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LETTER TO THE EDITOR

Long-term follow-up of a single institution pilot study of sirolimus, tacrolimus, and short course methotrexate for graft versus host disease prophylaxis in mismatched unrelated donor allogeneic stem cell transplantation

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Dear Editor,

Allogeneic stem cell transplantation (allo-SCT) is a potentially curative treatment for patients with hematologic malignancies [1]. However, overall survival (OS) is often compromised by treatment-related complications such as graft-versus-host disease (GVHD). Recipients of fully HLA-matched sibling donors (MSD) have an acute GVHD rates between 30 and 40% and severe life-threatening acute GVHD may occur in 15% of patients [2, 3]. Many patients who could potentially benefit from allo-SCT do not have a MSD or a matched unrelated donor (MUD) based on high-resolution molecular matching techniques [4]. Partially, HLA-mismatched unrelated donor (MMUD) transplantation is a viable option for patients lacking a fully matched donor; however, HLA mismatches significantly increase the risk of GVHD and confer an inferior OS [5].

Treatment for acute GVHD is not successful in all patients and may impact long-term survival. Patients with grade III–IV acute GVHD who do not respond to frontline therapy with corticosteroids have only a 30% 2-year overall survival [6]. Pharmacologic immunosuppression is the most commonly applied method for prevention of GVHD and has been traditionally performed using a calcineurin inhibitor in combination with a short course of methotrexate [7, 8]. These regimens effectively prevent severe GVHD in the majority of MSD and MUD transplantation. However, there is no uniformly accepted guideline for GVHD prophylaxis for MMUD transplants.

Sirolimus is an immunosuppressant derived from *Streptomyces hygroscopicus* [9], which is structurally similar to tacrolimus [10]. Sirolimus binds mammalian target of rapamycin (mTOR), that inhibits co-stimulatory pathway (e.g., CD28-AKT) and IL-2 driven pathway [11]. Sirolimus has potent anti-rejection activity in solid organ transplantation [12] and demonstrated activity as therapy of steroid-resistant GVHD [13]. Since tacrolimus and sirolimus have distinct mechanisms of action, combination therapy confers a synergistic effect [14, 15] to inhibit rejection in human organ allografting. Sirolimus does not cause nephrotoxicity and neurotoxicity, and is therefore less likely to cause synergistic adverse effects with a calcineurin inhibitor.

The combination of sirolimus, tacrolimus, and low-dose methotrexate was initially tested as GVHD prophylaxis in both matched related and limited numbers of mismatched related transplants, with reported rates of overall acute GVHD of 26% and severe acute GVHD (grades III–IV) of 13% [16]. Here, we aimed to study the activity of sirolimus/tacrolimus/low-dose methotrexate solely in MMUD transplantation and performed extended follow-up of patients registered in our pilot study.

This pilot study enrolled 25 recipients of MMUD allografts recruited at Yale University between 2008 and 2011. We monitored the efficacy of a regimen of sirolimus, tacrolimus, and methotrexate as GVHD prophylaxis for MMUD allo-grafting with extended follow-up until 2015. This study was approved by the Institutional Review Board of Yale University and

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patients consented in written form. Patients were older than 16 years of age and received a MMUD allograft defined as 8/10 or 9/10 HLA matching. Patients whose best available donor matched at 8/10 loci were required to have at least one of the mismatches at the DQ locus. For patients with best donor matched at 9/10 loci, single DQ mismatches were not permitted. All patients received G-CSF mobilized peripheral blood stem cells (PBSCs). A minimum of 2×10^6 CD34+ cells/kg were administered to recipients. Tacrolimus was administered initially by continuous infusion beginning day −3 until able to take oral medication reliably, targeting levels between 5 and 10 ng/ml. Tacrolimus was continued until day +120 post-transplant. Tacrolimus could be dose reduced or discontinued in the case of drug-related toxicity, or the development of disease recurrence. Sirolimus was administered as a 12-mg oral loading dose on day −3 followed by 4 mg daily. Sirolimus levels were obtained on day 0 and then at least twice weekly to maintain trough serum levels of 3–12 ng/ml. Sirolimus was continued until day +150 and could be dose reduced in the case of toxicity, or the development of disease recurrence. For recipients of reduced intensity conditioning, MTX was given on day +1 (10 mg/m^2) and day +3 (5 mg/m^2). For recipients of high-dose conditioning, a single dose of methotrexate (5 mg/m^2) was administered on day +1 only. Leucovorin (15 mg) was given every 6 h for 48 h after methotrexate therapy.

Baseline characteristics were summarized using descriptive statistics. Relapse-free survival (RFS) and OS were calculated using the Kaplan-Meier estimate. Cumulative incidence of non-relapsed mortality (NRM) and relapse were evaluated using a competing-risks model, with relapse and death as competing factors, respectively. Cumulative incidences of grade II–IV acute GVHD and chronic GVHD were estimated accounting for death and relapse as competing events. Acute GVHD and chronic GVHD were graded using NIH consensus criteria [17]. Venoocclusive disease (VOD) and thrombotic microangiopathy (TMA) were diagnosed based on criteria defined by the Blood and Marrow Transplant Clinical Trial Network [18]. All statistical analyses were performed using R version 3.3.2.

Patient characteristics are summarized in Table 1. Median age was 55 (20–72) years. The majority of patients were transplanted for myeloid malignancies (60%); 72% of patients received reduced intensity conditioning.

Median follow-up from the day of stem cell infusion (day 0) for surviving patients was 1496 days (607–2719 days). The cumulative incidence of grade II–IV acute GVHD occurring prior to day 100 was 24%, with no grade III–IV disease (Fig. 1a). Late-onset acute GVHD (defined as acute GVHD occurred beyond day 100 post-transplantation) occurred in an additional 16% of patients, typically following or shortly thereafter taper of immunosuppression, and was mostly stages III–IV. Two patients (8%) developed VOD and 3 (12%) developed thrombotic microangiopathic anemia (TMA). Immunosuppression was discontinued in 10 patients earlier than scheduled due to acute kidney injury (AKI) (7), TMA (1), or AKI + TMA (2). No patients developed pneumocystis pneumonia, cytomegalovirus pneumonitis, or infectious cause of death in the absence of GVHD. Substitution with alternate immunosuppression with mycophenolate or prednisone was sufficient in these patients to prevent breakthrough acute GVHD. The cumulative incidence of chronic GVHD was 32% (Fig. 1b). The 2-year incidences of NRM and relapse were 20.3 and 36%, respectively (Fig. 1c). Two-year RFS and OS were 43.3 and 51.3%, respectively (Fig. 1d). The 7.5 year RFS and OS were 38.5 and 41.1%, respectively. At 7.5 years of follow-up, only 3 of 9 patients were off immunosuppression.

Our report is a unique study of solely MMUD transplant recipients [16, 19]. The addition of sirolimus to tacrolimus and low-dose methotrexate effectively prevented severe acute GVHD within the first 3–4 months with a low rate of serious adverse events (i.e., TMA and VOD) and NRM. However, tacrolimus-/siroliumus-related toxicities, mostly renal

Table 1 Patient characteristics

N	25
Median age, in years (range)	55 (20–72)
Sex, *n*, M/F	15/10
Stem cell source, *n* (%) peripheral blood stem cells	25 (100)
Donor mismatched unrelated 7/8 match, *n* (%)	25 (100%)
Diagnosis	
AML, *n* (%)	11 (44)
• In CR1	• 1 (4)
• In CR2	• 5 (20)
• In CR3	• 1 (4)
• Primary induction failure	• 1 (4)
• Relapsed	• 2 (8)
APL (CR3), *n* (%)	1 (4)
CML-AP, *n* (%)	1 (4)
MDS	1 (4)
ALL, Ph-, n (%)	
• In CR1	2 (8)
• In CR2	1 (4)
ALL, Ph+, *n* (%)	1 (4)
MPN, *n* (%)	1 (4)
Hodgkin disease, relapsed	1 (4)
Recurrent lymphoplasmacytic lymphoma	1 (4)
Recurrent mantle cell lymphoma	1 (4)
NHL, *n* (%), relapsed and refractory	2 (8)
Aplastic anemia, *n* (%), therapy related	1 (4)
Conditioning regimen	
Myeloablative, *n* (%)	7 (28)
Reduced intensity, *n* (%)	18 (72)

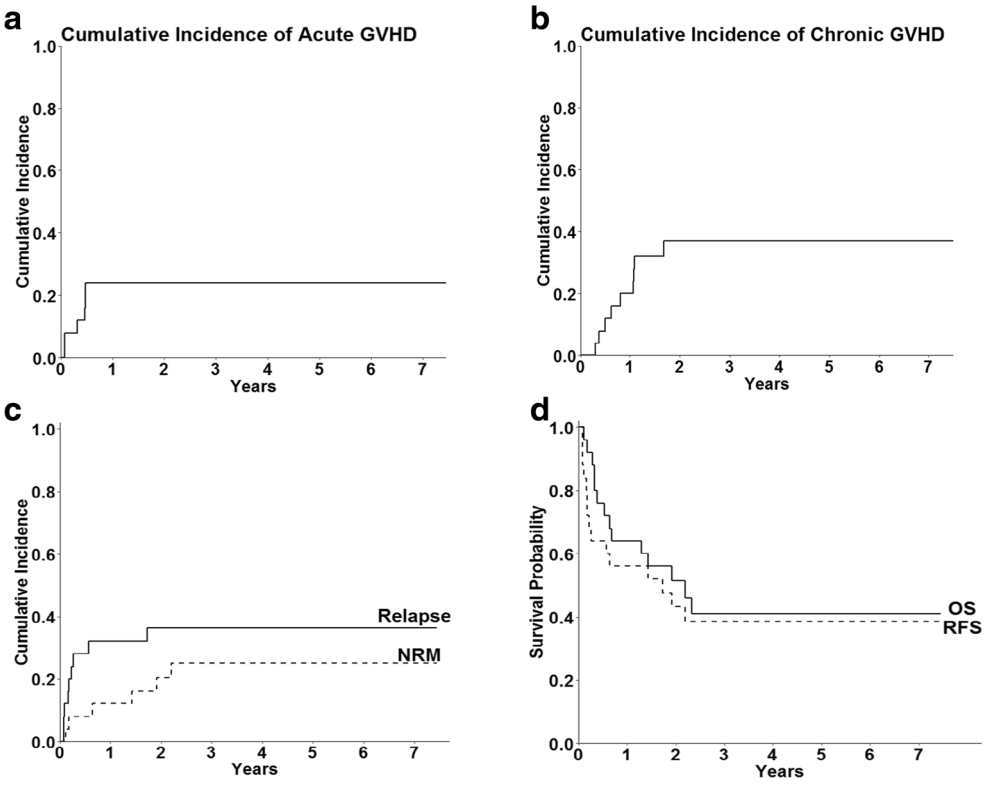

Fig. 1 Cumulative incidences of acute GVHD (**a**), chronic GVHD (**b**), relapse and non-relapsed mortality (NRM) (**c**), and overall survival (OS) and relapse-free survival (RFS) (**d**)

insufficiency, prevented the completion of the planned regimen in a significant proportion of patients. Nonetheless, early breakthrough acute GVHD did not occur after the omission or substitution of another agent for either tacrolimus or sirolimus. Unfortunately, the rate of late-onset acute GVHD was substantial, coincident with tapering of sirolimus or tacroliumus. Long-term immunosuppression has been required in most surviving recipients. As a result, our current practice is to continue sirolimus for a full year prior to consideration of taper in eligible patients.

The optimal method for transplantation for patients lacking a matched donor remains unclear. Alternative transplantation strategies to prevent GVHD for patients lacking a fully matched donor include the use of haploidentical grafts with immunosuppression incorporating post-transplant cyclophosphamide, T cell depletion, or use of umbilical cord blood grafts. Well-controlled direct comparative studies of such techniques have not been completed. T cell depletion techniques may not be applicable to all patients given requirements for intensive conditioning. The use of post-transplant cyclophosphamide for GVHD prevention with haploidentical grafts is associated with acceptable rates of acute GVHD, consistent engraftment, and very low rates of chronic GVHD, and offers promise to reduce the use of long-term immunosuppression in such patient populations [20]. A recent study using post-transplant cyclophosphamide in MMUD transplant patients reported a cumulative incidence of acute GVHD comparable to that in our study, but with less chronic GVHD and NRM [21]. The comparative efficacy of such methods with regard to disease control is unknown. Acknowledging several limitations, including the relatively small sample size of this study and the relatively high incidence of regimen modifications due to side effects, the long-term outcomes presented herein appear similar to reported outcomes of other GVHD prevention methods for patients undergoing MUD transplantation. We are hopeful that prolonged use of sirolimus may offer reduction in the rate of late-onset AGVHD and improve outcome.

Compliance with ethical standards

Ethical standards statement This study was in accordance with the ethical standards of the responsible institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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