Prolonged sirolimus administration after allogeneic hematopoietic cell transplantation is associated with decreased risk for moderate-severe chronic graft-*versus*-host disease

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ABSTRACT

Effective pharmacological strategies employed in allogeneic hematopoietic cell transplantation should prevent serious chronic graft-*versus*-host disease and facilitate donor-recipient immune tolerance. Based on demonstrated pro-tolerogenic activity, sirolimus (rapamycin) is an agent with promise to achieve these goals. In a long-term follow-up analysis of a randomized phase II trial comparing sirolimus/tacrolimus *versus* methotrexate/tacrolimus for graft-*versus*-host disease prevention in matched sibling or unrelated donor transplant, we examined the impact of prolonged sirolimus administration (≥ 1 year post-transplant). Median follow-up time for surviving patients at time of this analysis was 41 months (range 27-60) for sirolimus/tacrolimus and 49 months (range 29-63) for methotrexate/tacrolimus. Sirolimus/tacrolimus patients had significantly lower National Institutes of Health Consensus moderate-severe chronic graft-*versus*-host disease (34% *vs.* 65%; *P*=0.004) and late acute graft-*versus*-host disease (20% *vs.* 43%; *P*=0.04). While sirolimus/tacrolimus patients had lower prednisone exposure and earlier discontinuation of tacrolimus (median time to tacrolimus discontinuation 368 days *vs.* 821 days; *P*=0.002), there was no significant difference in complete immune suppression discontinuation (60-month estimate: 43% *vs.* 31%; *P*=0.78). Prolonged sirolimus administration represents a viable approach to mitigate risk for moderate-severe chronic and late acute graft-*versus*-host disease. Further study of determinants of successful immune suppression discontinuation is needed.

Introduction

The desired outcome of allogeneic hematopoietic cell transplantation (HCT) is cure of malignancy, minimization of graft-*versus*-host disease (GvHD)-associated morbidity, disability and treatment-related mortality, and achievement of donor-recipient immune tolerance manifested by complete discontinuation of immune suppressive medications without recurrent GvHD.

While successive advances in pharmacological GvHD prevention strategies have led to tangible reduction in acute GvHD, the burden of chronic GvHD largely remains unchanged with these approaches.[1-6] With the combination of tacrolimus (TAC)/methotrexate (MTX) or TAC/sirolimus (SIR), approximately 50% of patients will experience chronic GvHD after matched sibling donor HCT,[4] and a similar proportion are affected after unrelated donor HCT.[5] This represents a major obstacle to the success of HCT, as chronic GvHD remains a major source of late HCT-associated morbidity and death,[7-9] and is associated with prolonged immune suppressive therapy.[10] In contrast, *ex vivo* and *in vivo* T-cell depletion strategies (including ATG and post-transplant cyclophosphamide) are associated with lower risk of chronic GvHD,[11-13] and merit ongoing study.

The optimal type and duration of immune suppressive therapy to mitigate risk for serious chronic GvHD and effectively induce tolerance remains unknown. Several prior studies have examined variably defined prolonged courses of calcineurin inhibitors after HCT with these goals in mind.[14-18] While some studies have demonstrated reduction in chronic GvHD, others do not support this conclusion and fail to demonstrate benefits in ultimate immune suppression discontinuation. Taken together, these studies fail to provide convincing evidence of a beneficial effect of this approach.

Extensive pre-clinical evidence demonstrates that sirolimus (rapamycin) supports tolerance development,[19-26] and thus this agent holds promise to facilitate the above-stated goals of HCT. When early immune suppression taper goals were employed, a major phase III trial failed to demonstrate a significant advantage of SIR/TAC over MTX/TAC in prevention of chronic GvHD. In contrast, in an initial report of a randomized phase II trial, we demonstrated that prolonged SIR administration post-HCT (≥ 1 year post-HCT among SIR/TAC-treated patients *vs.* MTX/TAC-treated patients) led to a reduction in moderate-severe chronic GvHD and supported the reconstitution of functional regulatory T cells (Treg) after HCT.[27] In the present long-term follow-up analysis, we examined whether prolonged administration of sirolimus would result in durable reduction in chronic GvHD and increased rates of immune suppression discontinuation.

Methods

Parent trial

Patients were randomized to receive sirolimus/tacrolimus (SIR/TAC) *versus* methotrexate/tacrolimus (MTX/TAC) in a random-

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ized phase II trial of graft-*versus*-host disease (GvHD) prevention (*clinicaltrials.gov identifier: 00803010*). Trial inclusion and exclusion criteria, stratified randomization accounting for donor type and HCT recipient age, and patients' characteristics have been previously described.[27] The trial recommended the following immune suppression management: patients were eligible to initiate TAC taper at day 50 following HCT in the absence of acute GvHD. SIR, conversely, was intentionally continued through at least one year after HCT. Beyond this, the protocol did not mandate a taper schedule for TAC, SIR, systemic glucocorticoids, or other immune suppressive agents. The original report described the cumulative incidence of acute GvHD through day 100, estimated the incidence of overall and National Institutes of Health (NIH) moderate-severe chronic GvHD, relapse, non-relapse mortality, and overall survival, and described peripheral blood regulatory T cell (Treg) reconstitution and function after HCT.[27] A separate report described longitudinal recovery in patient-reported quality-of-life (QOL).[28]

Long-term follow up

In this long-term follow-up study, we collected comprehensive data on all trial patients to permit the following analyses.

1) Compliance with at least one year of sirolimus therapy among SIR/TAC patients: Time to discontinuation of SIR was summarized, and indications (intentional taper, toxicity, TMA per BMT CTN criteria,[29] malignancy relapse) were noted.

2) Incidence, severity, classification, and organ manifestations of chronic and late acute GvHD: individual chronic GvHD organ involvement and severity were recorded according to the NIH Consensus criteria for Diagnosis and Staging; overlap subtype had concurrent presence of acute GvHD features.[30,31] Data included both characteristics at chronic GvHD onset and maximum severity. Late acute GvHD solely had manifestations of acute GvHD, and severity was scored according to standard criteria.[32]

3) Burden of systemic glucocorticoid exposure: systemic glucocorticoid therapy was recorded in all patients. Prednisone exposure [individual time point-wise comparisons and cumulative exposure to prednisone (mg/kg/day x time)] was compared across SIR/TAC *versus* MTX/TAC groups. Data was summarized at standard time points (days post HCT): 120, 150, 180, 210, 240, 270, 360, 540, 720, 1080, 1440, and 1800 days. Only living patients without malignancy relapse were considered eligible for this analysis.

4) Incidence of TAC discontinuation (successful event defined as intentional taper and discontinuation of TAC; discontinuation of TAC for toxicity or malignancy relapse was not counted as success in this analysis) and complete immune suppression discontinuation (complete discontinuation of all immune suppressive agents including original immune suppressive prophylaxis, all systemic glucocorticoid therapy, and any other additional systemic immune suppressive agents added throughout study period).

5) Current estimates of malignancy relapse, non-relapse mortality (NRM), and overall survival. For the analysis of NRM, interaction of study group and conditioning regimen intensity was explored. Based on excess NRM among SIR/TAC patients treated at higher dose busulfan (co-enrollment on investigational trial with IV busulfan targeted to average daily AUC of 7500 μM/L*min/day together with fludarabine),[33] data are presented for all patients, and separately restricted to only those receiving standard dose busulfan-based regimens (IV busulfan targeted to average daily AUC of either 3500 or 5300 μM/L*min/day).

6) Sub-group analysis of patients with established chronic GvHD. Initial and subsequent lines of systemic immune suppressive therapy (including extra-corporeal photopheresis) were recorded. The occurrence and time to chronic GvHD resolution was recorded in cases that achieved complete resolution. Treatment success and failure-free survival definitions were modeled after previous literature.[34,35] Treatment success was defined as complete resolution of chronic GvHD and discontinuation of all immune suppression. Failure-free survival was defined as time to a composite event including death, relapse or additional systemic immune suppressive therapy beyond first-line chronic GvHD therapy.

Table 1. Late acute graft-*versus*-host disease characteristics and severity.

	SIR/TAC	MTX/TAC	*P*
Late acute GvHD subtype			
De novo	4	0	0.005
Persistent	1	3	
Recurrent	2	13	
Organ involvement			
Skin	5	6	NS
Gastrointestinal	4	7	
Liver	4	9	
Overall grade			
1	2	2	0.007
2	2	14	
3	0	0	
4	3	0	

GvHD: graft-versus-host disease.

Statistical analysis

Descriptive statistics included medians and ranges for continuous variables and frequencies for categorical variables. Categorical variables were compared using Fisher exact test, and continuous variables were compared using Wilcoxon rank-sum test. The cumulative incidence function was used to estimate outcomes including late acute GvHD, overall chronic GvHD, moderate-severe chronic GvHD, immune suppression discontinuation (all accounting for malignancy relapse and non-relapse death as competing risk events), malignancy relapse (with non-relapse death as competing risk event) and non-relapse death. Cumulative incidence of these outcomes was compared across groups using the Gray test.[36] Fisher exact test was used to compare chronic GvHD organ involvement and severity, as well as late acute GvHD organ severity across study groups. A multivariate model was developed to assess relationship of study variables: study group [(SIR/TAC *vs.* MTX/TAC), as well as patient (age), disease (diagnosis, CIBMTR risk category), and transplantation variables (donor type, donor/recipient CMV matching, donor/recipient gender matching, conditioning regimen, prior acute GvHD)] with risk for development of moderate-severe chronic GvHD: those with *P* value <0.25 were included in the initial multivariable model. The Kaplan-Meier method was used to estimate survival end points including failure-free survival and overall survival, and the log rank test was used to compare survival data across groups.

Results

Patients' characteristics

The trial design, included patients, and GvHD prophylaxis treatment plans have been previously described for the original trial.[27] Median follow-up time for surviving patients at the time of the initial study analysis was 20 months (range 4-32) for SIR/TAC, and 17 months (range 4-

32) for MTX/TAC. There were no significant differences between study arms for recipient or donor age, recipient/donor gender, diagnosis and pre-HCT remission status, CIBMTR risk category, donor type, or conditioning regimen (*P*=n.s. for all comparisons). The cumulative incidence of grade II-IV acute GvHD through day 100 post HCT showed a significant difference between study groups: 43% (95%CI: 27%-59%) in the SIR/TAC group, and 89% (95%CI: 72%-96%) in the MTX/TAC group; *P*<0.001. All patients (74 total, SIR/TAC n=37 and MTX/TAC n=37) were included in this long-term follow-up analysis. Median follow-up time for surviving patients at time of this analysis was 41 months (range 27-60) for SIR/TAC and 49 months (range 29-63) for MTX/TAC. Compliance with the planned one year or more of SIR therapy among SIR/TAC patients was excellent: One patient discontinued SIR at 161 days post HCT due to TMA (grade 1). Otherwise, SIR was not discontinued for toxicity in other cases. The median duration of SIR therapy among patients (n=27) surviving one year or more was 33 months (range 5-60 months).

Chronic graft-versus-host disease

The reduction in NIH Consensus moderate-severe chronic GvHD among SIR/TAC-treated patients persisted in this long-term follow-up analysis. The cumulative incidence of moderate-severe chronic GVHD is presented in Figure 1. A total of 9 patients in the SIR/TAC group experienced moderate-severe chronic GvHD *versus* 22 in the MTX/TAC group. On multivariate analysis, the SIR/TAC group had significantly lower risk for moderate-severe chronic GvHD (HR 0.32, 95%CI: 0.15-0.68; *P*=0.003). No other considered patient, disease, or HCT variables remained in the final multivariate model. Maximal chronic GvHD organ involvement and severity are presented in Figure 2. Most commonly affected organ sites were eyes, mouth, skin, and liver. The global severity score was significantly lower in SIR/TAC patients; however, no individual organ-specific comparisons were significantly different between groups. Overlap subtype of chronic GvHD was diagnosed in 7 SIR/TAC patients *versus* 14 MTX/TAC patients (*P*=0.15).

Late acute graft-versus-host disease

SIR/TAC patients had significantly lower incidence of late acute GvHD (Figure 3). Late acute GvHD severity and organ involvement is presented in Table 1. In keeping with

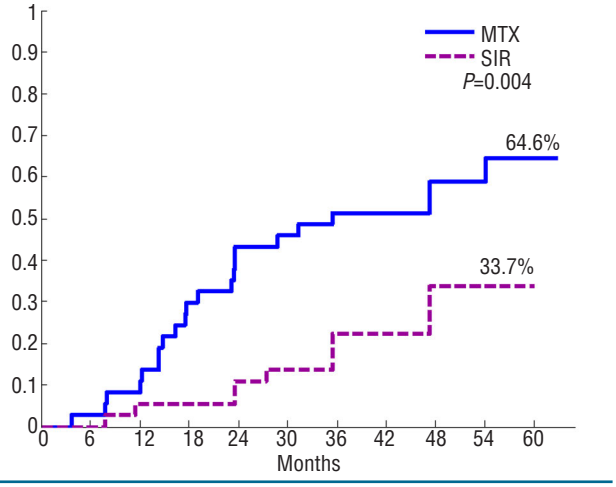
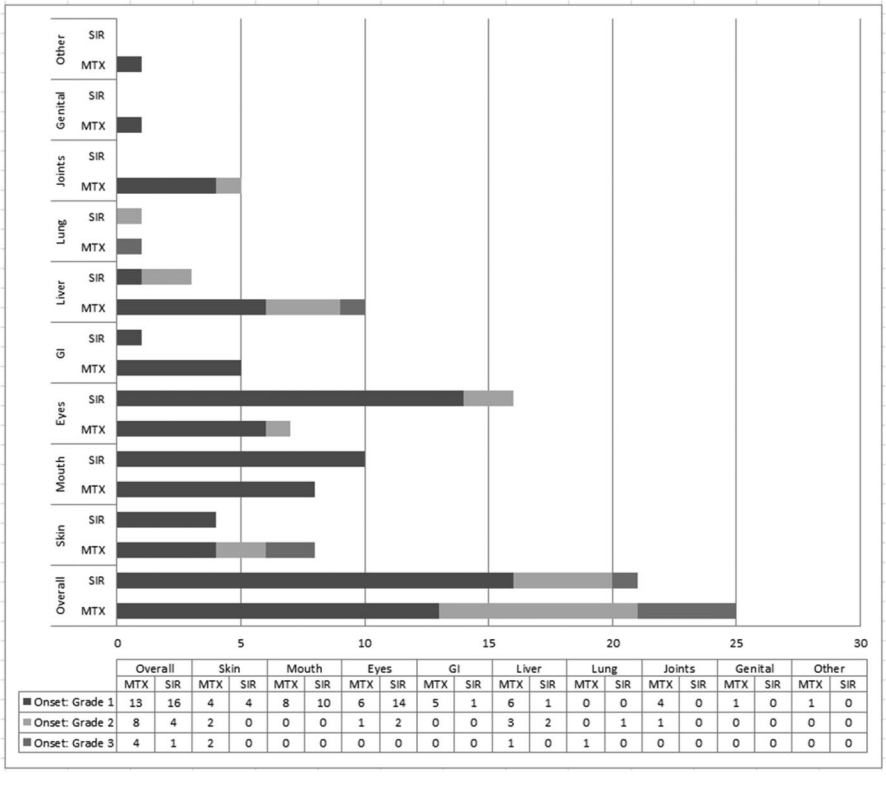


Figure 1. Cumulative incidence of NIH Consensus moderate-severe chronic graft-*versus*-host disease.



	Overall		Skin		Mouth		Eyes		GI		Liver		Lung		Joints		Genital		Other	
	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR	MTX	SIR
Onset: Grade 1	13	16	4	4	8	10	6	14	5	1	6	1	0	0	4	0	1	0	1	0
Onset: Grade 2	8	4	2	0	0	0	1	2	0	0	3	2	0	1	1	0	0	0	0	0
Onset: Grade 3	4	1	2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0

Figure 2. Comparative distribution of maximal chronic graft-*versus*-host disease organ involvement and severity.

greater classic acute GvHD within 100 days, the MTX/TAC group had a greater burden of persistent and recurrent late acute GvHD.

Systemic glucocorticoid therapy

Systemic glucocorticoid exposure (presented as mg/kg/day of prednisone) differed according to study group (Figure 4). Total exposure (SIR/TAC: median 20.1 mg/kg total exposure over study period, range 0-360 mg/kg *vs.* MTX/TAC: median 46.9 mg/kg total exposure over study period, range 0-474.8 mg/kg; *P*=0.18) was lower among SIR/TAC patients. Individual point-wise comparisons demonstrated significantly lower prednisone dose at day 540 (*P*=0.028) among SIR/TAC patients; however, no other point-wise comparisons were significantly different.

Immune suppression discontinuation

While a comparable number of SIR/TAC *versus* MTX/TAC patients successfully discontinued TAC in the absence of toxicity or malignancy relapse (SIR/TAC n=15 *vs.* MTX/TAC n=12), median time to TAC discontinuation was shorter (SIR/TAC 368 days *vs.* MTX/TAC 821 days; *P*=0.002) for the SIR/TAC group. The cumulative incidence of successful TAC discontinuation is presented in Figure 5.

In contrast, a greater number (n=8) of patients in the SIR/TAC group stopped TAC for toxicity (TMA n=6; thrombocytopenia n=1, and renal insufficiency n=1, respectively, without meeting criteria for TMA), compared to the MTX/TAC group (TMA n=1). One patient in each group stopped TAC for post-HCT malignancy relapse.

By 60 months of follow up, the cumulative incidence of complete discontinuation of all immune suppression in the absence of death or relapse was comparable for SIR/TAC *versus* MTX/TAC groups (SIR/TAC 43% *vs.* MTX/TAC 31%; *P*=0.78).

Overall survival, malignancy relapse, non-relapse mortality

There was no significant difference in overall survival between groups. Causes of death are outlined in Table 2. Patients in the SIR/TAC arm were less likely to die from

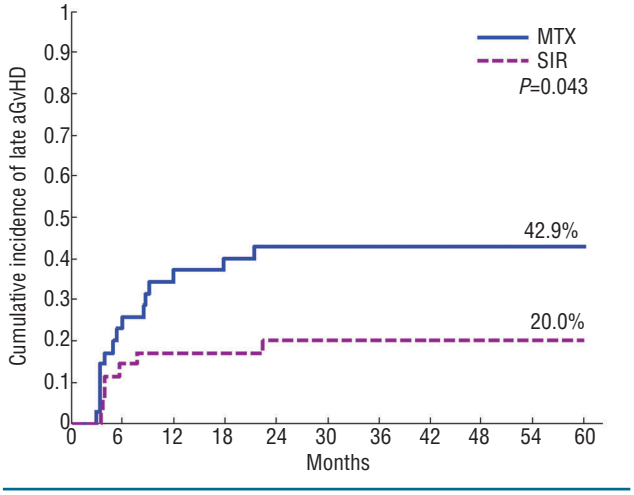
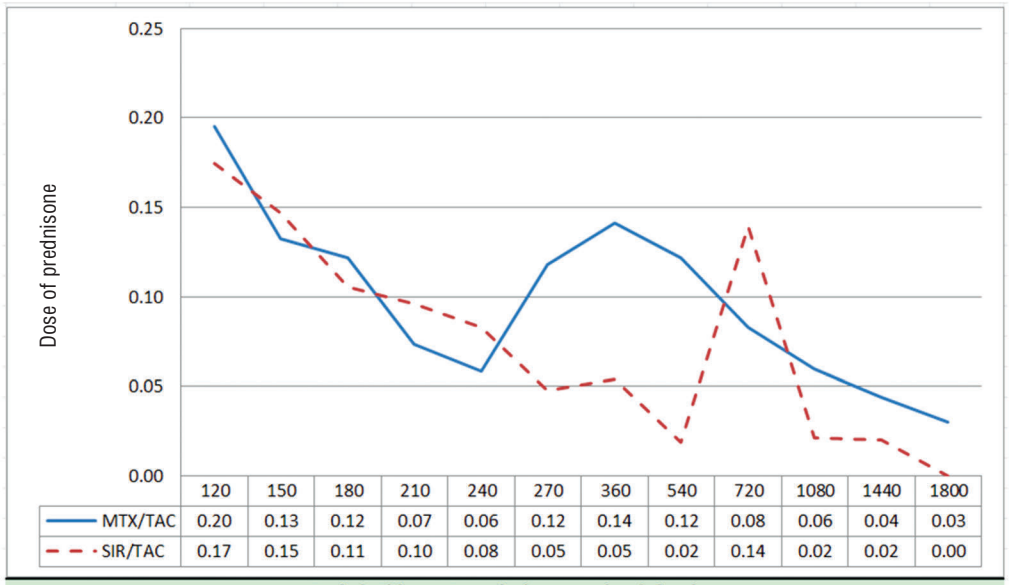


Figure 3. Cumulative incidence of late acute graft-*versus*-host disease.

Table 2. Causes of death.

Cause of death	N	SIR/TAC conditioning regimen	Days post-HCT	N	MTX/TAC conditioning regimen	Days post-HCT
Malignancy relapse	6			14		
GvHD	3			1		
Acute GvHD	0			0		
Late acute GvHD	2	Bu/pent, Flu/Mel	123,724	0		
Chronic GvHD	1	Flu/Mel	726	1	Bu (5300)/Flu	730
Sepsis	2			1		
sepsis, HHV6, MOSF	1	Bu (7500)/Flu	92	0		
sepsis, resp. failure	1	Bu (5300)/Flu	839	1	Bu (5300)/Flu	364
Organ failure	6			2		
GIB, renal/MOSF	1	Bu (7500)/Flu	227	0		
VOD/MOSF	1	Bu (5300)/Flu	58	0		
GIB, MOSF/sepsis	1	Bu (5300)/Flu	138	0		
Respiratory failure						
Pneumonia	1	Flu/Mel	986	0		
Influenza pneumonia	1	Bu (7500)/Flu	227	0		
RSV pneumonia	1	Flu/Mel	199	0		
Diffuse alveolar Hemorrhage	0			1	Bu (7500)/Flu	162
Liver failure	0			1	Bu (3500)/Flu	451

**Bu: busulfan; Flu: fludarabine; GIB: GI bleeding; GVHD: graft versus host disease; HCT: allogeneic hematopoietic cell transplantation; HHV6: human herpes virus 6; Mel: melphalan; MOSF: multi-organ system failure; pent: pentostatin; RSV: respiratory syncytial virus; VOD: hepatic veno-occlusive disease; 3500/5300/7500: targeted average daily busulfan exposure (μM/L*min/day).*



	120	150	180	210	240	270	360	540	720	1080	1440	1800
MTX/TAC	0.20	0.13	0.12	0.07	0.06	0.12	0.14	0.12	0.08	0.06	0.04	0.03
SIR/TAC	0.17	0.15	0.11	0.10	0.08	0.05	0.05	0.02	0.14	0.02	0.02	0.00

# of eligible patients (relapse or death free)

	Day											
	120	150	180	210	240	270	360	540	720	1080	1440	1800
MTX/TAC	29	29	26	26	26	26	26	23	21	18	12	3
SIR/TAC	34	31	31	30	28	28	26	24	24	15	8	1

Figure 4. Comparison of prednisone exposure over time. *Prednisone dose presented as mean values for SIR/TAC and MTX/TAC groups for each time point. Eligible patients at each point were free of relapse and death.

relapse. In keeping with data reported in the initial study report,[27] the cumulative incidence of relapse was lower in the SIR/TAC group (48-month estimate: SIR/TAC 19% *vs.* MTX/TAC 39%; *P*=0.06). As well, non-relapse mortality was increased in the SIR/TAC group (4- month estimate for total study population: SIR/TAC 32% *vs.* MTX/TAC 11%; *P*=0.03). Co-administration of escalated dose IV busulfan (7500 μM/L*min/day) together with fludarabine resulted in excess NRM among SIR/TAC (NRM: 3 of 5) patients, but not MTX/TAC (NRM: 1 of 11).[33] When restricted to standard dose conditioning (daily busulfan AUC of 5300 μM/L*min/day or less), no difference in NRM was observed (48-month estimate: SIR/TAC 10% *vs.* MTX/TAC 16%; *P*=0.98).

Subset analysis of cohort with established chronic graft-versus-host disease

While chronic GvHD therapy was not controlled on trial, subgroup analysis of those with chronic GvHD did not demonstrate differences to suggest that ongoing SIR treatment modified the natural history and therapeutic responsiveness of chronic GvHD. First, comparable numbers of patients per study arm received additional lines of systemic immune suppressive therapy for chronic GVHD (SIR/TAC n=7, and MTX/TAC n=5; *P*=0.15) beyond first-line treatment. Comparable agents were utilized in this setting as well: SIR/TAC (ECP 2, imatinib 2, MMF 5, pentostatin 1, rituximab 2) *versus* MTX/TAC (ECP 2, imatinib 1, MMF 2, sirolimus 3, rituximab 1). Next, there was no significant difference in the proportion of patients with chronic GvHD resolution by time of last follow up, or time from chronic GvHD onset to resolution. Finally, there was no significant difference in treatment success (cumulative incidence at 48 months: SIR/TAC 18% *vs.* MTX/TAC 21%; *P*=0.66), or failure-free survival (Figure 6).

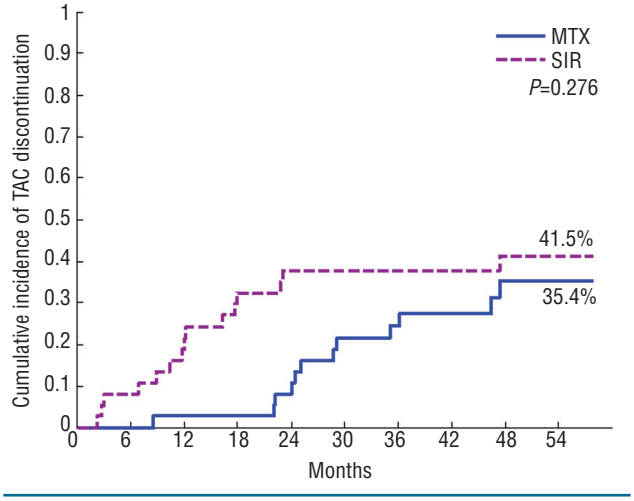


Figure 5. Cumulative incidence of successful TAC discontinuation

Discussion

Effective strategies that prevent serious chronic GvHD and facilitate donor-recipient immune tolerance are needed. Currently available pharmacological strategies not employing complete or selective T-cell depletion largely fail to achieve these goals, and prior studies examining prolonged administration of calcineurin inhibitors have not demonstrated consistent benefit. In addition, a major randomized phase III comparative trial has shown comparable rates of chronic GvHD (comparing SIR/TAC *vs.*

MTX/TAC) when earlier goals for immune suppression taper were implemented.[37] Based on extensive evidence supporting pro-tolerogenic activity of sirolimus (rapamycin), we determined whether prolonged administration of sirolimus would decrease risk for GvHD and enhance rates of successful complete immune suppression discontinuation.

These randomized controlled trial data support the concept that SIR administration for greater than one year is feasible and safe, and associated with beneficial reduction in moderate-severe chronic GvHD. This novel finding is of particular importance, as moderate-severe chronic GvHD is associated with increased mortality, symptom burden, and impaired quality of life.[7-9] The global 0-3 summary chronic GvHD score was significantly improved among SIR/TAC patients. While not significantly different, lung, liver, and gastrointestinal involvement and severity were decreased in the SIR/TAC group, and fewer cases had overlap subtype of chronic GvHD. While some controversy exists, current evidence supports adverse prognosis of overlap subtype of chronic GvHD.[31] There were no major differences in genital, joint, eye, or mouth involvement between study groups. Subgroup analysis of those with established chronic GvHD did not demonstrate any major differences to suggest that ongoing SIR treatment modified the natural history and therapeutic responsiveness of chronic GvHD.

Another important novel finding is the reduction in late acute GvHD among SIR/TAC patients, as prior retrospective studies predominantly suggest that this syndrome is associated with greater risk for mortality.[38-41] The observed excess of recurrent and persistent late acute in the MTX/TAC arm is in keeping with the greater burden of acute GvHD within 100 days post HCT for this group; however, determinants of late acute GvHD need further study. A national Chronic GvHD Consortium longitudinal observational study (*clinicaltrials.gov identifier: 01206309*) will provide new insight in the expected incidence of late acute and predictors for the development of this syndrome.

The analysis of immune suppression discontinuation largely did not support the hypothesis that prolonged SIR therapy would facilitate development of immune tolerance, as defined by successful complete discontinuation of immune suppression. While time to TAC discontinuation differed, there was no difference in ultimate incidence of both TAC and complete IS discontinuation between SIR/TAC and MTX/TAC groups. These data suggest that approximately one-third of patients will successfully discontinue all immune suppression by four years post-HCT in the absence of death or malignancy relapse under these treatment conditions. Further study follow up may be necessary to observe differences in rates of immune suppression discontinuation. It is possible that the use of TAC in both study groups antagonized the development of immune tolerance, and a calcineurin inhibitor-free prophylaxis regimen may be ideal. Unfortunately, currently available evidence does not support the safety of this approach.[42] We also acknowledge a more complete model of functional tolerance would include preservation of graft-*versus*-malignancy effects and immune competence for control of infection. The study was not designed to address these aspects of functional tolerance, and further investigation is needed.

The reduction in malignancy relapse is in keeping with some,43 but not all, prior reports,[44] suggesting a potential benefit of SIR-based immune suppression in HCT. While there was a decrease in malignancy relapse, non-relapse mortality was increased in the SIR/TAC group. Our analyses support the concept that SIR should not be given with escalated dose of busulfan (> average daily exposure of 5300 μM/L*min/day). Additional work is needed to further examine which patient, disease, and HCT characteristics are associated with excess mortality with SIR/TAC prophylaxis. A comprehensive analysis using a larger number of SIR/TAC-treated patients at our institution is planned to address this concern.

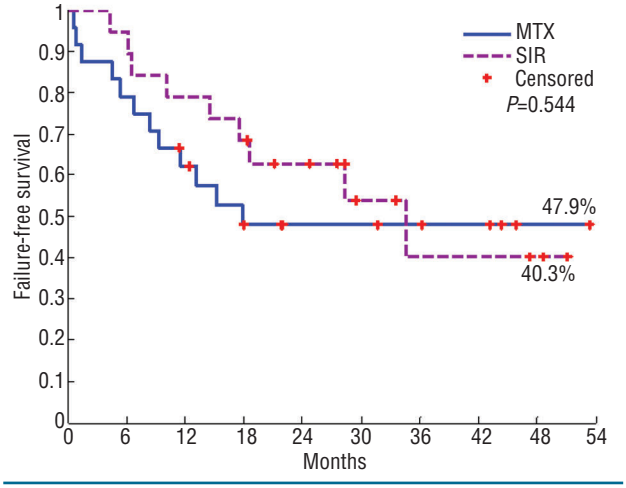


Figure 6. Failure-free survival for chronic graft-*versus*-host disease cohort.

This analysis had some limitations. First, this randomized phase II trial was not adequately powered to demonstrate conclusive benefit of SIR/TAC over MTX/TAC for acute GvHD prevention, and power for the secondary analyses studied here is limited. These findings are hypothesis-generating, however, and further study of prolonged SIR administration post HCT is indicated. Second, the trial only provided guidance in the initial taper of TAC for those without acute GvHD and also required SIR administration for one year or more post HCT; taper and discontinuation of immune suppression was otherwise not regulated by the trial, and therefore was subject to individual practices of treating clinicians. While previous evidence supports variation in practice of immune suppression discontinuation,[45] we anticipate that the duration of immune suppressive therapy was largely driven by GvHD activity rather than arbitrary factors. Thus, time to immune suppression discontinuation should serve as a useful indicator of immune tolerance development. However, we note that development of GvHD after immune suppression discontinuation is common, and therefore the true measure of tolerance is sustained freedom from GvHD after immune suppression discontinuation. Next, peripheral blood samples were not obtained beyond day 360 in the parent trial, and therefore further examination of Treg reconstitution, activity, and association with late clinical outcomes was not possible in this long-term follow-up study. In addition, this trial included only 8/8 or more HLA-matched sibling or unrelated

donors, used exclusively chemotherapy-based conditioning regimens, did not include mismatched or other alternative donors, and specifically excluded ATG (or other *in vivo* or *ex vivo* T-cell depletion), as well as the use of cyclophosphamide containing regimens (to mitigate risk for hepatic veno-occlusive disease). Thus, the observed data cannot be generalized to these conditions. Finally, we acknowledge that other approaches may decrease risk for chronic GvHD and promote immune tolerance. Ex vivo T-cell depletion and post-transplantation cyclophosphamide hold promise,[11,13] as do *in vivo* T-cell depletion strategies such as ATG.[12,46] Furthermore, the use of marrow *versus* peripheral blood is associated with less chronic GvHD.[5]

In summary, these data support that prolonged sirolimus administration mitigates risk for moderate-severe chronic GvHD, late acute GvHD, and decreases systemic glucocorticoid exposure. Further investigation into determinants of immune suppression discontinuation and subsequent GvHD is needed to advance in this field.

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Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96(6):2062-2068.
2. Perkins J, Field T, Kim J, Kharfan-Dabaja MA, et al. A randomized phase II trial comparing tacrolimus and mycophenolate mofetil to tacrolimus and methotrexate for acute graft-versus-host disease prophylaxis. Biol Blood Marrow Transplant. 2010; 16:937-947.
3. Ratanatharathorn V, Nash RA, Przepiorka 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; 92:2303-2314.
4. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus vs. tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. Blood. 2014;124(8):1372-1377.
5. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012;367(16):1487-1496.
6. Kornblit B, Maloney DG, Storer BE, et al. A randomized phase II trial of tacrolimus, mycophenolate mofetil and sirolimus after non-myeloablative unrelated donor transplantation. Haematologica. 2014; 99(10):1624-1631.
7. Arai S, Jagasia M, Storer B, et al.Global and organ-specific chronic graft-versus-host disease severity according to the 2005 NIH Consensus Criteria. Blood. 2011; 118(15):4242-4249.
8. Pidala J, Kim J, Anasetti C, et al. The global severity of chronic graft-versus-host disease, determined by National Institutes of Health consensus criteria, is associated with overall survival and non-relapse mortality. Haematologica. 2011;96(11):1678-1684.
9. Pidala J, Kurland B, Chai X, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on baseline data from the Chronic GVHD Consortium. Blood. 2011;117(17):4651-4657.
10. Stewart BL, Storer B, Storek J, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. Blood. 2004;104(12):3501-3506.
11. Devine SM, Carter S, Soiffer RJ, et al. Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the blood and marrow transplant clinical trials network protocol 0303. Biol Blood Marrow Transplant. 2011; 17(9):1343-1351.
12. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10(9):855-864.
13. Luznik L, Bolanos-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. 2010; 115(16):3224-3230.
14. Burroughs L, Mielcarek M, Leisenring W, et al. Extending postgrafting cyclosporine decreases the risk of severe graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation. Transplantation. 2006;81(6):818-825.
15. Kansu E, Gooley T, Flowers ME, et al. Administration of cyclosporine for 24 months compared with 6 months for prevention of chronic graft-versus-host disease: a prospective randomized clinical trial. Blood. 2001;98(13):3868-3870.
16. Lonnqvist B, Aschan J, Ljungman P, Ringden O. Long-term cyclosporin therapy may decrease the risk of chronic graft-versus-host disease. Br J Haematol. 1990; 74:547-548.
17. Mengarelli A, Iori AP, Romano A, et al. One-year cyclosporine prophylaxis reduces the risk of developing extensive chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplantation. Haematologica. 2003;88(3):315-323.
18. Ruutu T, Volin L, Elonen E. Low incidence of severe acute and chronic graft-versus-host disease as a result of prolonged cyclosporine prophylaxis and early aggressive treatment with corticosteroids. Transplant Proc. 1988;20(3):491-493.
19. Powell JD, Lerner CG, Schwartz RH. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. J Immunol. 1999;162(5):2775-2784.
20. Zheng Y, Collins SL, Lutz MA, et al. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. J Immunol. 2007;178(4):2163-2170.
21. Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. Blood. 2005;105(12):4743-4748.
22. Coenen JJ, Koenen HJ, van Rijssen E, et al. Rapamycin, not cyclosporine, permits thymic generation and peripheral preservation of CD4+ CD25+ FoxP3+ T cells. Bone Marrow Transplant. 2007;39(9):537-545.
23. Gao W, Lu Y, El Essawy B, et al. Contrasting effects of cyclosporine and rapamycin in de novo generation of alloantigen-specific regulatory T cells. Am J Transplant. 2007; 7(7):1722-1732.
24. Hackstein H, Taner T, Logar AJ, Thomson AW. Rapamycin inhibits macropinocytosis and mannose receptor-mediated endocytosis by bone marrow-derived dendritic cells. Blood. 2002;100(3):1084-1087.
25. Taner T, Hackstein H, Wang Z, Morelli AE, Thomson AW. Rapamycin-treated, alloantigen-pulsed host dendritic cells induce ag-specific T cell regulation and prolong graft survival. Am J Transplant. 2005; 5(2):228-236.
26. Turnquist HR, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. J Immunol. 2007; 178(11):7018-7031.
27. Pidala J, Kim J, Jim H, et al. A randomized phase II study to evaluate tacrolimus in combination with sirolimus or methotrexate after allogeneic hematopoietic cell transplantation. Haematologica. 2012; 97(12):1882-1889.
28. Jim HS, Barata A, Small BJ, Jacobsen PB, Pidala J. Quality of life associated with sirolimus for prevention of graft-versus-host disease: results from a randomized trial. Haematologica. 2014;99(3):548-553.
29. Ho VT, Cutler C, Carter S, et al. Blood and

marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2005; 11(8):571-575.
30. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945-956.
31. Pidala J, Vogelsang G, Martin P, et al. Overlap subtype of chronic graft-versus-host disease is associated with an adverse prognosis, functional impairment, and inferior patient-reported outcomes: a Chronic Graft-versus-Host Disease Consortium study. Haematologica. 2012;97(3):451-458.
32. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-828.
33. Perkins JB, Kim J, Anasetti C, et al. Maximally tolerated busulfan systemic exposure in combination with fludarabine as conditioning before allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2012; 18(7):1099-1107.
34. Inamoto Y, Storer BE, Lee SJ, et al. Failure-free survival after second-line systemic treatment of chronic graft-versus-host disease. Blood. 2013;121(12):2340-2346.
35. Martin PJ, Storer BE, Carpenter PA, et al. Comparison of short-term response and long-term outcomes after initial systemic treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2011; 17(1):124-132.
36. Gray R. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Annals Of Statistics 1988;16:1141-1154.
37. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus versus tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic hematopoietic cell transplantation. Blood. 2014;124(8):1372-1377.
38. Jagasia M, Giglia J, Chinratanalab W, et al. Incidence and outcome of chronic graft-versus-host disease using National Institutes of Health consensus criteria. Biol Blood Marrow Transplant. 2007;13(10):1207-1215.
39. Vigorito AC, Campregher PV, Storer BE, et al. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood. 2009;114(3):702-708.
40. Cho BS, Min CK, Eom KS, et al. Feasibility of NIH consensus criteria for chronic graft-versus-host disease. Leukemia. 2009; 23(1):78-84.
41. Arora M, Nagaraj S, Witte J, et al. New classification of chronic GVHD: added clarity from the consensus diagnoses. Bone Marrow Transplant. 2009;43(2):149-153.
42. Johnston L, Florek M, Armstrong R, et al. Sirolimus and mycophenolate mofetil as GVHD prophylaxis in myeloablative, matched-related donor hematopoietic cell transplantation. Bone Marrow Transplant. 2012;47(4):581-588.
43. Armand P, Gannamaneni S, Kim HT, et al. Improved survival in lymphoma patients receiving sirolimus for graft-versus-host disease prophylaxis after allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning. J Clin Oncol. 2008;26(35):5767-5774.
44. Armand P. The Addition Of Sirolimus To The Gvhd Prophylaxis Regimen In Reduced Intensity Allogeneic Stem Cell Transplantation For Lymphoma: A Multicenter Randomized Trial American Society of Hematology Annual Meeting and Exposition. New Orleans, LA; 2013.
45. Pidala J, Lee SJ, Quinn G, Jim H, Kim J, Anasetti C. Variation in management of immune suppression after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2011; 17(10):1528-1536.
46. Bacigalupo A, Lamparelli T, Barisione G, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006;12(5):560-565.