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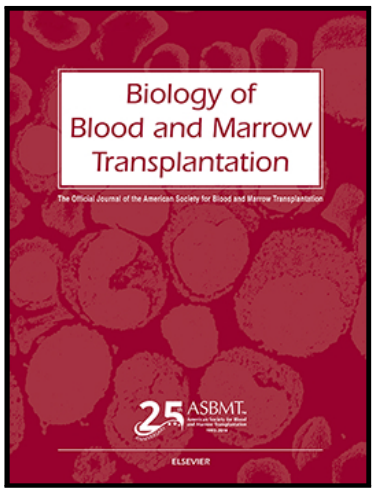

Comparative study of mizoribine and mycophenolate mofetil combined with a calcineurin inhibitor-based immunosuppressive regimen in patients with alternative donor hematopoietic cell transplantation

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Comparative study of mizoribine and mycophenolate mofetil combined with a calcineurin inhibitor-based immunosuppressive regimen in patients with alternative donor hematopoietic cell transplantation

Mizoribine *vs* mycophenolate mofetil for hematopoietic cell transplantation

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Highlight

1. A high CMV DNA peak load predicts a persistent and refractory disease course.
2. Treatment with MZR can reduce the severity of CMV infection in the presence of ganciclovir.
3. MZR shows good immunosuppressive as well as antiviral effects in the setting of HCT.

Abstract

Background and Objective: Cytomegalovirus (CMV) infection and graft versus host disease (GvHD) remain the major causes of nonrelapse mortality (NRM) in patients following alternative donor HCT. Mizoribine (MZR) showed an anti-CMV effect in addition to its immunosuppressive effect in patients with renal transplantation. In this study, we aimed to evaluate the efficacy and safety of MZR combined with CNIs as a method of prophylactic immunosuppression in recipients following alternative donor HCT.

Methods: Eighty patients were enrolled in the study and randomized to the MZR (n = 40) and MMF (n = 40) cohorts before transplant conditioning. Analyses involved a comparison of the

outcomes between the two cohorts as well as risk analyses of early NRM and severe CMV infection.

Results: In contrast to MMF, MZR resulted in a lower but statistically nonsignificant median CMV DNA peak load (p = 0.075), significantly fewer episodes of persistent/refractory infection (OR = 0.12), and a lower failure rate of CMV treatment (OR = 0.82), but the occurrence of hyperuricemia was significantly increased (OR = 2.75). The transplant efficacy was comparable between the two cohorts regarding engraftment, the development of secondary poor graft function (sPGF) and GvHD, and the estimated OS and PFS. The 1-y NRM of the MZR cohort was not different from that of the MMF cohort, while the rate of 1-y NRM caused by viral infections was reduced in the MZR cohort and was of borderline statistical significance (p = 0.05). In the multivariate analysis, lower doses of CD34+ cells in grafts (HR = 3.65) and persistent/refractory CMV infections (*vs* w/o CMV infection: HR = 7.31; *vs* CMV infection that was not persistent/refractory: HR = 4.46) were predictors of increased 1-y NRM. The use of MMF (*vs* MZR cohort: OR = 11.54) and grade Ⅱ to Ⅳ acute GvHD (OR = 15.32) were independent risk factors for developing persistent/refractory CMV infections.

Conclusions: When combined with CNIs, MZR functioned well in terms of both immunosuppression and the reduction of the severity of CMV infection; however, further studies are warranted to verify whether it could be used as a potential immunosuppressant for alternative donor HCT.

Keywords: mizoribine; mycophenolate mofetil; CMV infection; hematopoietic cell transplantation.

Introduction

Calcineurin inhibitor (CNI) - based regimens using cyclosporine (CsA) or tacrolimus with methotrexate (MTX) have been commonly used to prevent graft versus host disease (GvHD) in patients undergoing matched sibling donor hematopoietic cell transplantation (HCT) [1, 2], while more intensive immunosuppression involving the addition of mycophenolate mofetil (MMF) and *in vivo* T-cell depletion (anti-thymocyte globulin, ATG) to the standard regimen is usually needed for recipients undergoing alternative donor HCT [3, 4]. However, the enhanced strategy carries increased risks of delayed immune reconstitution, opportunistic infections and mortality [5]. Cytomegalovirus (CMV) reactivation remains the most important infectious complication for alternative donor HCT, occurring in as many as 60% of in-risk recipients and subsequently leading to an increased risk of nonrelapse mortality (NRM) [6, 7].

Mizoribine (MZR), a nucleoside analogue that was developed as an immunosuppressive agent, selectively inhibits the proliferation of lymphocytes via inhibition of inosine monophosphate dehydrogenase (IMPDH) in the same manner as MMF [8], and it also inhibits lymphocyte proliferaton via inhibition of guanosine monophosphate synthetase, which is not affected by MMF [9]. In addition, MZR shows synergistic anti-CMV activity with antiviral agents such as ganciclovir and ribavirin via depletion of guanosine nucleotides [10]. In a meta-analysis of 1149 renal transplants in Asian patients in randomized controlled trials and cohort studies, although the efficacy of MZR and MMF in the cohort were equal, a safety advantage was observed for recipients who received MZR rather than MMF, as the former resulted in significantly fewer episodes of leukopenia, gastrointestinal disorder, and especially CMV infection; however, the occurrence of hyperuricemia was increased significantly in patients who received MZR [11].

Based on the findings obtained in the field of solid organ transplantation, we conducted a

randomized comparative trial to assess whether MZR could be substituted for MMF and be used in combination with CNIs as GvHD prophylaxis in recipients following alternative donor HCT.

Methods

Study Design

The study was designed to evaluate the efficacy and safety of MZR compared with that of MMF combined with CNI and MTX, which served as prophylactic immunosuppressive agents, in recipients with a high risk of GvHD and CMV reactivation. Study inclusion required the following: (1) patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or high-grade myelodysplastic syndrome (MDS) who were in the first complete remission at the time of transplantation; (2) patients undergoing myeloablative conditioning treatment followed by a graft from either a haplo-identical donor or a matched unrelated donor; and (3) patients with a Karnofsky score > 70. Patient exclusion criteria were: (1) patients with a history of allergy, hypersensitivity or serious reaction to either MZR or MMF; (2) patients with serious renal dysfunction and/or serious liver dysfunction. For sample-size estimation, we supposed the difference in the episodes of CMV infection of 25% between the two treatment arms as clinically relevant, and set the type Ⅰ error (α) at a level of 0.05 and the type Ⅱ error (β) of 0.20 (the power was 0.80). Based on the look-up table method, the intended number of sample-size in each arm was 45, but only 40 cases were enrolled in each arm until the end. Once enrolled, the subject was allocated to a treatment arm according to the random number generated by the Excel table RAND() function.

Transplant Procedures

The preparative regimen for AML patients consisted of intravenous (IV) busulfan (Bu, 3.2 mg/kg

per day for 3 days), cyclophosphamide (40 mg/kg per day for 2 days), fludarabine (30 mg/m^2 per day for 3 days), cytarabine (2 g/m^2 per day for 3 days) and rabbit antithymocyte globulin (Thymoglobuline®, 2 mg/kg per day for 4 days). Total body irradiation (10 Gy divided into 3 doses administered once a day over 3 days) in place of Bu was used in the above AML regimen for conditioning patients with ALL, while the addition of decitabine (20 mg/m^2 per day for 5 days) to the above AML regimen was used for conditioning patients with MDS.

Patients were given a hematopoietic graft from a haploidentical family or a matched unrelated donor on day 0. A combination of IV CsA or tacrolimus with MTX (15 mg/m^2 on day +1, 10 mg/m^2 on days +3, +6, +9) and oral MZR (3 mg/kg per day, MZR cohort) or oral MMF (15 mg/kg per day, MMF cohort) was used for GvHD prophylaxis. IV CsA or tacrolimus was replaced with orally administered CsA or tacrolimus once patients were able to resume a normal diet, which was tapered beginning at day +150 in the absence of GvHD. The study drug, MZR or MMF, was discontinued at day +60 in the absence of GvHD; it could also be reduced, suspended or withdrawn at any time due to the failure of GvHD prevention, poor graft function and/or relapse of leukemia.

Prophylactic and Preemptive Therapy against CMV

Recipients who were CMV seropositive would receive prophylactic anti-CMV therapy with ganciclovir at a dosage of 5 mg/kg twice daily for 7 days in the peritransplant period. During engraftment, recipients were monitored for CMV infection via real-time polymerase chain reaction assays (PCR) of plasma twice a week. If the screening test was positive (i.e. the viral DNA levels > 1000 copies/ml in plasma samples), ganciclovir was immediately administered twice daily for at least 2 weeks until the test yielded a negative result.

Patient Characteristics

Eighty consecutive patients were enrolled in this study between March 2014 and March 2017 who had a median age of 27.5 years (range, 3-57 years) and a median follow-up time of 50.7 months (range, 33.8-71.3 months) for patients who survived until the end of the study. Patients were randomized to either the MZR (n = 40) or MMF (n = 40) cohort before transplant conditioning. Clinical characteristics were comparable between the two cohorts and are detailed in Table 1. Each patient was assessed for survival, graft function, CMV infection, GvHD, disease progression or relapse, study drug-related toxicities, and cause of death.

Definitions

Overall survival (OS) was the duration between HCT and death from any cause or the last follow-up for patients who survived until the end of the study. Progression-free survival (PFS) was defined as the interval of time from HCT through relapse/progression of any underlying diseases, death from any cause, or the last follow-up for patients who remained alive or who were relapse/progression free. NRM was defined as death from any cause other than recurrence of underlying diseases. Neutrophil engraftment was defined as the detection of an absolute neutrophil count $\geq 0.5 \times 10^9$/L for three consecutive days; platelet engraftment was defined as the detection of a platelet count $\geq 20 \times 10^9$/L over 7 days independent of transfusion. Secondary poor graft function (sPGF) was defined as the occurrence of cytopenia (neutrophil count $< 0.5 \times 10^9$/L or platelet count $< 20 \times 10^9$/L) and hypoplastic/aplastic bone marrow after primary engraftment with full donor chimerism. Acute GvHD (aGvHD) was clinically graded from 0 to Ⅳ based on the standard criteria [12] and chronic GvHD (cGvHD) was diagnosed as either absent, limited, or extensive according to the Seattle clinical criteria [13]. CMV infection was defined as the

detection of high viral DNA levels of over 1000 copies/ml in plasma samples in two consecutive PCR [14]. Persistent CMV infection was defined as persistent DNAemia for over 3 weeks despite treatment with the available antivirals [15]. Refractory CMV infection was defined as CMV DNAemia that showed an increase (i.e., > 1 $\log_{10}$ increase in the CMV DNA level corresponding to the peak viral load within the first week and that corresponding to the peak viral load at $\geqslant$ 2 weeks) after at least 2 weeks of appropriate antiviral therapy [15]. Study drug-related toxicities occurring within 3 months of transplant were graded according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0.

Statistical Methods

The Wilcoxon rank sum test was used to compare the medians of continuous variables. Chi-square tests were used to compare the frequency distributions of categorical variables and ordinal variables. A Kaplan-Meier curve was used to estimate survival and NRM. The cumulative incidences of GvHD and the relapse of underlying disease were estimated and plotted with the cmprsk package (R software). The prognostic variables for NRM were evaluated by Cox regression analysis. Risk factors for persistent/refractory CMV infection were evaluated by logistic regression analysis. All tests were 2-tailed, and a difference was considered significant at $p \leq 0.05$.

Results

Study drug-related AEs

Within 100 days after transplantation, study drug-related toxicities of grade 3 or greater were observed in 16 (40%) *vs* 14 (35%) patients from the MZR and MMF cohorts, respectively. As shown in Table 2, patients treated with MZR were at increased risk incidence of grade 3 or greater

hyperuricemia which was in contrast with that observed for patients treated with MMF (27.5% *vs* 10%, odds ratio (OR) = 2.75, 95% confidence interval (CI): 0.96-7.92, p = 0.045), while the incidences of other grade 3 or greater events, including hyperbilirubinemia (5% *vs* 5%), glucose intolerance (2.5% *vs* 10%), iron overload (7.5% *vs* 10%), adrenal insufficiency (2.5% *vs* 2.5%) and autoimmune hemolysis (0 *vs* 2.5%), were comparable.

Engraftment, sPGF and GvHD

All patients in the MZR and MMF cohorts achieved primary engraftment, with median recovery times of 14.5 (range, 10-23) *vs* 13 (11-22) days (p = 0.480) for neutrophils and 19.5 (12-109) *vs* 17 (8-159) days (p = 0.407) for platelets, respectively. However, there was 1 patient in the MZR cohort and 2 in the MMF cohort who did not achieve platelet recovery until 100 days after HCT, and there were 3 patients in the MZR cohort and 4 in the MMF cohort who never achieved adequate platelet engraftment, i.e., a platelet count > 50 × 10^9/L. This resulted in a cumulative percentage of patients in the MZR cohort with a platelet count > 50 × 10^9/L of 45% ± 7.9% *vs* 47.5% ± 7.9% for the MMF cohort (p = 0.654) at 30 days; the percentage of patients was 82.5% ± 6% for the MZR cohort *vs* 72.5% ± 7.1% (p = 0.704) for the MMF cohort at 100 days (see Figure 1A). Among the patients who achieved both neutrophil and platelet primary engraftment within 100 days (n = 77), 9 and 11 patients in the MZR and MMF cohorts, respectively, experienced sPGF with cumulative incidences of 23.1% ± 6.7% *vs* 28.8% ± 7.4%, respectively (p = 0.514, see Figure 1B).

The probabilities of developing grade Ⅰ to Ⅳ (50% ± 7.9% *vs* 62.5% ± 7.7%, p = 0.267), Ⅱ to Ⅳ (27.5% ± 7.1% *vs* 37.9% ± 7.7%, p = 0.279; see Figure 1C), and Ⅲ to Ⅳ aGvHD (7.5% ± 4.2% *vs* 12.8% ± 5.4%, p = 0.451) at day 100 were comparable between the patients in the MZR and MMF

cohorts, respectively. For patients in the MZR and MMF cohorts surviving more than 100 days after HCT, the probability of developing cGvHD was 42% ± 8.7% *vs* 41.5% ± 9.1%, respectively (p = 0.710, see Figure 1D), and the probability of developing extensive cGvHD was 28% ± 8% *vs* 26.3% ± 8.2%, respectively (p = 0.924).

Infections

Twenty-two patients in the MMF cohort and 19 in the MZR cohort developed CMV infection, with incidences of 55% *vs* 47.5% (p = 0.502) and median onset times at 40 (21-64) *vs* 42 (15-63) days after transplantation, respectively (p = 0.937). When subjected to ganciclovir treatment, the MMF cohort demonstrated a statistically non-significant higher median CMV DNA peak load (8451 (1788-180613) *vs* 4571 (1462-27188) copies/ml, p = 0.075) compared with the MZR cohort (see Figure 1E), and patients with a higher DNA peak load (cutoff = 9000 copies/ml) were more likely to progress to persistent/refractory infection (OR = 5, 95% CI: 1.22-20.46, p = 0.026). Finally, 4 of the 41 infected cases were not resolved, and all of them involved patients in the MMF cohort; the treatment failure rate was 18.2% *vs* 0% for the MMF and MZR cohorts, respectively (OR = 1.22, 95% CI: 1.00-1.49, p = 0.021). Seventeen out of 41 infected patients died, of whom 3 and 1 (all in the MMF cohort) died of CMV-associated pneumonia and intestinal infection, respectively.

According to the univariate analysis (Table 3), treatment with MMF (p = 0.001), the presence of grade Ⅱ to Ⅳ aGvHD (p < 0.0001) and the use of high-dose corticosteroids (defined as the use of methylprednisolone for at least 14 days at a daily dose greater than 1 mg/kg) (p = 0.026) were associated with an increased risk of persistent/refractory CMV infection. In the logistical regression analysis (Table 3), treatment with MMF (OR = 11.54, 95% CI: 2.42-55.05, p = 0.002)

and grade Ⅱ to Ⅳ aGvHD (OR = 15.32, 95% CI: 3.77-62.23, p = 0.0001) were identified as independent risk factors.

Infections caused by viruses other than CMV were documented in 11 patients in the MZR cohort *vs* 10 patients in the MMF cohort, including 5 *vs* 5 patients with *Epstein-Barr virus* infection and 6 *vs* 5 patients with *varicella zoster virus* infection, respectively. The estimated one-year mortality due to various viral infections was decreased and of borderline statistical significance for the MZR cohort compared to that for the MMF cohort (2.9% ± 2.9% *vs* 15.4% ± 5.8%, p = 0.050).

Bacterial bloodstream infections occurred in 9 patients in the MZR cohort *vs* 12 patients in the MMF cohort. Invasive fungal disease (probable) developed in 6 patients in the MZR cohort *vs* 3 patients in the MMF cohort. Infiltrative pulmonary tuberculosis was seen in one patient in the MZR cohort.

Survival

Thirty-one patients died; 11 patients died due to relapse and 20 died due to treatment-related complications. Both the estimated OS and PFS at 5 years after HCT (5-y OS and 5-y PFS, hereafter) for recipients in the MZR cohort seemed to be slightly better than those for recipients in the MMF cohort (67.5% ± 7.4% *vs* 55% ± 7.9%, p = 0.205, and 67.5% ± 7.4% *vs* 55% ± 7.9%, p = 0.221, respectively; see Figure 1F), even though the cumulative incidences of relapse after HCT for the two cohorts were similar (16.7% ± 6.3% and 18.1% ± 6.7%, respectively (p = 0.850)). As shown in Table 4, nonrelapse-related deaths in the MZR and MMF cohorts were due to GvHD (n = 3 *vs* 4) and infections (n = 4 *vs* 9), while the estimated NRM at one year after HCT (1-y NRM, hereafter) was 13.2% ± 5.5% *vs* 28.2% ± 7.2% (p = 0.095), respectively (see Figure 1G).

Risk predictors of 1-y NRM

According to the univariate analysis (Table 5), the 1-y NRM in patients with persistent/refractory CMV infections was significantly increased compared with that in those without CMV infection (47.4% ± 11.5% *vs* 12.1% ± 4.3%, p = 0.001) or those with CMV infection that was not persistent/refractory (47.4% *vs* 14.3% ± 7.6%, p = 0.018). Patients infused with lower doses of CD34+ cells in grafts (cutoff = 2.8×10^6/kg) (36.2% ± 10.3% *vs* 14.5% ± 4.7%, p = 0.026) and patients experiencing grade Ⅱ to ⅣaGvHD (34.6% ± 9.3% *vs* 13.9% ± 4.9%, p = 0.019) were also shown to develop high 1-y NRM. Other factors, including age, gender, underlying disease, donor type, the use of female donors for male recipients, high levels of serum ferritin (cutoff = 1000 μg/L) at the time of HCT, high doses of CD3+ cells in grafts (cutoff = 1×10^8/kg), and extensive cGvHD were not significantly predictive for 1-y NRM.

According to the multivariate analysis (Table 5), two predictors of increased 1-y NRM were identified: lower doses of CD34+ cells in grafts (HR = 3.65, 95% CI: 1.35-9.88, p = 0.011) and persistent/refractory CMV infections (*vs* without infection: HR = 7.31, 95% CI: 2.21-24.13, p = 0.001; *vs* CMV infection that was not persistent/refractory: HR = 4.46, 95% CI: 1.20-16.67, p = 0.025).

Discussion

In this study, we found that MZR used in combination with CNIs was associated with a reduction in the incidence and mortality of persistent/refractory CMV infections following alternative donor HCT, while the transplant efficacy was comparable between the two treatment arms in terms of engraftment, the development of sPGF and GvHD, and the estimated OS and PFS.

MZR has been used as an immunosuppressant for the prevention of rejection in renal transplantation in some Asian countries [8]. After considering and testing the clinical efficacy and

safety, we first used MZR for the treatment of HCT as a GvHD prophylactic agent. This study was designed to be a randomized comparative trial; however, there are significant limitations. First, we did not monitor the serum concentrations of MZR or MMF and therefore could not further accurately determine their effects. Second, we did not include a high-dose group in the trial, as previous studies have illustrated that, unlike a daily dose of 1-3 mg/kg, high-dose MZR or MZR at a high trough level is correlated with a decreased risk of acute rejection and CMV infection in renal transplantation [16-19]. Furthermore, the scheme used in this study did not record episodes of diarrhea in the AE files because of concerns about not accurately determining the true causes of diarrhea in the HCT setting; another study found that treatment with MZR was associated with fewer episodes of diarrhea compared with treatment with MMF in renal transplantation [11].

Nevertheless, the current study suggested that MZR showed good immunosuppressive as well as antiviral effects in the setting of HCT, which is similar to the results in a series of reports on renal transplantation [11, 16-19]. GvHD is the most important immune event that must be overcome in HCT, similar to graft rejection in solid organ transplantation. MZR at a dosage of 3 mg/kg per day in this study was sufficient to achieve a similar immunosuppressive effect as a conventional regimen containing MMF; therefore, it could be hypothesized that better outcomes could be achieved with a higher dose MZR treatment. For CMV infection, the prevalence in each treatment arm was similar, but the severity of infection was significantly different, which was manifested in the MZR treatment arm as a lower median CMV DNA peak load (p = 0.075), significantly fewer episodes of persistent/refractory infection (OR = 0.12) and a significantly lower failure rate for CMV treatment (OR = 0.82). The advantage of MZR was confirmed in the logistic regression analysis, as treatment with MZR was a favorable factor for the amelioration of severe CMV

infections. We further found that higher CMV DNA peak loads predicted a persistent and refractory disease course, which was seldom reported in previous studies, and persistent/refractory CMV infections were one of the predictors of increased 1-y NRM.

GvHD is primarily a donor-derived T cell-mediated disease. The action of MZR is directed toward prolonged suppression of T cell function via its active form mizoribine 5′-monophosphate [8, 9]. Thus, it is not difficult to understand why MZR combined with CNIs is a reliable prophylactic regimen for allogeneic HCT. The current study identified the presence of grade Ⅱ to ⅣaGvHD as a risk factor for severe CMV infection, which was consistent with the fact that GvHD and its treatment may result in impaired immune reconstitution, leading to an increased risk of life-threatening infections.

Unlike MMF, MZR inhibits guanosine monophosphate in addition to IMPDH and thus may increase the ratios of antiviral agents to guanosine in treated cells, resulting in a strong synergistic augmentation of the antiviral activity of agents such as ganciclovir and acyclovir [10]. This may partially explain why taking MZR alone in the study did not significantly reduce the incidence of CMV infection but effectively controlled the severity of CMV infection in the presence of ganciclovir.

In conclusion, combined with CNIs, MZR functioned well both in immunosuppression and the reduction of the severity of CMV infection and could be used as an alternative to MMF following alternative donor HCT. MZR at a daily dose of 3 mg/kg in this study was well tolerated despite the fact that hyperuricemia was commonly documented. Further investigations will be performed to assess the efficacy and safety of high-dose MZR in allogeneic HCT as well as to determine the mechanism underlying the effect of MZR on immune reconstitution.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

1. Storb R, Deeg HJ, Whitehead J, Appelbaum F, Beatty P, Bensinger W, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. N Engl J Med. 1986 Mar 20;314(12):729–735.
2. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998 Oct 1; 92(7):2303–2314.
3. Niederwieser D, Maris M, Shizuru JA, Petersdorf E, Hegenbart U, Sandmaier BM, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood. 2003 Feb 15;101(4):1620-1629. doi: 10.1182/blood-2002-05-1340.
4. Wagner JE, Thompson JS, Carter SL, Kernan NA; Unrelated Donor Marrow Transplantation Trial. Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial. Lancet. 2005 Aug 27-Sep 2;366(9487):733-741.
5. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. Curr Opin Hematol. 2012 Jul;19(4):324-335. doi: 10.1097/MOH.0b013e328353bc7d.
6. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. Blood 2016 May 19; 127(20), 2427–2438. doi: 10.1182/blood-2015-11-679639
7. Atay D, Akcay A, Erbey F, Ozturk G. The impact of alternative donor types on viral infections in pediatric hematopoietic stem cell transplantation. Pediatr Transplant. 2018 Mar;22(2). doi: 10.1111/petr.13109.
8. Ishikava H. Mizoribine and mycophenolate mofetil. Curr Med Chem. 1999 Jul;6(7):575-597.
9. Tsuzuki K. Role of mizoribine in renal transplantation. Pediatr Int. 2002 Apr;44(2):224-231
10. Kuramoto T, Daikoku T, Yoshida Y, Takemoto M, Oshima K, Eizuru Y, et al. Novel anticytomegalovirus activity of immunosuppressant mizoribine and its synergism with ganciclovir. J Pharmacol Exp Ther. 2010 Jun;333(3):816-21. doi: 10.1124/jpet.109.160630.
11. Xing S, Yang J, Zhang X and Zhou P. Comparative efficacy and safety of mizoribine with mycophenolate mofetil for Asian renal transplantation - A meta-analysis. Clin Biochem. 2014 May;47(7-8):663-669. doi: 10.1016/j.clinbiochem.2014.01.014.
12. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825-828.

13. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980; 69:204-217.
14. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis.2002 Apr 15;34(8):1094–1097. doi: 10.1086/339329.
15. Chemaly RF, Chou S, Einsele H, Griffiths P, Avery R, Razonable RR, et al. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. Clin Infect Dis. 2019 Apr 8;68(8):1420-1426. doi: 10.1093/cid/ciy696.
16. Oshiro Y, Nakagawa K, Hoshinaga K, Aikawa A, Shishido S, Yoshida K, et al. A Japanese multicenter study of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroid in renal transplantation (the fourth report). Transplant Proc. 2013 May;45(4):1476-80. doi: 10.1016/j.transproceed.2013.03.016.
17. Yoshimura N, Ushigome H, Nobori S, Suzuki T, Sakai K, Koshino K, et al. Excellent results of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroids in renal transplant recipients--4-year results. Transplant Proc. 2013 May;45(4):1472-5. doi: 10.1016/j.transproceed.2013.01.055.
18. Yoshimura N, Ushigome H, Akioka K, Nobori S, Suzuki T, Sakai K, et al. The beneficial effect of high-dose mizoribine combined with cyclosporine, basiliximab, and corticosteroids on CMV infection in renal transplant recipients. Clin Exp Nephrol. 2013 Feb;17(1):127-33. doi: 10.1007/s10157-012-0669-4.
19. Ushigome H, Uchida K, Nishimura K, Akioka K, Fukuda Y, Yuzawa K, et al. Efficacy and Safety of High-Dose Mizoribine Combined With Cyclosporine, Basiliximab, and Corticosteroids in Renal Transplantation: A Japanese Multicenter Study. Transplant Proc. 2016 Apr;48(3):794-8. doi: 10.1016/j.transproceed.2015.12.117.

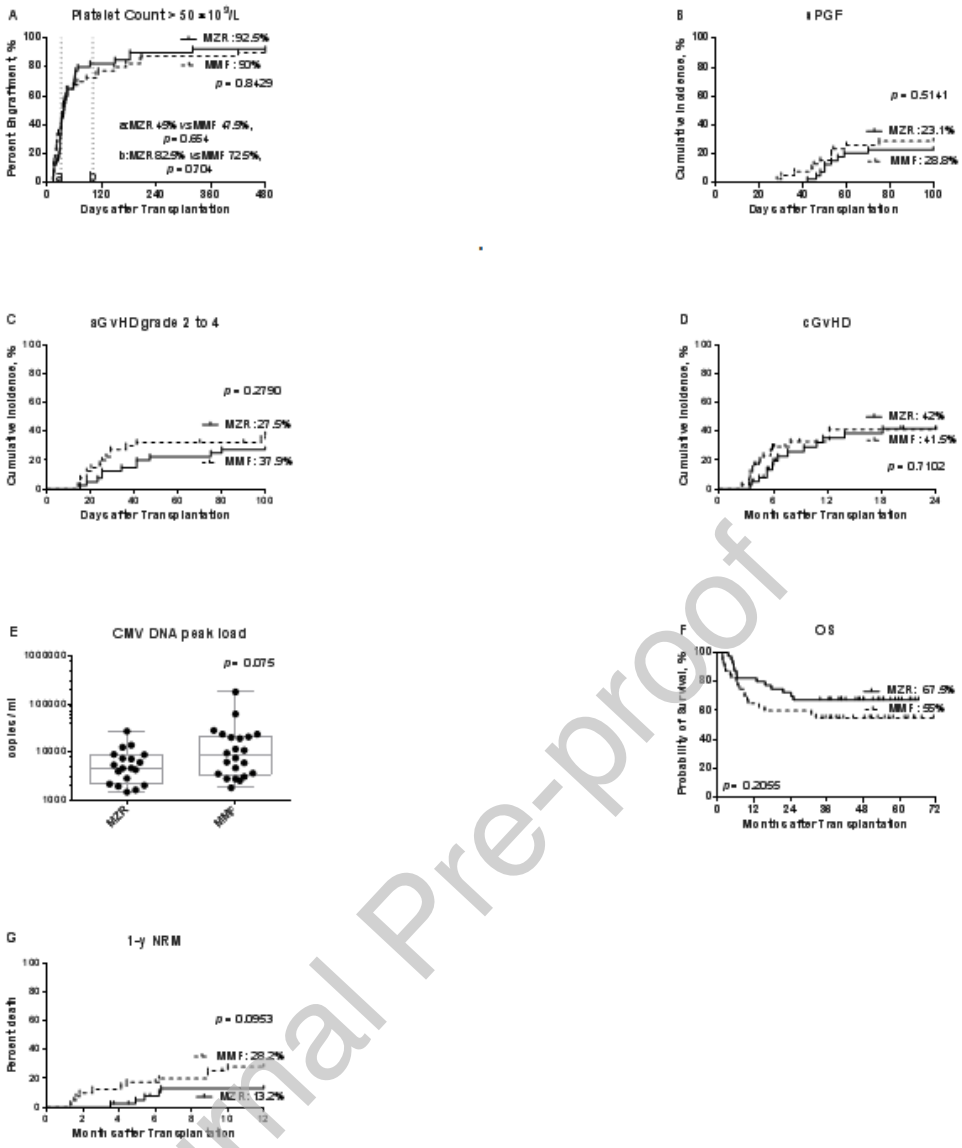

Figure 1. Comparison of treatment outcomes between the MZR cohort and the MMF cohort.
1A demonstrated the cumulative percentage of patients with a platelet count > 50 × 10^9/L, in which the *a* and *b* lines added at X axis refer to the target percentages at 30 d and 100 d after transplantation, respectively. The plots also demonstrated the cumulative incidences of sPGF at 100 d (B), grade Ⅱ to Ⅳ aGvHD (C) and cGvHD (D), the median CMV DNA peak loads among infected patients (E), and the probabilities of OS (F) and 1-y NRM (G).

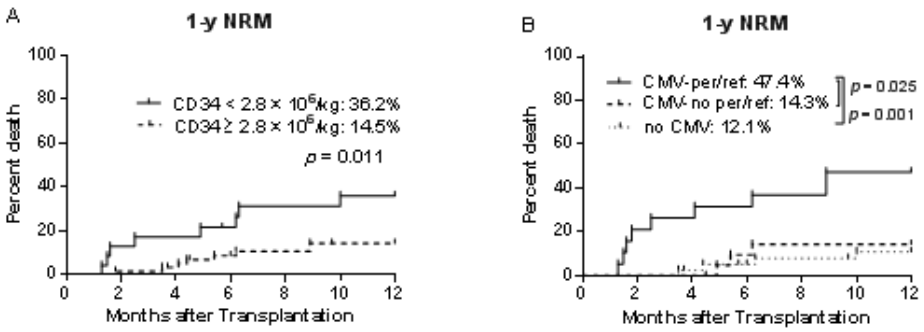

Figure 2. 1-y NRM adjusted for risk factors.

The plots demonstrated the impacts of the dosage of CD34+ cell in grafts (A) and episode of persistent/refractory CMV infection (B) on the probability of 1-y NRM.

Table 1. Clinical Characteristics

Characteristics	MZR	MMF	*p* value
Number of patients	40	40	
Median follow-up from HCT in surviving patients, in months (range)	48.2 (33.8-66.3)	51.75 (34.4-71.3)	0.725
Median age at HCT, in years (range)	27.5 (3-57)	28.5 (6-54)	0.847
Gender, male, *n*, (%)	21 (53)	18 (45)	0.502
Underlying disease, *n*, (%)			0.669
AML	19 (48)	23 (58)	
ALL	16 (40)	13 (33)	
MDS	5 (13)	4 (10)	
Median serum ferritin at the time of HCT, μg/L, (range)	903 (28-4998)	723 (57-5221)	0.204
Median KPS score at the time of HCT, (range)	90 (80-90)	90 (80-90)	1.000
Donors			0.491
haplo-identical family donor, n, (%)	26 (65)	23 (58)	
unrelated donor, n, (%)	14 (35)	17 (43)	
Female donor - male recipient pair, *n*, (%)	7 (18)	3 (8)	0.176
Doses of graft cells, median, (range)			
mononuclear cells ($\times 10^8$/kg)	9.00 (5.53-19.36)	8.00 (5.73-13.00)	0.115
CD34+ cells ($\times 10^6$/kg)	3.25 (1.87-7.60)	3.62 (2.08-8.76)	0.343
CD3+ T cella ($\times 10^8$/kg)	1.26 (0.28-5.03)	1.24 (0.55-2.67)	0.923

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HCT: hematopoietic cell transplantation; MDS: myelodysplastic syndrome; KPS: Karnofsky performance status.

Table 2. Study drug-related AEs (grade 3 or greater)

AEs	MZR (%)	MMF (%)	*p* value
Number of patients	40	40	
Endocrine disorders			
Adrenal insufficiency	1 (2.5)	1 (2.5)	*ns*
Immune system disorders			
Autoimmune hemolysis	0 (0)	1 (2.5)	*ns*
Metabolic disorder			
Hyperuricemia	12 (27.5)	4 (10)	0.045
Hyperbilirubinemia	2 (5)	2 (5)	*ns*
Glucose intolerance	1 (2.5)	4 (10)	*ns*
Iron overload	3 (7.5)	4 (10)	*ns*
Sum:			
Cases of AEs of grade 3 or greater	16 (40)	14 (35)	*ns*
Number of AEs of grade 3 or greater	19	16	

AE: adverse event; ns: non-statistical

Table 3. Logistic regression analysis of persistent/refractory CMV infections.

	Univariate analysis		Logistic regression analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age: ⩾35 years *vs* <18 years	1.64 (0.40-6.76)	0.498		
⩾35 years *vs* 18-34 years	1.64 (0.50-5.35)	0.410		
Gender: male *vs* female	1.89 (0.66-5.45)	0.234		
Underlying disease: AML *vs* ALL	3.13 (0.91-10.75)	0.095		*ns*
AML *vs* MDS	4 (0.45-35.23)	0.153		*ns*
Donors: haplo. *vs* unrelated	2.98 (0.88-10)	0.105		*ns*
SF at the time of transplantation: ⩾ 1000 μg/L vs < 1000 μg/L	0.98 (0.35-2.78)	0.968		
Doses of graft cells: CD34+ cells < 2.8×10^6/kg *vs* ⩾ 2.8×10^6/kg	1.2 (0.39-3.65)	0.755		
CD3+ cells < 1×10^6/kg *vs* ⩾ 1×10^6/kg	1.19 (0.39-3.65)	0.755		
Grade Ⅱ to Ⅳ aGvHD: with *vs* w/o	11.43 (3.44-37.98)	< 0.0001	15.32 (3.77-62.23)	0.0001
High-dose corticosteroids*: used *vs* no	5.67 (1.84-17.49)	0.002		*ns*
Treatment with MMF *vs* MZR	8.22 (2.16-31.27)	0.001	11.54 (2.42-55.05)	0.002

aGvHD: acute graft versus host disease; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CI: confidence interval; MDS: myelodysplastic syndrome; NRM: non-relapse mortality; ns: non-significant; OR: odds ratio; SF: serum ferritin

*: defined as use of methylprednisolone for at least 14 days at a daily dose greater than 1 mg/kg.

Table 4. Causes of death.

Causes of death	MZR (No.)	MMF (No.)	All patients (No.)
CMV associated disease	0	4	4
viral infections other than CMV	1	2	3
invasive fungal disease, probable	2	2	4
bacterial sepsis	1	1	2
acute GvHD	2	0	2
chronic GvHD	1	4	5
relapse	6	5	11

GvHD: graft versus host disease

Table 5. Univariate and multivariate Cox analysis of 1-y NRM.

	Univariate		Multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age: <18 years *vs* 18-34 years	0.53 (0.14-1.97)	0.346		
<18 years *vs* ≥35 years	0.42 (0.10-1.67)	0.216		
18-34 years *vs* ≥35 years	0.80 (0.27-2.37)	0.690		
Gender: male *vs* female	1.05 (0.39-2.80)	0.923		
Underlying disease: AML *vs* ALL	1.29 (0.46-3.64)	0.635		
AML *vs* MDS	1.86 (0.40-8.65)	0.426		
ALL *vs* MDS	1.56 (0.25-9.92)	0.636		
Donors: haplo. *vs* unrelated	1.42 (0.52-3.87)	0.496		
Female donor to male recipient *vs* others	1.67 (0.39-7.21)	0.490		
SF at the time of transplantation: ≥ 1000 μg/L vs < 1000 μg/L	0.78 (0.29-2.09)	0.615		
Doses of graft cells: CD34+ cells < 2.8×10^6/kg *vs* ≥ 2.8×10^6/kg	2.89 (1.17-11.04)	0.026	3.65 (1.35-9.88)	0.011
CD3+ cells ≥ 1×10^6/kg *vs* < 1×10^6/kg	1.23 (0.42-3.62)	0.705		
CMV infection: P/R *vs* w/o CMV infection	7.93 (2.35-26.75)	0.001	7.31 (2.21-24.13)	0.001
P/R *vs* CMV infection that was not P/R	4.23 (1.28-12.76)	0.018	4.46 (1.20-16.67)	0.025
CMV infection that was not P/R *vs* w/o CMV infection	1.35 (0.29-6.42)	0.692		
Viral infections other than CMV infection: with *vs* w/o	1.50 (0.47-4.73)	0.493		
Bacterial bloodstream infections: with *vs* w/o	1.00 (0.35-2.88)	0.999		
Invasive fungal diseases, probable: with *vs* w/o	2.69 (0.64-11.30)	0.178		
Grade Ⅱ to Ⅳ aGvHD: with *vs* w/o	3.61 (1.23-10.59)	0.019		*ns*
Extensive cGvHD: with *vs* w/o	1.58 (0.26-9.37)	0.618		
Treatment with MZR *vs* MMF	0.43 (0.16-1.16)	0.095		*ns*

aGvHD: acute graft versus host disease; ALL: acute lymphoblastic leukemia; AML: acute

myeloid leukemia; CI: confidence interval; HR: hazard ratio; MDS: myelodysplastic syndrome; NRM: non-relapse mortality; ns: non-significant; P/R: persistent/refractory CMV infections; SF: serum ferritin.