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

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Running title:
Prolonged sirolimus administration after transplant

Key words:
Sirolimus, chronic graft vs. host disease, immune suppression discontinuation

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Abstract:

Effective pharmacologic strategies employed in allogeneic hematopoietic cell transplantation should prevent serious chronic graft vs. 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 vs. methotrexate/tacrolimus for graft vs. 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 NIH Consensus moderate-severe chronic graft vs. host disease (34% vs. 65%, p=0.004) and late acute graft vs. 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 vs. 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 vs. 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 pharmacologic GVHD prevention strategies have led to tangible reduction in acute GVHD, the burden of chronic GVHD largely remains unchanged with these approaches. 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, and a similar proportion are affected after unrelated donor HCT. This represents a major obstacle to the success of HCT, as chronic GVHD remains a major source of late HCT-associated morbidity and death, and is associated with prolonged immune suppressive therapy. 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, 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. 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, 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. 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) vs. methotrexate/tacrolimus (MTX/TAC) in a randomized phase II trial of graft vs. host disease (GVHD) prevention (NCT00803010). Trial inclusion and exclusion criteria, stratified randomization accounting for donor type and HCT recipient age, and patient characteristics have been previously described. 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 1 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 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. A separate report described longitudinal recovery in patient-reported quality of life (QOL).

**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 1 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, 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 GHVD features. 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.
(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 vs. 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 7500µM/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 5300µM/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.

**Statistical methods:**

Descriptive statistics included medians and ranges for continuous variables and frequencies for categorical variables. Categorical variables were compared using the Fisher’s exact test, and
continuous variables were compared using the 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. The Fisher’s 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 endpoints including failure-free survival and overall survival, and the log-rank test was used to compare survival data across groups.

Results:

**Patient characteristics**

The trial design, included patients, and GVHD prophylaxis treatment plans have been previously described for the original trial. 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=NS for all comparisons). The cumulative incidence of grade II-IV acute GVHD through day 100 post-HCT did significantly differ 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 ≥ 1 year 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 ≥ 1 year was 33 months (range 5 – 60 months).

**Chronic graft vs. 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 vs. 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 vs. 14 MTX/TAC patients (p=0.15).

**Late acute graft vs. 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 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 20.1mg/kg total exposure over study period, range 0-360 mg/kg vs. MTX/TAC: median 46.9mg/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 vs. 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 vs. 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 relapse. In keeping with data reported in the initial study report, 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 (48 month estimate for total study population: SIR/TAC 32\% vs. MTX/TAC 11\%, p = 0.03). Co-administration of escalated dose IV busulfan (7500µM/L·min/day) together with fludarabine resulted in excess NRM among SIR/TAC (NRM: 3/5) patients, but not MTX/TAC (NRM: 1/11). 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 GVHD**

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) vs. 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).

**Discussion:**

Effective strategies that prevent serious chronic GVHD and facilitate donor-recipient immune tolerance are needed. Currently available pharmacologic 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. As well, 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 GI 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. 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. 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 (NCT01206309) 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, the ultimate incidence of both TAC and complete IS discontinuation was not different between SIR/TAC and MTX/TAC groups. These data suggest that approximately a third of patients will successfully discontinue all immune suppression by 4 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. As well, we acknowledge a more complete model of functional tolerance would include preservation of graft vs. 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, but not all prior reports, suggesting a potential benefit of SIR-based immune suppression in HCT. While malignancy relapse was decreased, 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 5,300µM/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.

We note the following limitations to this analysis: 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 ≥ 1 year 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, 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. As well, this trial included only ≥ 8/8 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- 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 can’t 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, as do in-vivo T cell depletion strategies such as ATG. As well, the use of marrow vs. peripheral blood is associated with less chronic GVHD.

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 the field.
**Authorship contributions:**

JP – produced study design, conducted the trial, analyzed data, and wrote the manuscript

JK – contributed to study design, performed statistical analysis, and contributed to writing of the manuscript

MA, EA, BCB, HFF, TF, HJ, MAK-D, FLL, AM, TN, LO-B, LP, MR, and CA - contributed to study design, data analysis, and writing of the manuscript

**Conflict of Interest Statement:**

The authors have no conflicts of interest to disclose.

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References:


### Table 1: Late acute GVHD characteristics and severity

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Table 2: Causes of death

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*Bu – busulfan; Flu – fludarabine; GIB - GI bleeding; GVHD – graft vs. 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).
**Figures:**

Figure 1: Cumulative incidence of NIH Consensus moderate-severe chronic GVHD.

Figure 2: Comparative distribution of maximal chronic GVHD organ involvement and severity.

Figure 3: Cumulative incidence of late acute GHVD

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.*

Figure 5: Cumulative incidence of successful TAC discontinuation

Figure 6: Failure-free survival for chronic GVHD cohort
The graph shows two survival curves labeled MTX and SIR. The p-value is 0.004.
<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Skin</th>
<th>Mouth</th>
<th>Eyes</th>
<th>GI</th>
<th>Liver</th>
<th>Lung</th>
<th>Joints</th>
<th>Genital</th>
<th>Other</th>
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The graph shows the dose of prednisone over time for two different regimens: MTX/TAC (blue line) and SIR/TAC (red dashed line). The x-axis represents days from 120 to 1800, and the y-axis represents the dose of prednisone.

The table below the graph provides the number of eligible patients (relapse or death free) for each regimen at different time points:

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<th>Day</th>
<th>MTX/TAC</th>
<th>SIR/TAC</th>
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