Better outcomes of modified myeloablative conditioning without antithymocyte globulin versus myeloablative conditioning in cord blood transplantation for hematological malignancies: A retrospective (development) and a prospective (validation) study

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Cord blood transplantation (CBT) is an effective option for treating hematological malignancies, but graft failure (GF) remains the primary cause of therapy failure. Thus, based on myeloablative conditioning (MAC) of busulfan with cyclophosphamide (Bu/Cy) or total body irradiation with Cy (TBI/Cy), fludarabine (Flu) was added to Bu/Cy and cytarabine (CA) to TBI/Cy for a modified myeloablative conditioning (MMAC). To compare the prognosis of MMAC with MAC, we conducted a retrospective study including 58 patients who underwent CBT with MAC or MMAC from 2000 to 2011. Neutrophil and platelet engraftment rate, overall survival (OS) and disease free survival (DFS) were significantly higher in the MMAC group (adjusted hazard ratio [HR], 2.58, 2.43, 0.36 and 0.37; p < 0.01, p = 0.01, p = 0.02 and p = 0.02, separately). Nonrelapse mortality (NRM) was comparable (p = 0.183). To validate the outcomes noted in the MMAC group, we conducted a prospective single-arm clinical trial including 188 patients who underwent CBT with MMAC from 2011 to 2015. Engraftment rate, survival and NRM of the MMAC group in the prospective trial (MMAC-P) were similar to the MMAC group in the retrospective study (MMAC-R). This study is the first to demonstrate the superiority of MMAC to MAC in CBT for hematological malignancies.

Cord blood (CB) is an alternative option to standard graft sources for hematopoietic stem cell transplantation (HCT), and has been increasingly used in adults with hematological malignancies. There is considerable evidence that CB is a promising option for patients who lack a human leukocyte antigen (HLA)-matched related or unrelated donor.1 Several studies comparing results of CBT and either bone marrow or peripheral blood stem cell transplantation showed similar results regarding OS and leukemia-free survival, despite slower hematopoietic recovery and higher incidence of GF for CB transplant recipients.2,3 GF is a life-threatening complication of all kinds of HCT and occurs more frequently after CBT than after transplants using other standard graft sources.4 Some authors have

Key words: myeloablative conditioning, cord blood transplantation, antithymocyte globulin, hematological malignancies, engraftment

Abbreviations: ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; ATG: antithymocyte globulin; Bu: busulfan; CA: cytarabine; CB: cord blood; CBT: cord blood transplantation; CSA: cyclosporine; Cy: cyclophosphamide; DFS: disease-free survival; DSA: donor-specific anti-HLA antibodies; GF: graft failure; GRFS: GVHD-free/relapse-free survival; GVHD: graft-vs.-host disease; GVL: graft versus leukemia; HCT: hematopoietic stem cell transplantation; HLA: human leukocyte antigen; HR: hazard ratio; MAC: myeloablative conditioning; MDS: myelodysplastic syndrome; MMAC: modified myeloablative conditioning; MMF: mycophenolate mofeti; MTX: methotrexate; NRM: nonrelapse mortality; OS: overall survival; TBI: total body irradiation

Additional Supporting Information may be found in the online version of this article.

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reported that the overall incidence of GF after CBT is between 10% and 20%.\(^3\)-\(^5\) GF can be due to immunological rejection of donor cells by residual host lymphocytes, the presence of donor-specific anti-HLA antibodies (DSA), or nonimmunologic mechanisms such as poor stem cell viability and viral infections.\(^6\)-\(^7\) Particularly, factors associated with GF in CBT can be inherently low stem cell doses, HLA mismatch, relative immaturity of CB lymphocytes and the increasing use of reduced intensity conditioning with CBT.\(^8\)-\(^9\) Ioannis et al. studied the T-cell immune reconstitution after CBT and found that in the early post-transplant period, the predominant pathway of T-cell reconstitution was mediated by the uniformly naive T cells transferred from the cord blood or recipient T cells that had survived conditioning.\(^10\) Therefore, we speculate that the GF and delay of immune reconstitution of patients with MAC after CBT may be due to the deficiency of conditioning intensity, which will relatively increase the residual host lymphocytes. Patient lymphocytes will not only cause the immunological rejection but also compete with the naive T cells for the space in bone marrow.

Furthermore, the use of antithymocyte globulin (ATG) and methotrexate (MTX) as part of graft-versus-host disease (GVHD) prophylaxis may also be the cause of GF and prolonged hematopoietic recovery. The use of ATG could impair the early development of donor-derived T cells due to the persistence of these antibodies in the patient for several weeks after administration especially if given too close to graft infusion.\(^11\) Several studies have compared the outcomes between CBT with or without ATG, and the use of ATG was associated with lower OS and higher NRM, regardless of the lower incidence of GVHD.\(^12\)-\(^13\) Similarly, use of MTX-containing regimen for GVHD prophylaxis has been associated with delayed engraftment and increased risk of graft failure in patients with hemoglobinopathies and malignant diseases transplanted with an HLA-identical sibling CB unit.\(^14\)-\(^15\)

Thus, we conducted a retrospective study to compare the outcomes of MMAC (Flu/Bu/Cy and CA/TBI/Cy) with MAC (Bu/Cy and TBI/Cy). The GVHD prophylaxis of MMAC included cyclosporine (CSA) and mycophenolate mofetil (MMF) while the GVHD prophylaxis of MAC also included ATG or MTX. We then conducted a prospective single-arm clinical trial to validate the findings of the retrospective study.

### Materials and Methods
#### Patient and donor selection
The retrospective study was conducted at the Department of Hematology, Affiliated Provincial Hospital of Anhui Medical University from April, 2000 to November, 2011. The trial enrolled 58 patients who underwent a single-unit CBT as a first HCT. The MAC regimen consisted of Bu/Cy (Bu, 0.8 mg/kg per dose intravenously every 6 hr for 4 days; Cy, 60 mg/kg for 2 days) or TBI/Cy (TBI, 3 Gy twice daily for 2 days; Cy, 120 mg/kg). The MMAC regimen included the addition of Flu (30 mg/m² for 4 days) to Bu/Cy or CA (2 g/m² for 2 days) to TBI/Cy. The prospective study included data from 188 patients who underwent a single-unit CBT as a first HCT following the MMAC regimen of Flu/Bu/Cy (n = 83) or CA/TBI/Cy (n = 105) from December, 2011 to December, 2015. All patients suffered from acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) requiring allogeneic HCT. Patients who received a related HLA-matched HCT or haploidentical transplantation or a double-unit CBT were excluded. The choice of conditioning regimen was made according to the discussion of our transplantation team. Our protocol complied with the Declaration of Helsinki and was approved by the Anhui Medical University Institutional Review Board, and all patients included in the study provided informed consent. The validation trial was registered at www.chictr.org.cn as # ChiCTR-ONRC-11001430.

The CB unit selection was based primarily on the total nucleated cell number among 4/6 to 6/6 HLA-matched units. HLA-A and HLA-B antigens were identified by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing. Anti-HLA antibody screening was not performed. Cord blood units were obtained through the Chinese Cord Blood Bank Network.

#### GVHD prophylaxis and treatment and supportive care
All patients were given a combination of CsA and MMF for GVHD prophylaxis. CsA was started (2.5–3 mg/kg/day) on Day −1 and continued until patients were able to take CsA orally with target trough levels of 200–250 ng/mL for at least one month, and then CsA blood concentration was kept at about 150–250 ng/mL for at least 6 months. MMF (25–30 mg/kg/day, p.o. three times a day) was started on Day +1 until Day +28 or neutrophil recovery. Thirteen patients of
Neutrophil engraftment was defined as the achievement of an ANC ≥0.5 × 10^9/L for three consecutive days and platelet recovery was defined as the achievement of a platelet count ≥20 × 10^9/L unsupported by platelet transfusions for 7 days. Primary graft failure was defined as the failure to reach an ANC ≥0.5 × 10^9/L for at least three consecutive days or a mixed chimerism of donor cells ≥10% on Day 42 following transplantation based on chimerism analysis using polymorphic genetic markers. The assessment of pre-engraftment syndrome, grading of acute GVHD (aGVHD) and grading of chronic GVHD (cGVHD) was performed according to standard criteria. Patients surviving in remission for least 100 days after transplantation were regularly evaluated for chronic GVHD. Relapse was defined based on morphological evidence of disease in the peripheral blood, marrow or extra-medullary sites. NRM was defined as death after CBT without disease progression or relapse. The OS was measured from the time of CBT to the time of death from any cause or last recorded follow-up. Post-transplant disease-free survival (DFS) was defined as the time interval from CBT to relapse, death or the last contact. GVHD-free/relapse-free survival (GRFS) was defined as the time interval from CBT to grade II to IV aGVHD, systemic therapy-requiring cGVHD, relapse or death.

Statistical analyses
Patient and transplantation characteristics from different groups were compared using the Mann–Whitney nonparametric U test for continuous variables and the χ^2 test or the Fisher-exact test for the categorical variables. Engraftment, NRM, relapse, aGVHD and cGVHD were analyzed using Gray’s method. NRM was calculated considering relapse as a competing risk. The Fine-Gray proportional hazards model was used in multivariate analyses for engraftment, NRM, relapse, aGVHD and cGVHD. OS, DFS and GRFS rate were calculated with the Kaplan–Meier method and compared using log-rank tests. Factors with significance or borderline significance (p < 0.2) in the univariate analysis were subjected to a multivariate analysis using the Cox proportional hazards model. Statistical analyses were performed using SPSS software, version 21.0. The α level of all tests and the p values was set at 0.05.

Results
Characteristics of patients and cord bloods
The characteristics of patients and transplants are illustrated in Tables 1 and 2 separately for retrospective study and prospective study. In the retrospective study, the median age was 11 years (2–42) and 41 (72.4%) of the patients were male, 34 (75.9%) were defined as a high risk and the median follow-up period for surviving patients was ~6.3 years. The patient’s sex, diagnosis, disease status, extent of HLA match and cell dose showed no significant differences between the MAC group and the MMAC group. However, there were considerable differences with respect to patient’s age, body weight, GVHD prophylaxis and conditioning types (Bu-based or TBI-based). Patients with MAC were younger and lighter. Thirteen patients in MAC group received ATG as part of GVHD prophylaxis, and the other 11 patients received MTX, while ATG and MTX were omitted in MMAC group (p < 0.05). Additionally, patients in MAC group received more Bu-based conditioning (p < 0.05). In the prospective study, the median age was 13 years (2–47), 146 (65.8%) of the patients were male and 188 (84.7%) were defined as high risk and the median follow-up period for surviving patients was ~3.2 years. There were no statistical differences between MMAC-R group and MMAC-P group in patients’ sex, age, body weight, disease status, nuclear cell count of grafts and extent of HLA match. However, there were more patients with ALL in MMAC-P group and the median follow-up period for surviving patients was shorter (p < 0.05 and p = 0.00, separately).

Neutrophil and platelet engraftment
In the retrospective study, neutrophil engraftment rate by 30 days was significantly higher in MMAC group (Fig. 1a; 97.1% vs. 62.5%, p < 0.01). This difference was also significant in univariate analysis (Supporting Information, Table S1; HR, 2.39; 95%CI, 1.29–4.45; p < 0.01). Besides, more loci of HLA mismatch and shorter pretransplant therapy period were associated with lower neutrophil engraftment rate with borderline significance (p < 0.2). With the extent of HLA mismatch and pretransplant therapy period as the confounding factors, MMAC showed remarkable association with higher incidence of neutrophil engraftment (Supporting Information, Table S1; adjusted HR, 2.58; 95%CI, 1.39–4.79; p < 0.01) in the multivariate analysis.

Platelet engraftment rate by 120 days was superior in the MMAC group (Fig. 1b; 82.4% vs. 50.0%, p < 0.05). In the univariate analysis, MMAC, heavier weight (weighted 45 kg or heavier) and more CD34+ cells were associated with higher platelet engraftment rate with borderline significance (p < 0.2) (p = 0.01, p = 0.19 and p = 0.18, separately). In the multivariate analysis including these three factors, MMAC showed significant association with the increase of platelet engraftment (Supporting Information, Table S1; adjusted HR, 2.43; 95%CI, 1.23–4.79; p = 0.01).

In the validation study, there were no statistical differences between MMAC-P group and MMAC-R group in neither neutrophil engraftment rate nor platelet engraftment rate (96.3% vs. 97.1% and 88.8% vs. 82.4%, p = 0.28 and
Table 1. Patients and grafts characteristics for retrospective study

|                        | MAC       | MMAC      | p       |
|------------------------|-----------|-----------|---------|
| **Patient number**     | 24        | 34        |         |
| **Median age (range), years** | 8 (2–42) | 14 (3–37) | 0.013¹ |
| ≤20                    | 22        | 27        |         |
| >20                    | 2         | 7         | 0.367   |
| **Median weight (range), kg** | 26 (12–55)| 42 (12–66)| 0.005¹ |
| ≤45                    | 22        | 23        |         |
| >45                    | 2         | 11        | 0.066   |
| **Male sex, n (%)**    | 17 (71)   | 25 (74)   | 1       |
| **Sex (donor/patient), n (%)** |         | 0.354     |         |
| Male/male              | 8 (33.3)  | 8 (23.5)  |         |
| Male/female            | 3 (12.5)  | 7 (20.6)  |         |
| Female/male            | 8 (33.3)  | 16 (47.1) |         |
| Female/female          | 4 (16.7)  | 2 (5.9)   |         |
| Missing data           | 1 (4.2)   | 1 (2.9)   |         |
| **Diagnosis, n (%)**   |           | 0.371     |         |
| ALL                    | 15 (62.5) | 16 (47.1) |         |
| AML or MDS             | 9 (37.5)  | 18 (52.9) |         |
| **Disease status, n (%)** |         | 0.831     |         |
| CR1                    | 15 (62.5) | 19 (55.9) |         |
| CR2                    | 6 (25)    | 11 (34.5) |         |
| ≥CR3 or NR             | 3 (12.5)  | 4 (11.8)  |         |
| **Disease risk**       |           | 0.539     |         |
| Intermediate           | 7 (29.2)  | 7 (20.6)  |         |
| High                   | 17 (70.8) | 27 (79.4) |         |
| **Pretransplant therapy period** |         | 0.733     |         |
| Median, days           | 199 (98–1542) | 218 (80–2545) |         |
| ≤200                   | 12 (50.0) | 16 (47.1) |         |
| >200                   | 11 (45.8) | 18 (52.9) |         |
| Unknown                | 1 (4.2)   | 0         | 0.913   |
| **No of HLA-A, B, DR mismatched, n (%)** |         | 0.090     |         |
| 0                      | 1 (4.2)   | 4 (11.8)  |         |
| 1                      | 20 (83.3) | 19 (55.9) |         |
| ≥2                     | 3 (12.5)  | 11 (34.5) |         |
| **Cell compositions in allograft** |         | 0.099     |         |
| Infused nuclear cells 10⁷/kg | 4.99 (2.70–16.24) | 4.03 (1.96–9.60) |         |
| ≤3.99                  | 7         | 17        |         |
| >3.99                  | 17        | 17        | 0.188   |
| Infused CD34+ cells 10⁵/kg | 2.45 (0.90–21.11) | 2.43 (1.04–5.24) | 0.548   |
| ≤2.38                  | 10        | 16        |         |
| >2.38                  | 14        | 18        | 0.890   |
| **Conditioning, n (%)** |           | 0.002¹    |         |
| Bu based               | 22 (91.7) | 17 (50.0) |         |
| TBI based              | 2 (83.3)  | 17 (40.0) |         |
| GVHD prophylaxis, n (%) |           | 0.000¹    |         |
| CSA/MMF/ATG            | 13 (54.5) | 0         |         |
\( p = 0.56, \) separately), which demonstrated the efficacy and constancy of MMAC in improving engraftment.

**Nonrelapse Mortality**

NRM of the MMAC group was lower than the MAC group in the retrospective study (Fig. 1c; 26.5% vs. 41.7%, \( p = 0.18 \)). While in the prospective study, the rate of NRM was further reduced in the MMAC-P group (Fig. 3c; 17.8% vs. 26.5%, \( p = 0.22 \)).

We also analyzed the causes of NRM. Ten patients in MAC group, 9 in MMAC-R group and 38 in MMAC-P group died without relapse. The main factors leading to NRM were infection and GVHD. There were no statistical differences between these three groups in each cause.

**Relapse and GVHD**

The cumulative incidence of relapse by 3 years was lower in MMAC Group (5.9% vs. 12.5%, \( p = 0.37 \)). However, in the validation study, relapse rate was higher in the MMAC-P Group (23.4% vs. 5.9%, \( p = 0.03 \)). Univariate analysis showed that MMAC-P, more patients with ALL, younger age (aged 20 years or younger), worse disease status (CR3 or NR), male and more infused nuclear cells (infused 3.99 × 10^9/kg or more) were associated with higher rate of relapse. One locus of HLA mismatch was associated with lower relapse rate (Supporting Information, Table S4). In the multivariate analysis, more patients with AML/MDS and one locus of HLA mismatch were related to lower relapse rate (Supporting Information, Table S4; adjusted HR, 0.33 and 0.42; \( p = 0.03 \) and 0.04, separately). While male showed significant relation to higher relapse rate (Supporting Information, Table S4; adjusted HR, 2.83; \( p = 0.02 \)).

Grade II to IV aGVHD, Grade III to IV aGVHD and cGVHD showed no statistical differences in both retrospective study and validation study.

**Survival**

In the retrospective study, 3 years of OS and DFS were significantly improved in the MMAC group (Figs. 2a and 2b; 67.6% vs. 45.8% and 67.6% vs. 45.8%, \( p < 0.05 \) and \( p < 0.05 \), separately). These differences were also significant in univariate analysis (Supporting Information, Table S2; HR, 0.45 and 0.45; 95%CI, 0.20–1.02 and 0.20–1.00; \( p = 0.06 \) and 0.05, separately) with borderline significance (\( p < 0.2 \)). Besides, grade III to IV aGVHD was associated with the reduction of OS and DFS (Supporting Information, Table S2; HR, 5.89 and 5.58; 95%CI, 2.23–15.58 and 2.10–14.79; \( p = 0.00 \) and 0.00, separately). With grade III to IV aGVHD as a confounding factor, MMAC showed a remarkable relation with the increase of OS (Supporting Information, Table S2; HR, 0.36 and 0.37; 95%CI, 0.16–0.84 and 0.16–0.84; \( p = 0.02 \) and 0.02, separately). In the validation study, 3 years of OS and DFS were almost the same between the MMAC-P group and MMAC-R group (Figs. 4a and 4b; 68.3% vs. 67.6% and 68.3% vs. 58.9%, \( p = 0.52 \), separately).

Three years of GRFS was lower in the MAC group than MMAC group (Fig. 2c; 45.8% vs. 67.6%; \( p = 0.09 \)) in the retrospective study. In the prospective study, three years of GRFS was almost the same between MMAC-P group and MMAC-R group (Fig. 4c; 54.1% vs. 67.6%, \( p = 0.28 \)).

**Immune reconstitution**

In this study, we also analyzed the immune reconstitution of T cells and NK cells one month after transplantation. In the retrospective study, the proportion of CD3+ cells and CD8+ T cells accounting for lymphocytes was slightly higher in the MMAC group than MAC Group (57.7% vs. 35.2% and 40.0% vs. 20.8%, \( p = 0.16 \) and \( p = 0.25 \), separately). And there were significant differences in the proportion of CD4+ T cells and NK cells to lymphocytes between MMAC group and MAC Group (17.9% vs. 5.4% and 33.9% vs. 14.2, \( p = 0.01 \) and \( p < 0.05 \), separately).

In the validation study, there were no statistical differences between MMAC-P group and MMAC-R group in the proportion of CD3+ cells, CD4+ T cells, CD8+ T cells and NK cells accounting to lymphocytes (49.2% vs. 57.7%, 13.9% vs. 17.9%, 21.4% vs. 40.0% and 41.6% vs. 33.9%; \( p = 0.71 \), \( p = 0.25 \), \( p = 0.16 \) and \( p = 0.84 \), separately).

**Discussion**

In this study, we compare the prognosis of MMAC (Flu/Bu/ Cy or CA/TBI/Cy) with MAC (Bu/Cy or TBI/Cy) in CBT for hematological malignancies (ALL/AML/MDS), and there are three main findings: MMAC significantly improved engraftment rate and overall survival and at the same time avoided the increase of NRM.

We found that MMAC without ATG or MTX for GVHD prophylaxis showed remarkable association with the increase of engraftment rates for both neutrophil and platelet (adjusted HR, 2.58 and 2.43; \( p < 0.01 \) and \( p = 0.01 \), separately). Similar to our results, some authors have reported that lower conditioning intensity is associated with delayed...

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Table 1. Patients and grafts characteristics for retrospective study (Continued)

|                | MAC        | MMAC      | \( p \)  |
|----------------|------------|-----------|----------|
| CSA/MMF/MTX    | 11 (45.5)  | 0         |          |
| CSA/MMF        | 0          | 34 (100)  |          |
| Follow-up period (range), days\(^2\) | 3570 (2035–4864) | 2248 (1914–3088) | 0.039\(^\dagger\) |

\(^1\)Statistically significant.

\(^2\)Follow-up period was for surviving patients.
Table 2. Patients and grafts characteristics for prospective study

|                          | MMAC-R   | MMAC-P   | p     |
|--------------------------|----------|----------|-------|
| Patient number           | 34       | 188      |       |
| Median age, years (range)| 14 (3–37)| 13 (2–47)| 0.845 |
| ≤ 20                     | 27       | 143      |       |
| > 20                     | 7        | 45       | 0.838 |
| Median weight, kg (range)| 42 (12–66)| 42 (10–100)| 0.786 |
| ≤ 45                     | 23       | 104      |       |
| More than 45             | 11       | 84       | 0.251 |
| Male sex, n (%)          | 25 (74)  | 121 (64.4)| 0.401 |
| Male/female              | 8 (23.5) | 57 (30.3) |       |
| Female/male              | 16 (47.1)| 64 (34.0) |       |
| Female/female            | 2 (5.9)  | 33 (17.6) |       |
| Missing data             | 1 (2.9)  | 0        |       |
| Diagnosis, n (%)         | 0.021^   |          |       |
| ALL                      | 16 (47.1)| 130 (69.1)|      |
| AML or MDS               | 18 (52.9)| 58 (30.9) |       |
| Disease status, n (%)    | 0.647    |          |       |
| CR1                      | 19 (55.9)| 101 (53.7)|      |
| CR2                      | 11 (34.5)| 53 (28.2) |       |
| ≥ CR3 or NR              | 4 (11.8) | 34 (18.1) |       |
| Disease risk             | 0.436    |          |       |
| Intermediate             | 7 (20.6) | 27 (14.4) |       |
| High or very high        | 27 (79.4)| 161 (85.6)|      |
| Pretransplant therapy period |       |          |       |
| Median, days             | 218 (80–2545)| 227 (15–5449)| 0.367 |
| ≤ 200                    | 16       | 80       |       |
| > 200                    | 18       | 108      | 0.764 |
| No of HLA-A, B, DR mismatched, n (%) | 0.889 |          |       |
| 0                        | 4 (11.8) | 28 (14.9) |       |
| 1                        | 19 (55.9)| 100 (53.2)|       |
| ≥ 2                      | 11 (34.5)| 60 (31.9) |       |
| Cell compositions in allografts (range) |       |          |       |
| Infused nuclear cells 10^7/kg | 4.03 (1.96-9.60)| 3.91 (1.98-17.27)| 0.744 |
| ≤ 4.89                   | 17       | 99       |       |
| > 4.89                   | 17       | 89       | 0.921 |
| Infused CD34+ cells 10^5/kg | 2.43 (1.04–5.24)| 2.31 (0.40-10.55)| 0.735 |
| ≤ 2.82                   | 16       | 97       |       |
| > 2.82                   | 18       | 91       | 0.764 |
| Conditioning, n (%)      | 0.657    |          |       |
| Bu based                 | 17 (50.0)| 105 (55.9)|      |
| TBI based                | 17 (40.0)| 83 (44.1) |       |
| GVHD prophylaxis         | 1        |          |       |
| CSA/MMF                  | 34       | 188      |       |
| Follow-up period (range), days² | 2248 (1914–3088)| 824 (397–1237)| 0.000^ |

^Statistically significant.
²Follow-up period was for surviving patients.
immune reconstitution and a higher incidence of GF.\textsuperscript{8,9} Notably, conditioning type (TBI-based or Bu-based) was significantly different between the MAC group and MMAC group in the retrospective study, and TBI has always been a favorable factor in improving neutrophil engraftment in CBT.\textsuperscript{22,23} To identify the relationship between conditioning type and neutrophil engraftment rate in this study, we did a univariate analysis, and there was no statistical difference ($p = 0.77$). The different proportion of TBI-based conditioning in the two groups was not relatively related to the difference of neutrophil engraftment rate.

The improvement of engraftment may be attributed to the pharmacological function of Flu and CA. Flu is a nucleoside analog with significant immunosuppressive activity and has

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**Figure 1.** Engraftment and NRM after CBT in MAC group and MMAC group.

**Figure 2.** Survival after CBT in MAC group and MMAC group.

**Figure 3.** Engraftment and NRM after CBT in MMAC-R group and MMAC-P group in validation study.
been substituted for Cy to develop high-intensity conditioning with low toxicity.\textsuperscript{24} Besides, Flu inhibits DNA repair and thus is synergistic with alkylators that cause breaks in the DNA strand. The use of Flu in MAC for the CBT recipients, especially those with lower TNC does show improved neutrophil and platelet recovery.\textsuperscript{15} CA is a cell cycle-specific antineoplastic agent that affects cells only in S-phase. Although its mechanism of action is not entirely understood, its metabolite cytarabine triphosphate is believed to inhibit the action of DNA polymerase. It may also function as a false substrate when incorporated into DNA. Numbers of studies have demonstrated the superiority of CA added to TBI/Cy.\textsuperscript{25–28} Furthermore, granulocyte colony-stimulating factor is believed to increase the susceptibility of myeloid leukemic cells to CA, thereby contributing to decreased relapse rate.\textsuperscript{27,28} The addition of Flu and CA to the conventional MAC enhances the intensity of the conditioning program and further eradicates the residual host lymphocytes, which may not only “create enough space” for the engraftment of CB-derived T cells but also weaken the immunological rejection induced by the host T cells surviving conditioning.

The omission of ATG and MTX may protect the naïve T cells. Predominant T-cell reconstitution in the early post-transplant period is mediated by the CB-derived naïve T cells which are at a small number and easily rejected.\textsuperscript{10} The use of ATG could impair the early development of donor-derived T cells due to the persistence of these antibodies in the patient for several weeks after administration especially if given too close to graft infusion.\textsuperscript{11} Similarly, use of MTX is also associated with delayed engraftment and increased risk of graft failure.\textsuperscript{12,13} Reducing the intensity of the GVHD prophylaxis regimen by avoiding the use of ATG and MTX will relatively “protect” the naïve T cells from the toxicity of these two drugs and achieved a higher rate of engraftment.\textsuperscript{29} In this study, we also found that CD4+ T cells and NK cells of MMAC group grew faster than MAC group at one month after transplantation, which may be related with the omission of ATG in MMAC group. T-cell immune reconstitution has been associated with the use of ATG shortly before or after infusion of CB as ATG will increase the depletion of T cells and prohibit the proliferation of CB.\textsuperscript{30} The reduction of ATG after CBT could achieve an early CD4+ T-cell immune reconstitution and no exposure to ATG results in even exceptional immune reconstitution potential, as recently shown.\textsuperscript{31} NK cells typically recover within the first month after transplant and are therefore one of the first lymphocytes to recover after transplant, regardless of stem-cell source.\textsuperscript{32} Due to the delay of T-cell immune recovery, NK cells are a vital lymphocyte subset exerting GVL effects and have been associated with superior OS, less CMV infection and reduced relapse rate.\textsuperscript{15} Zhao \textit{et al.} have reported that NK-cell immune reconstitution after haplo-identical HCT was affected by conditioning regimen, immune suppression therapy and level of T-cell depletion.\textsuperscript{34} However, factors influencing NK-cell recovery after CBT remain unclear, and more studies are needed to elucidate the rapid growth of NK cells in MMAC group.

In recent years, increased attention has been paid to the presence of DSA. Recent studies have shown the close relationship between DSA in the recipient and graft failure in HCT.\textsuperscript{35,36} Owing to the lack of related data in our database, we did not perform analysis of the role of DSA in neither the MAC group nor the MMAC group. A large-scale prospective multicenter study including the analysis of DSA is needed.

We showed that MMAC was significantly associated with the improvement of OS and DFS, which may be attributed to the low incidence of relapse and NRM of the MMAC group. It is widely acknowledged that relapse generally results from residual malignant cells that survived the preparative regimen and are not eliminated by the graft-versus-leukemia (GVL) effect. The reduction of relapse rate in the MMAC group was relatively the result of the antileukemia effects of both the conditioning regimens and the cord blood. On the one hand, with the addition of Flu and CA, MMAC can eradicate the residual host lymphocytes more thoroughly, which make it difficult for the host malignant cells to survive from the conditioning regimen. On the other hand, CB has a powerful GVL effect which is crucial in eliminating the drug-resistant...
malignant cells. Milano et al. reported that among patients with pretransplantation minimal residual disease, OS after CBT was as favorable as HCT with HLA-matched unrelated donor and much higher than HCT with HLA-mismatched unrelated donor, and the probability of relapse was lower in the cord-blood group than in either of the other groups, which indicated that cord blood may have a stronger GVL effect than other graft sources. In this study, we found that both relapse rate and cGVHD rate were lower in the MMAC group, which seemed to be inconsistent with the previous reports as some authors believed that GVL effect was closely related to cGVHD. Nevertheless, most of these studies were designed for HCT with matched sibling donors, haploidentical-related donors and unrelated donors, the GVL effects of CBT may work in a different way. Despite the possible GVL effect, cGVHD was reported to have an adverse effect on NRM and was the main cause of poor quality of life after transplantation. However, low probability of cGVHD has always been one of the advantages of CB, and many studies reported that the relapse rate in CBT was similar to or lower than in other kinds of HCT, which reflected the clinical separation of cGVHD and the GVL effect in CB. Furthermore, our previous study showed that long-term survivors with CBT had less cGVHD and higher quality of life compared to HCT in HLA-identical sibling's donors. Thus, our protocol of MMAC without ATG not only makes full use of the GVL effects of CBT but also keeps its advantage of low probability of cGVHD.

In terms of the complications, MMAC was not associated with an increase in the cumulative incidence of 3-year NRM when compared with MAC (Supporting Information, Table S3; 26.5% vs. 41.7%, p = 0.18) and causes of NRM were comparable between the two groups. Also, in the validation study, NRM of the MMAC-P group was similar to that of the MMAC-R group (Supporting Information, Table S3; 17.8% vs. 26.5%, p = 0.22). There are no more fatal complications induced by the intensification of the conditioning regimen. The cumulative incidence of grade II to IV aGVHD and grade III to IV aGVHD were identical between the MAC group, the MMAC-R group and the MMAC-P group, which indicated that the complications of MMAC were comparable to MAC.

The relapse rate of the MMAC group was lower than the MAC Group (5.9% vs. 12.5%, p = 0.37). However, in the validation study, relapse rate was higher in the MMAC-P Group (23.4% vs. 5.9%, p = 0.03), which may be related to the higher proportion of patients with ALL in the MMAC-P Group (69.1% vs. 47.1%, p = 0.02). Some authors have reported that compared to patients with AML/MDS, those with ALL have a higher incidence of extramedullary relapse. In this study, we also found that patients with AML/MDS had a lower relapse rate than patients with ALL (adjusted HR, 0.33; p = 0.03), which indicated that diagnosis of ALL was an independent risk factor for relapse in CBT. However, our center is the largest and most experienced center for CBT in China, different methods have been developed to prevent and treat post-CBT relapse, such as monitoring WT1 in the early phase after CBT and injecting interferon instantly when the value of WT1 is beyond the upper level set in our center. More works are needed to further modify our CBT protocol and lower down the relapse rate of patients with ALL.

In conclusion, MMAC significantly improves the neutrophil engraftment rate, the platelet engraftment rate, 3 years of OS and DFS without increasing NRM in single-unit CBT for hematological malignancies (ALL/AML/MDS). However, the retrospective study has all the inherent biases and the sample size is small, and the prospective study is non-randomized in consideration of ethical principles. A larger prospective multicenter study is needed to verify the outcomes shown in this study.

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