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Sirolimus, tacrolimus and antithymocyte globulin as GVHD prophylaxis in HLA-mismatched unrelated donor hematopoietic cell transplantation: a single institution experience

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Allogeneic hematopoietic cell transplantation (allo-HCT) is curative for various hematological malignancies with improved outcomes observed over the past years.^{1,2} Offering a HLA-mismatched allo-HCT to patients lacking a suitable HLA-matched related (MRD) or unrelated donor (MUD) is feasible, but results in high non-relapse mortality (NRM) and inferior overall survival (OS).³ Adding antithymocyte globulin (ATG) improves post-allograft outcomes by reducing incidence and severity of acute (aGVHD) and chronic GVHD (cGVHD).⁴ Accordingly, ATG has become essential for aGVHD prophylaxis in mismatched unrelated donor (MMUD) allo-HCT.^{5,6} No consensus exists on a uniformly accepted standard GVHD prophylaxis regimen for MMUD allografting. Regimen choice is generally based on physician and/or center preference. Sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR), is used in combination with tacrolimus for aGVHD prophylaxis. A randomized phase 2 study comparing tacrolimus plus sirolimus (TAC-SIR) or methotrexate (TAC-MTX) in 74 patients receiving an allograft from a MRD (SIR = 17, TAC = 18) or a MUD (SIR = 20, TAC = 19) donor showed lower incidence of grade II–IV aGVHD with TAC-SIR but no difference in OS.⁷ A larger multicenter phase 3 trial comparing TAC-SIR to TAC-MTX failed to show a difference in incidence of grade II–IV aGVHD or OS, though a trend toward less clinically severe grade III–IV aGVHD was described with TAC-SIR.⁸ TAC-SIR showed faster neutrophil and platelet engraftment and less severe oropharyngeal mucositis.⁸ Adding ATG to TAC-SIR is feasible. Al-Kadhimi *et*

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*al.*⁹ evaluated TAC–SIR–ATG in a single-arm phase 2 study of 47 unrelated donor allo-HCT (HLA 8/8 match = 47%, HLA 7/8 match = 53%) showing an incidence of grade II–IV aGVHD of 23%, cGVHD 33%, 2-year NRM 32% and 1-year OS of 65%. Another phase 2 trial using TAC–SIR–ATG⁹ was prematurely discontinued after enrolling 32 of 60 intended patients owing to graft failure (12.5%) and 3 associated deaths.¹⁰ We report our experience using TAC–SIR–ATG in MMUD allo-HCT recipients at our institution.

This retrospective study evaluated 27 consecutive patients who received TAC–SIR–ATG as GVHD prophylaxis for MMUD allo-HCT between August 2008 and September 2013. This study was approved by the Institutional Review Board of University of South Florida, and patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki. Patients were 18 years of age and received a MMUD allograft defined as 7/8 HLA-matching at antigen(s) or allele(s) levels. All received G-CSF mobilized PBSCs. Patients receiving umbilical cord blood transplantation or GVHD prophylaxis other than TAC–SIR–ATG were excluded.

We evaluated the efficacy of TAC–SIR–ATG as GVHD prophylaxis on cumulative incidence of aGVHD and cGVHD, NRM, relapse, relapse-free survival (RFS) and OS. The incidence of EBV reactivation using real-time PCR (Quiagen Artus EBV RG PCR ASR, Hamburg, Germany) and use of rituximab was assessed considering the inherent risk of developing post-transplant lymphoproliferative disorder (PTLD). CMV reactivation was assessed by PCR (Quiagen Artus). TAC started on day –3 at 0.01 mg/kg (ideal body weight), initially as IV and later converted to oral formulation. Starting dose of SIR was 12 mg (day –1) followed by 4 mg/day starting on day 0. The goal level range for TAC was 3–7 ng/mL and for SIR was 8–14 ng/mL. All received ATG (rabbit thymoglobulin, Sanofi, Paris, France) at a total dose of 7.5 mg/kg: 1 mg/kg (day –3) followed by 3.25 mg/kg/day (days –2 and –1). EBV was monitored weekly from day 0 until day +100. Based on our practice, a titer exceeding 1000 copies/mL prompted initiation of rituximab 375 mg/m². Persistent EBV viremia, where quantitative PCR showed less than a one-log reduction, would have prompted three additional weekly rituximab doses. For CMV, we followed a preemptive treatment approach where anti-CMV therapy was initiated if CMV copies exceeded 1000/ml or at lower levels if clinically indicated.

Baseline characteristics were summarized using descriptive statistics including mean, median, s.d. and range for continuous measures and frequencies and proportion for categorical measures. Probabilities for RFS and OS were calculated using the Kaplan–Meier estimate. Cumulative incidence of NRM and relapse were evaluated using a competing-risks model by Gray,¹¹ with relapse and death as competing factors, respectively. Similarly, cumulative incidences of grade II–IV aGVHD and cGVHD were estimated accounting for death and relapse as competing events. Neutrophil engraftment was defined as the first of 3 consecutive days of an ANC ≥ 500/μL. Platelet engraftment was defined as the first of 7 days without platelet transfusion. Also, aGVHD and cGVHD were graded using 1996 consensus criteria¹² and National Institute of Health (NIH) consensus.¹³ Sinusoidal obstructive syndrome (SOS) and thrombotic microangiopathy (TMA) were diagnosed based on criteria by the Blood and Marrow Transplant Clinical Trial Network¹⁴ and McDonald *et al.*,¹⁵ respectively.

Median age was 56 (24–67) years. The majority were allografted for a myeloid malignancy (63%) using a myeloablative regimen (74%). Median number of infused CD34+ cells ($\times 10^6$ /recipient weight in kg) was 8.6 (3.2–23.0). These and other characteristics are summarized in Table 1.

Median follow-up from the day of cell infusion (day 0) for all surviving patients was 13.04 (2.92–55.0) months. There were no primary graft failures. Median time-to-ANC and platelet engraftment were 14 (12–24) days and 15 (10–27) days, respectively. One patient's platelets never dropped $< 20\,000/\mu\text{L}$. The 100-day incidence of grade II–IV aGVHD was 56% (95% confidence interval (CI) = 35–72%); and for grade III–IV aGVHD was 11% (95% CI = 3–26%). Incidence of cGVHD (NIH-moderate and severe) was 23% (95% CI = 9%–41%). Two-year NRM was 16% (95% CI = 5–33%) (Figure 1a). There were only 4 non-relapse deaths owing to GVHD ($n = 1$), viral infection ($n = 2$) and unknown ($n = 2$).

Two-year cumulative incidence of relapse was 28% (95% CI = 12–46%) (Figure 1a). Two-year RFS and OS (Figure 1b) were 57% (95% CI = 36–73%) and 80% (95% CI = 58–91%), respectively. At 1-year follow-up, only 2 (10%) of 20 evaluable patients were off immune suppression. Two (7%) patients developed SOS on days +35 and +68, and 2 (7%) other developed TMA on days +43 and +70, respectively. None of the non-relapse deaths were attributable to SOS or TMA. Preemptive rituximab was prescribed in 78% but none developed PTLT. Median time-to-first EBV reactivation was 26 (18–100) days. Within the first 100 days, 8 (30%) developed CMV reactivation, at a median of 34 (16–91) days, requiring therapy. All eight patients were CMV seropositive preallografting.

TAC–SIR–ATG is a feasible GVHD prophylaxis regimen for MMUD allo-HCT. An encouraging OS occurred despite a relatively older population (median age of 56 years) and over half of them (56%) having intermediate- or high-risk disease by CIBMTR criteria. Older age is known to result in inferior survival after allo-HCT. Moreover, this encouraging OS is explained in part by the relatively low 2-year NRM of 16%.

Administration of TAC–SIR–ATG as GVHD prophylaxis resulted in high incidence of EBV reactivation, requiring rituximab administration. Our incidence of EBV reactivation (78%) was certainly higher than reported by Al-Kadhimi *et al.*⁹ (20%). One explanation is the higher (67%) cumulative dose of ATG used at our center (7.5 vs 4.5 mg/kg). None of the patients who reactivated EBV developed or died from PTLT.

Cumulative incidence of grade II–IV aGVHD in our study was 56% (95% CI = 35–72%), certainly higher than the 23.4% grade II–IV incidence of aGVHD reported by others.⁹ However, all of our patients received MMUD grafts (19% had two mismatches; Table 1), whereas in the study by Al-Kadhimi *et al.*⁹ 47% were allografted from MRDs, who are inherently at lower risk of developing aGVHD. This is also the case for Khaled *et al.*¹⁰ who reported a cumulative incidence of aGVHD of 37.3%; but 56% of enrolled patients were allografted from a HLA MUDs. With regards to cGVHD, our study compared favorably to Khaled *et al.*¹⁰ who reported extensive cGVHD in 17 (68%) of 25 evaluable patients. One notable difference among the studies was the ATG dose. In our study, a total of 7.5 mg/kg was prescribed, whereas the total ATG dose in Khaled *et al.*¹⁰ and Al-Khadimi *et al.*⁹ were

only 4.5 mg/kg, respectively. In Al-Kadhimi *et al.*,⁹ the cumulative incidence of cGVHD (all grades) was 33% (mild = 8/16, moderate/severe = 8/16).

Use of TAC–SIR–ATG resulted in timely ANC and platelets engraftment and we did not observe graft failure. Our study shows a 7% incidence of SOS and 7% incidence of TMA with TAC–SIR–ATG. One patient who developed SOS died from relapsed AML and another is alive after 30+ months. Moreover, two patients who developed TMA are alive at 26 and 35 months post-transplantation. Given the potential morbidity and mortality from SOS or TMA, transplant team members must be familiar with best practice management of these complications.

We acknowledge several limitations, including the study's retrospective nature and the relatively small sample size. Although it is arguable that our analysis has a relatively short follow-up, one should note that negative post-transplant outcomes, notably NRM, commonly occur in the first year post-transplantation. In our program, SIR has become the preferred agent for GVHD prophylaxis in comparison to methotrexate or mycophenolate mofetil⁷ and adding ATG was the reasonable approach towards improving GVHD control in MMUD allo-HCT. Notwithstanding these limitations, the encouraging OS seen with TAC–SIR–ATG in MMUD is intriguing and requires confirmation. Optimal ATG dose is another important question that remains unanswered.

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Figure 1.
(a) Cumulative incidence of NRM and relapse. (b) OS.

Table 1
Patient-, disease- and treatment-related characteristics

Variables	TAC–SIR–ATG (N = 27)
Recipient median (range) age, years	56 (24–67)
Recipient gender, N(%)	Female = 15 (56%) Male = 12 (44%)
Recipient/donor gender matching, N(%)	M/M = 9 (33%) M/F = 3 (11%) F/M, F = 15 (56%)
Preparative regimen MAC or RIC	MAC = 20 (74%) RIC = 7 (26%)
Primary disease, N(%)	Myeloid = 17 (63%) Lymphoid = 9 (33%) Others = 1 (4%)
CIBMTR disease risk, N(%)	High = 5 (19%) Int = 10 (37%) Low = 12 (44%)
Recipient CMV serology, N(%)	Seropositive = 21 (78%)
HLA matching ^a 7/8, N(%)	MHC Class I: A = 10 (37%) B = 9 (33%) C = 3 (11%) MHC class II: DRB1 = 0 Others: A, C = 1 (4%) A, DQB1 = 4 (15%)
Median (range) CD34 cell dose ($\times 10^6$ /recipient weight in kg) cells	8.6 (3.2–23.01)

Abbreviations: CIBMTR = Center for International Blood and Marrow Transplant Research; F = female; Int = intermediate; M = male; MAC = myeloablative conditioning; RIC = reduced intensity conditioning; TAC–SIR–ATG = tacrolimus/sirolimus/antithymocyte globulin. Myeloid malignancies consisted of AML, myelodysplastic syndrome and CML. Lymphoid malignancies consisted of ALL, CLL, Hodgkin lymphoma and non-Hodgkin lymphomas. Other diagnoses not included in the lymphoid or myeloid group (myelofibrosis = 1). MAC regimen: fludarabine IV plus pharmacokinetically targeted busulfan IV to an area under the receiver operating characteristic curve (AUC) of 5300 $\mu\text{mol} \times \text{min}$ per dose ($\times 4$). RIC regimens or myeloablative but reduced toxicity: fludarabine IV plus pharmacokinetically targeted busulfan IV to an AUC of 3500 $\mu\text{mol} \times \text{min}$ per dose ($\times 4$), fludarabine IV plus cyclophosphamide, fludarabine IV plus melphalan and fludarabine IV plus 2 Gy total body irradiation.

^aDenotes antigen- or allele-level mismatch.