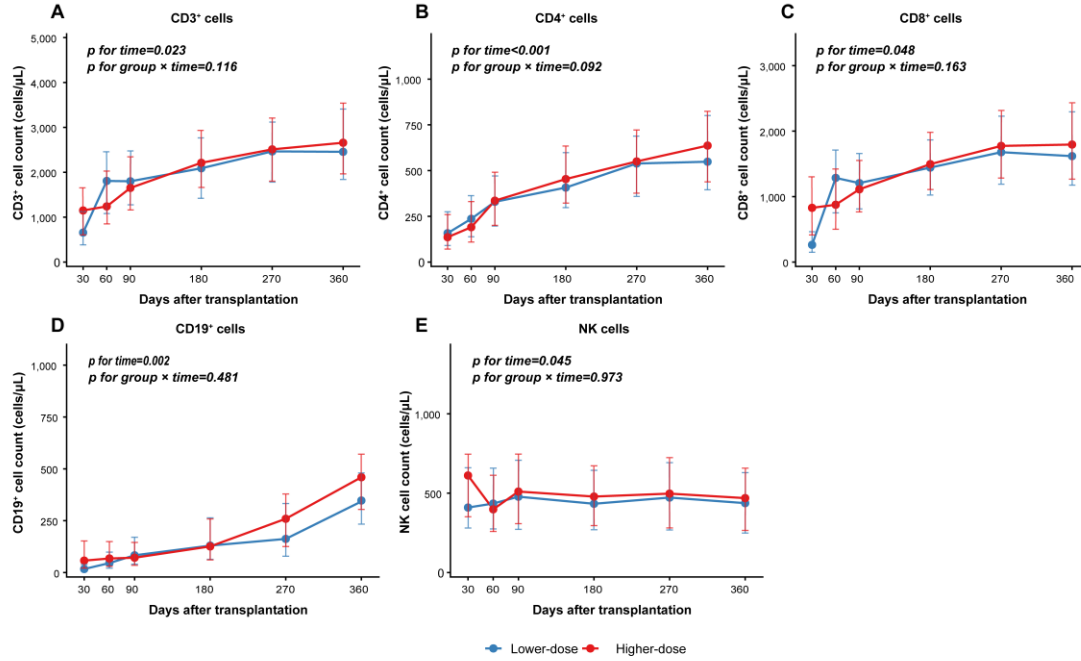


Figure S2. Longitudinal trajectories of lymphocyte subsets after transplantation. (A) CD3⁺ Cell. (B) CD4⁺ Cell. (C) CD8⁺ Cell. (D)CD19⁺ Cell. (E)NK Cell.

Lymphocyte subset counts were transformed using $\log(\text{count} + 1)$ and analyzed using linear mixed-effects models. Models included treatment group, categorical post-transplant time, the group-by-time interaction, age, sex, and cumulative rATG dose category, with a patient-specific random intercept. Points and error bars represent back-transformed estimated marginal means and 95% confidence intervals.

Abbreviations: ATG, anti-thymocyte globulin; NK, natural killer.

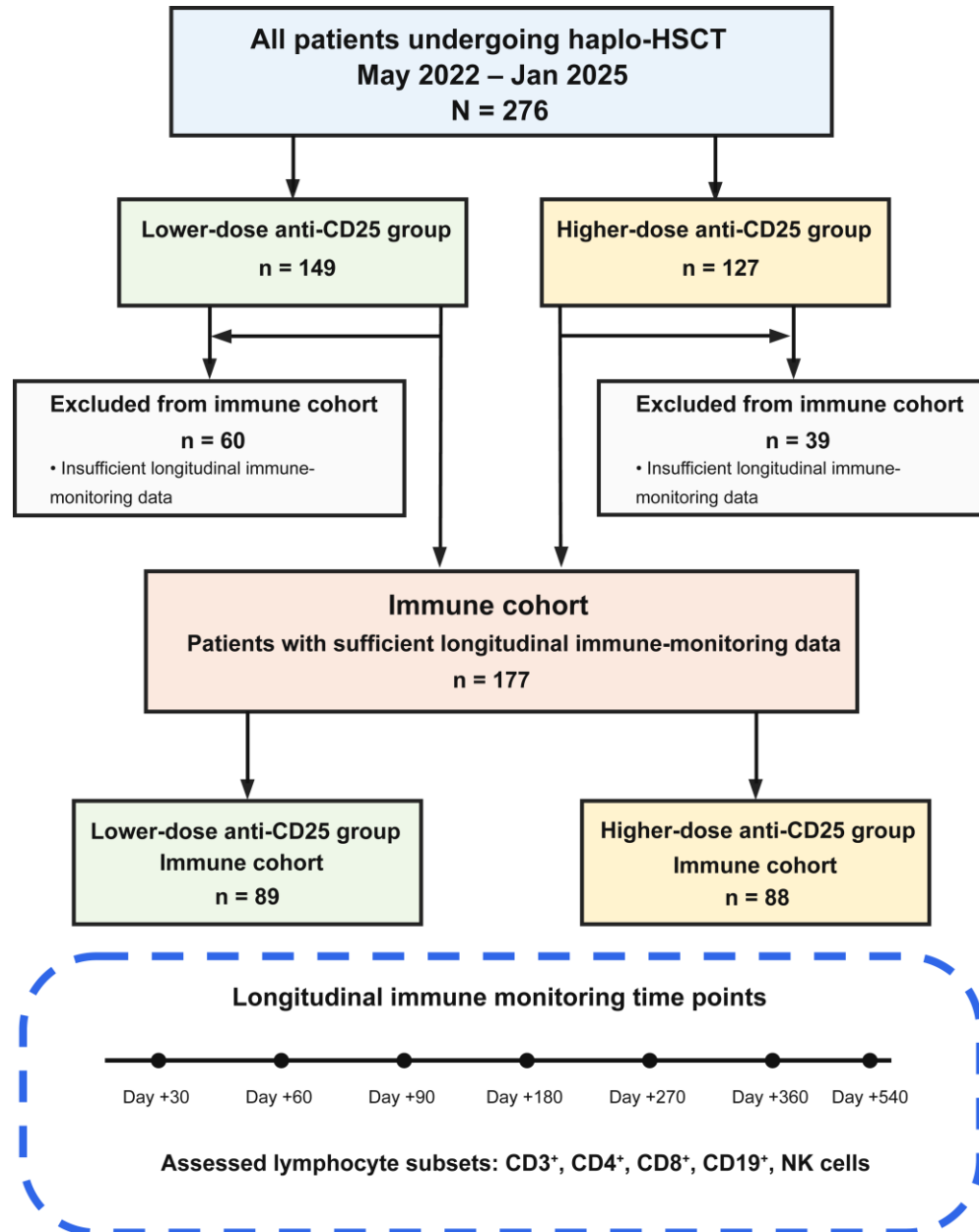


Figure S3. Selection of patients for the immune-reconstitution cohort and schedule of longitudinal immune monitoring. Among the 276 patients who underwent haploidentical hematopoietic stem cell transplantation between May 2022 and January 2025, 149 received the lower-dose anti-CD25 strategy, and 127 received the higher-dose anti-CD25 strategy. Patients with sufficient serial flow-cytometry measurements across the predefined assessment periods were included in the immune-reconstitution cohort. The final immune-reconstitution cohort comprised 177 patients, including 89 in the lower-dose group and 88 in the higher-dose group. The remaining 99 patients, including 60 in the lower-dose group and 39 in the higher-dose group, were not included because sufficient longitudinal immune-monitoring data were unavailable. Immune assessments were performed at approximately days +30, +60, +90, +180, +270, +360, and +540 and included CD3⁺, CD4⁺, CD8⁺, CD19⁺, and natural killer cells. Abbreviations: haplo-HSCT, haploidentical hematopoietic stem cell transplantation; NK, natural killer.

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