A Phase I/II GVHD Prevention Trial Combining Pacritinib with Sirolimus-Based Immune Suppression

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I. Background and Rationale

Allogeneic hematopoietic cell transplantation (HCT) is a curative intervention for patients with high risk hematologic malignancies and blood disorders. The success and survival of HCT is limited by graft-versus-host disease (GVHD), which significantly contributes to transplant-related mortality. Janus kinase 2 (JAK2) and downstream STAT3 activation are biologically relevant in orchestrating T cell alloresponses and pathologic Th17 development[1]. We have demonstrated that CD4+ T-cell STAT3 activity is significantly elevated early after HCT among patients who go on to develop grade II-IV acute GVHD[2]. We have also shown that alloreactive Th17 cells are significantly increased in target-organs at time of GVHD diagnosis[2]. Selective JAK2 inhibition prevents allosensitization and induces a durable tolerant state, yet preserves immunity toward nominal viral antigens[1]. Neutralization of JAK2 reduces pathogenic STAT3-directed Th1/Th17 differentiation, and permits STAT5-mediated regulatory T-cell development[1]. Others have demonstrated that JAK2 blockade improves survival and reduces the severity of GVHD in MHC-mismatched murine models[3,4]. More recently, JAK1/2 inhibition demonstrated clinical benefit in a series of patients with steroid refractory acute GVHD[3].

Pacritinib (PAC) is a highly selective JAK2 inhibitor, with significantly less off-target effects on JAK1 or JAK3[5-8]. This is advantageous during tolerance induction, as selective JAK2 inhibition allows for common gamma chain cytokine activity required for Treg differentiation[1]. While JAK1/JAK2 inhibition limits anti-viral immune clearance and may increases susceptibility to opportunistic infections[9,10]; selective JAK2 blockade permits anti-viral activity by human T-cells and is not linked to infectious complications[1,5,7]. Moreover, robust clinical trial data shows that PAC does not induce myelosuppression in patients with myelofibrosis – which distinguishes PAC from other existing JAK2 inhibitors. As reported at the 2015 annual ASCO meeting, PAC was administered at a dose of 400mg orally daily and showed significant benefit compared with best available therapy.

Sirolimus is a specific inhibitor of the mTOR (mammalian target of rapamycin) pathway, where alloreactive T cells are selectively dependent upon this signaling route[11]. Sirolimus fosters the differentiation of regulatory T cells (Treg) by favoring their activation through the IL-2/JAK1/3/STAT5 pathway[12] and inhibiting the phosphorylation of STAT3[13], which can antagonize the expression of FOXP3 (Figure 1). STAT5 is vital to Treg development and function. This T helper cell subset down-regulates GVHD[12]. We have demonstrated that while rapamycin partially inhibits RORgammaT expression in allosensitized human T-cells, full elimination of Th17 differentiation is achieved by combining mTOR blockade with JAK2/STAT3 inhibition[2]. A phase II trial at Moffitt Cancer Center (MCC) demonstrated that prophylaxis with sirolimus plus low-dose tacrolimus (SIR/TAC) was superior to methotrexate (MTX) and high dose TAC in preventing acute GVHD and promoting Tregs[14,15]. Despite the success of SIR/TAC, nearly 50% of patients still developed grade II-IV acute GVHD. SIR/TAC is an ideal platform to combine JAK2 inhibition with PAC, as both strategies favor Treg development and reduce Th17. Selective JAK2 inhibition also promotes immune tolerance[1]. Moreover, our data supports that combined inhibition of JAK2 and mTOR synergistically suppresses alloreactive human T-cells. **A phase I/II, single arm, trial of PAC and our standard GVHD prophylaxis regimen of SIR/TAC is proposed, as a means to ablate STAT3 signaling, promote immune tolerance, and polarize Treg development over Th1 and Th17.**

Hypothesis: The selective JAK2 inhibitor, pacritinib, combined with sirolimus plus low-dose tacrolimus, will be safe in allogeneic HCT recipients and significantly reduce STAT3 activation and subsequent alloreactivity.

Study objectives:
Phase I: 1) Determine the lowest biologically active dose of pacritinib that is safe and well tolerated when combined with SIR/TAC.

Phase II: 2) Determine if PAC/SIR/TAC suppresses STAT3 activity in circulating CD4+ T-cells at day +21, 3) Investigate whether PAC/SIR/TAC reduces the cumulative incidence of grade II-IV acute GVHD by day +100, compared our published rate of 43% with SIR/TAC alone[14,15], 4) Investigate the impact of PAC/SIR/TAC on Treg, Th1, and Th17 differentiation after allogeneic HCT, and 5) Determine how PAC/SIR/TAC impacts CD28 and IL-2 receptor signal transduction by measuring S6 (mTOR), H3 ser10 (Aurora kinase), and STAT5 phosphorylation in CD4+ T-cells at days +21 and +100.

Background:

The role of JAK2/STAT3 activation and Th17 cells in acute GVHD

Naïve CD4+ T cells are plastic and may develop into various functional phenotypes (Th1, Th2, Th17, Treg) through specific cytokine-mediated intracellular signaling events. Th17 cells are implicated in murine and human GVHD[2,16,17]. IL-6 mediates Th17 development by activating JAK2/STAT3 and influencing the expression of RORgammaT[18]. Conversely, IL-2 signaling through STAT5 favors Foxp3 expression and subsequent immune tolerizing Treg development[19]. Studies in murine acute GVHD have shown that mice transplanted with CD4+ T cells deficient in IL-17 have significantly reduced mortality[20]. We and others have demonstrated that tissue invading Th17 cells are significantly increased at time of acute GVHD diagnosis in humans as well[2]. Additionally, we have demonstrated that increased tissue-resident Th17 cells correlates with GVHD severity and portends a poor response to upfront therapy with glucocorticoids (Figure 1A)[2]. Deletion of STAT3 in donor T-cells favors Treg development and improves the survival of transplanted mice[21]. We have shown that CD4+ T-cell STAT3 activation by IL-6 is significantly increased early after allogeneic HCT among asymptomatic patients who later develop grade II-IV acute GVHD (Figure 1B,C)[2]. This observation uniquely identified JAK2 and downstream STAT3 as clinically dynamic, druggable targets in acute GVHD. The degree of IL-6-induced CD4+ T-cell STAT3 phosphorylation was 11% for healthy non-transplant controls, 32% for those who did not develop acute GVHD, and 70% for recipients who did develop acute GVHD by day +100. This trial biologically targets JAK2/STAT3 activation by combining pacritinib, a selective JAK2 inhibitor, with SIR/TAC to reduce the CD4+ T-cell STAT3 phosphorylation to at least 35%. This target coincides with the STAT3 phosphorylation range observed among HCT recipients who did not develop acute GVHD in our prior exploratory study. The association of CD4+ T-cell STAT3 phosphorylation and acute GVHD risk is under prospective validation, but currently offers guidance in determining the degree of on-target pathway suppression for HCT recipients on this trial. We will strengthen our current investigation by determining if JAK2/STAT3 pathway suppression reduces the cumulative incidence of acute GVHD. The trial will be expanded by 17 patients to study the effect of PAC/SIR/TAC on GVHD prevention, if 9 or less of the 24 initial patients develop acute GVHD by day +100.

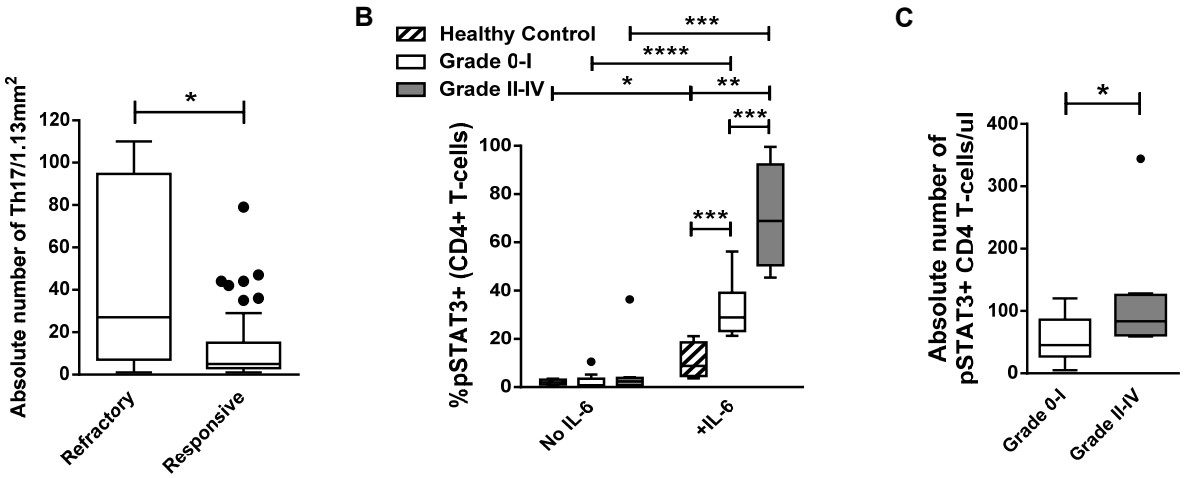

Figure 1: CD4+ T-cell STAT3 activity is increased among alloHCT recipients before acute GVHD onset. (A) Tissue-resident Th17 cells are increased in among patients with glucocorticoid-refractory GVHD. (B,C) Day +21 CD4+ STAT3 activation (%phosphorylation and absolute number) is significantly increased in asymptomatic allograft recipients that go on to develop grade II-IV acute GVHD by day +100 (N=18 patients). *P<.05, **P=.001-.01, ***P=.0001-.001, ****P<.0001.

Selective JAK2 inhibition induces allotolerance, while sparing anti-viral responses

Selective JAK2 inhibition with TG101348, a nonclinical chemical tool, ablates IL-6 mediated phosphorylation of STAT3 in human T-cells (Figure 2A)[1]. TG101348 did not prevent IL-2 activation of STAT5, which is required by Treg and cytotoxic T lymphocytes (Figure 2B)[1]. We have demonstrated that selective JAK2 inhibition with TG101348 significantly reduces the proliferative response of DC-allostimulated T-cells (5-day alloMLR, DC:T-cell ratio 1:30, Figure 2C)[1]. This suppressive effect is still observed upon re-challenge with fresh allogeneic DCs from the original stimulating party, without additional exposure to the JAK2 inhibitor (Figure 2D)[1]. TG101348 also inhibits secondary responses by T cells, which have already been sensitized to alloantigen in a primary mixed lymphocyte reaction (MLR) without the JAK2 inhibitor (Figure 2E)[1]. Conversely, responses to stimulation by influenza virus matrix peptide (fluMP) remain intact (Figure 2F)[1]. Altogether, we identified that JAK2 inhibition induces durable allotolerance without compromising antiviral activity.

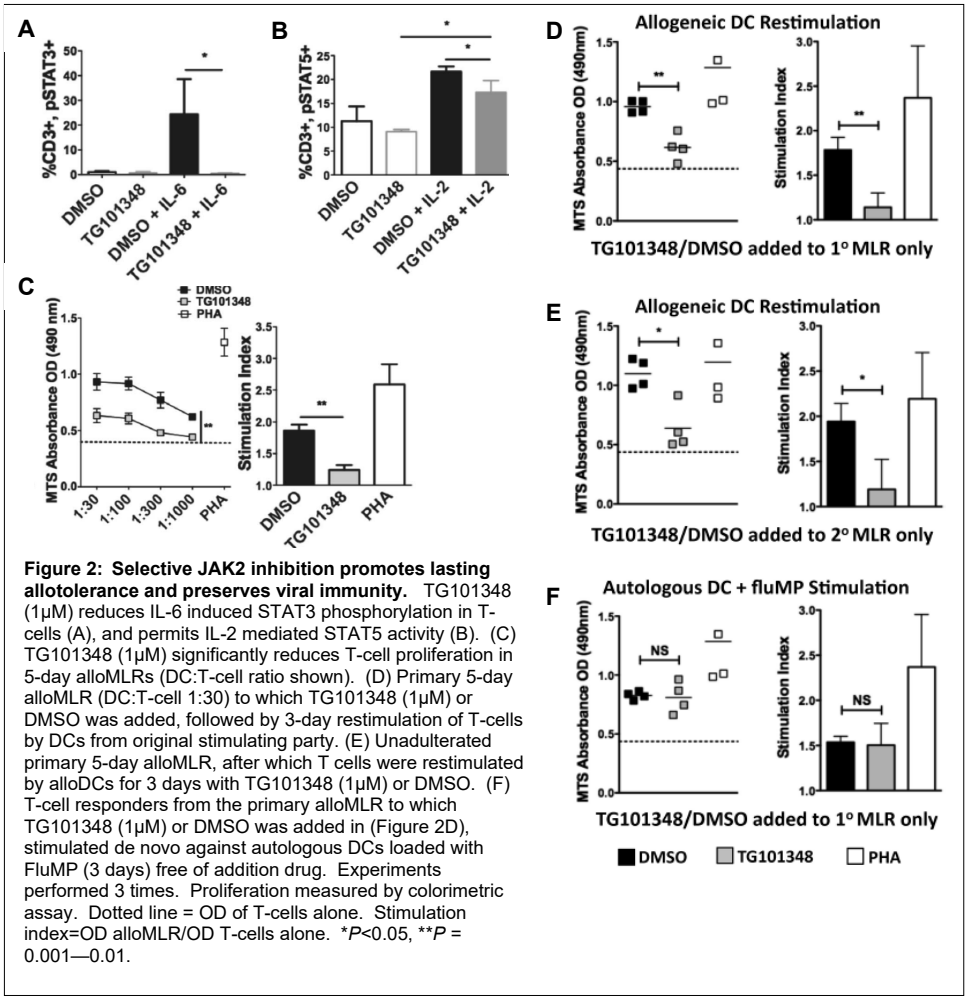

Figure 2: Selective JAK2 inhibition promotes lasting allotolerance and preserves viral immunity. TG101348 (1μM) reduces IL-6 induced STAT3 phosphorylation in T-cells (A), and permits IL-2 mediated STAT5 activity (B). (C) TG101348 (1μM) significantly reduces T-cell proliferation in 5-day alloMLRs (DC:T-cell ratio shown). (D) Primary 5-day alloMLR (DC:T-cell 1:30) to which TG101348 (1μM) or DMSO was added, followed by 3-day restimulation of T-cells by DCs from original stimulating party. (E) Unadulterated primary 5-day alloMLR, after which T cells were restimulated by alloDCs for 3 days with TG101348 (1μM) or DMSO. (F) T-cell responders from the primary alloMLR to which TG101348 (1μM) or DMSO was added in (Figure 2D), stimulated de novo against autologous DCs loaded with FluMP (3 days) free of addition drug. Experiments performed 3 times. Proliferation measured by colorimetric assay. Dotted line = OD of T-cells alone. Stimulation index=OD alloMLR/OD T-cells alone. *P<0.05, **P = 0.001—0.01.

PAC is a highly selective JAK2 inhibitor, with no suppressive effect on JAK1 at therapeutic concentrations[5-8,22-24]. This is favorable in the transplant setting, as

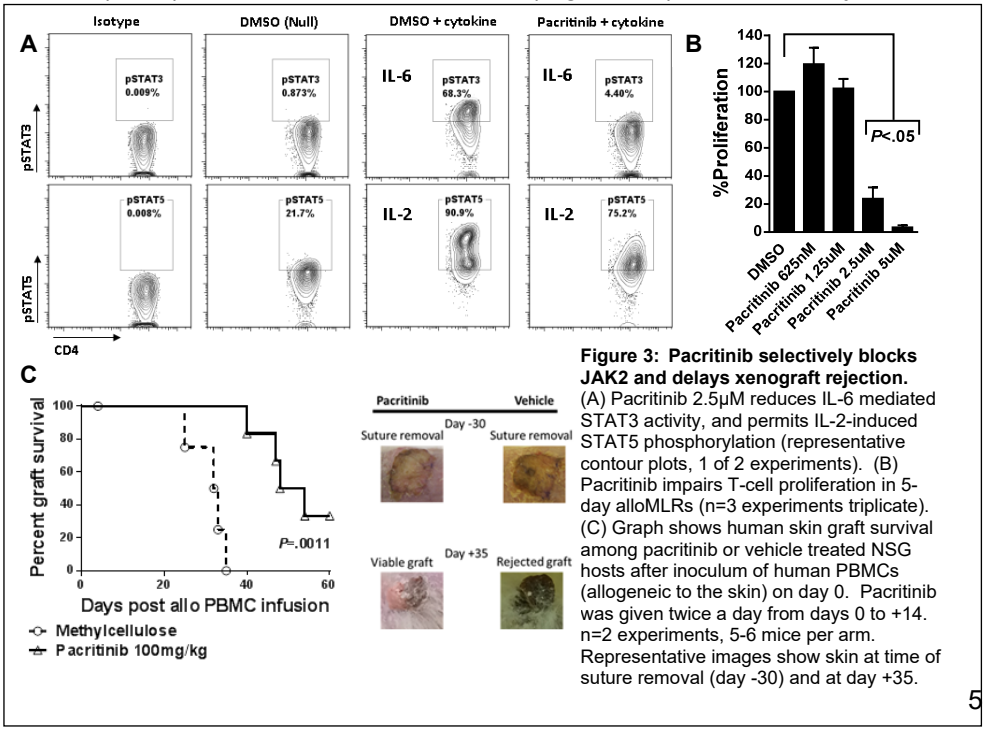

Figure 3: Pacritinib selectively blocks JAK2 and delays xenograft rejection. (A) Pacritinib 2.5μM reduces IL-6 mediated STAT3 activity, and permits IL-2-induced STAT5 phosphorylation (representative contour plots, 1 of 2 experiments). (B) Pacritinib impairs T-cell proliferation in 5-day alloMLRs (n=3 experiments triplicate). (C) Graph shows human skin graft survival among pacritinib or vehicle treated NSG hosts after inoculum of human PBMCs (allogeneic to the skin) on day 0. Pacritinib was given twice a day from days 0 to +14. n=2 experiments, 5-6 mice per arm. Representative images show skin at time of suture removal (day -30) and at day +35.

agents like ruxolitinib that inhibit JAK1 are associated with reduced in vivo clearance of murine viral pathogens[9]. We confirmed that PAC selectively reduces IL-6 stimulated STAT3 phosphorylation, while permitting IL-2-mediated STAT5 activity in human T-cells (Figure 3A). We also demonstrate that PAC significantly reduces alloreactive T-cell proliferation in MLRs at 2.5-5μM (Figure 3B). To investigate the effect of JAK2 inhibition on human T-cell alloresponses in vivo, a human skin / NSG (NOD/SCID/gamma chain deficient) mouse xenograft model was used. This experimental approach was ideal, given that skin is a highly immunogenic target organ of acute GVHD. A 1x1 cm split-thickness human skin graft was transplanted onto the animal dorsally, followed by intraperitoneal injection of $5x10^6$ allogeneic peripheral blood mononuclear cells (PBMC) 30 days after surgery. Irradiation was not required for human PBMC engraftment. PAC was administered at 100mg/kg twice a day by oral gavage for 15 days beginning at time of PBMC injection. The treatment significantly delayed allograft rejection by the human donor PBMCs, compared with vehicle control (Figure 3C). Representative images from grafted mice show human skin at time of suture removal on day -30, and later at day +35, from both PAC- and vehicle-treated mice. The viable, PAC-treated graft shows mild pigmentation changes at day +35, while the vehicle-treated graft is completely necrotic with thick hyperkeratosis (Figure 3C). **Collectively, these data clearly identify JAK2 as a therapeutic target to control donor alloreactivity after alloHCT.**

JAK2 inhibition spares Treg while suppressing Th1/Th17 responses.

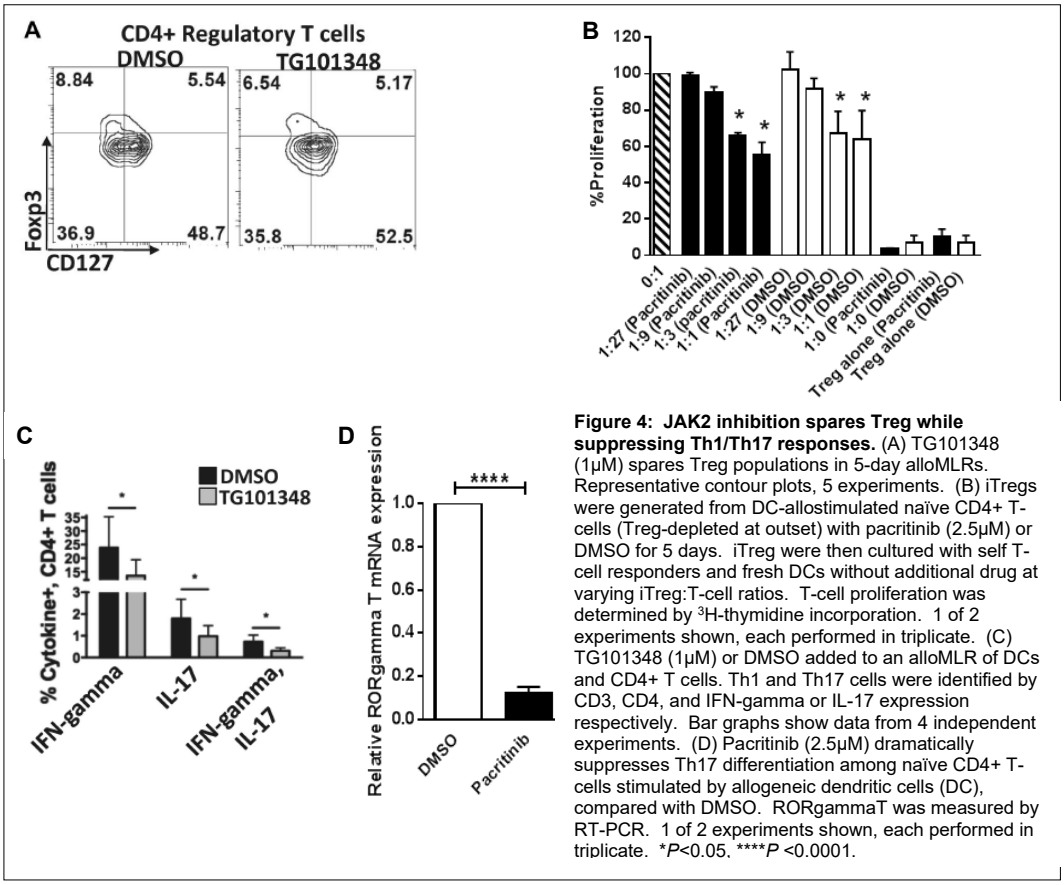

Figure 4: JAK2 inhibition spares Treg while suppressing Th1/Th17 responses. (A) TG101348 (1μM) spares Treg populations in 5-day alloMLRs. Representative contour plots, 5 experiments. (B) iTregs were generated from DC-allostimulated naïve CD4+ T-cells (Treg-depleted at outset) with pacritinib (2.5μM) or DMSO for 5 days. iTreg were then cultured with self T-cell responders and fresh DCs without additional drug at varying iTreg:T-cell ratios. T-cell proliferation was determined by ^{3}H-thymidine incorporation. 1 of 2 experiments shown, each performed in triplicate. (C) TG101348 (1μM) or DMSO added to an alloMLR of DCs and CD4+ T cells. Th1 and Th17 cells were identified by CD3, CD4, and IFN-gamma or IL-17 expression respectively. Bar graphs show data from 4 independent experiments. (D) Pacritinib (2.5μM) dramatically suppresses Th17 differentiation among naïve CD4+ T-cells stimulated by allogeneic dendritic cells (DC), compared with DMSO. RORgammaT was measured by RT-PCR. 1 of 2 experiments shown, each performed in triplicate. *P<0.05, ****P <0.0001.

We previously demonstrated that TG1010348 permits the development of human allo-antigen specific, natural Tregs in vitro (Figure 4A)[1]. Similarly, PAC permits the differentiation of inducible Treg (iTreg) from isolated naïve CD4+ T-cells stimulated with allogeneic DCs. The PAC-generated iTreg retain suppressive function comparable to the iTregs grown in the presence of DMSO (Figure 4B). This suggests that JAK2 is not required for iTreg function. With regard to Th1 and Th17 development, we observed that TG1010348 significantly decreases IFN-gamma and IL-17 respectively among DC-allostimulated CD4+ T-cells (Figure 4C)[1]. Our preliminary data shows that JAK2 inhibition with PAC significantly suppresses expression of RORgammaT, a key Th17 differentiation factor (Figure 4D). **The immune effects directed by JAK2 inhibition are favorable following allo-HCT, as tolerizing Tregs securely develop while pathogenic Th1 and Th17 cells are greatly impaired.**

Combined blockade of JAK2 and mTOR offers synergistic control over alloreactive T-cells

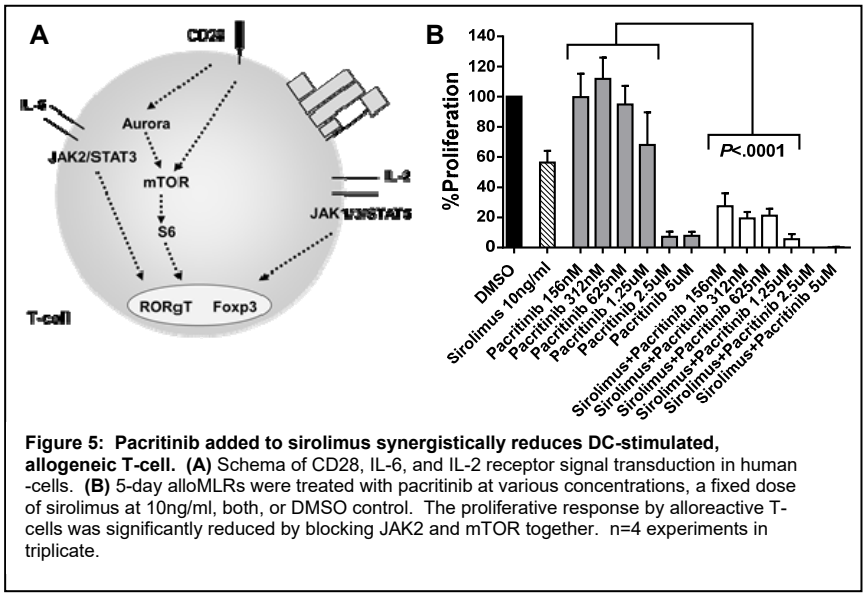

Figure 5: Pacritinib added to sirolimus synergistically reduces DC-stimulated, allogeneic T-cell. (A) Schema of CD28, IL-6, and IL-2 receptor signal transduction in human -cells. **(B)** 5-day alloMLRs were treated with pacritinib at various concentrations, a fixed dose of sirolimus at 10ng/ml, both, or DMSO control. The proliferative response by alloreactive T-cells was significantly reduced by blocking JAK2 and mTOR together. n=4 experiments in triplicate.

CD28 costimulation of T-cells requires signaling via mTOR and Aurora kinase. mTOR is a known therapeutic target in preventing acute GVHD (Figure 5A). As described, IL-6 signaling contributes to GVHD by polarizing Treg recovery over pathologic Th1 or Th17 (Figure 5A). Our data supports that combined inhibition of JAK2 and mTOR is synergistic, and significantly reduces the proliferative response of allostimulated human T-cells (Figure 5B). This concept will be studied in this innovative clinical trial, where PAC will be combined with sirolimus-based immune suppression to suppress these pathways concurrently and prevent GVHD after alloHCT.

The Moffitt experience with sirolimus-based acute GVHD prevention

A randomized phase II clinical trial conducted at MCC included 74 patients randomized 1:1 to a GVHD prophylaxis regimen of SIR/TAC or MTX/TAC[25]. The median patient age was 49 [23-69], with recipients receiving related or unrelated 8/8 HLA-matched peripheral blood HSC allografts[25]. Conditioning consisted of our standard, reduced-toxicity regimen of pharmacologically targeted busulfan and fludarabine[25]. The 100-day cumulative incidence of grade 2-4 acute GVHD for SIR/TAC was 43%, and 89% for MTX/TAC, $p<0.0001$[25]. A multicenter, phase III trial of SIR/TAC versus MTX/TAC among HLA-matched related alloHCT recipients by the BMT CTN did not identify a significant difference in the incidence of grade II-IV acute GVHD between the two prophylaxis regimens (26 versus 34%)[26]. However a post hoc analysis showed a potential trend in less cases of severe, grade III-IV acute GVHD with SIR/TAC compared to MTX/TAC[27]. It is important to note that the Moffitt SIR/TAC regimen targets a higher therapeutic range for sirolimus with a goal of 5-14ng/ml[25] as opposed to 3-12ng/ml[27]. Our intensified dosing strategy may in part explain the clinical advantage seen with SIR/TAC versus MTX/TAC in our Moffitt GVHD prevention trial. Additionally, we observed a greater frequency of acute GVHD on both arms of our SIR/TAC versus MTX/TAC study compared to the BMT CTN trial[25,26]. This is likely explained by 1) our inclusion of unrelated allografts and 2) our practice of aggressively using endoscopic procedures to evaluate for stage I upper gastrointestinal GVHD in the setting of nausea and anorexia[25,28]. No significant differences in survival, toxicities, or thrombotic microangiopathy (TMA) were observed between SIR/TAC and MTX/TAC in the Moffitt phase II trial[25]. Our trial also produced a favorable biologic impact on Treg expansion using SIR/TAC over MTX/TAC[25]. Despite the significant decrease in acute GVHD, 43%[25] of patients receiving SIR/TAC still developed this immune complication – underscoring the need for further optimization of this approach. The goal of PAC/SIR/TAC GVHD prophylaxis will be to ablate JAK2 and mTOR signaling early after HCT and reduce acute GVHD.

Clinical development of pacritinib, a selective JAK2 inhibitor

PAC was investigated in a phase I study of relapsed or refractory lymphoma[7]. A total of 34 patients were treated on a dose escalation strategy, from 100 to 600mg daily for 28 days[7]. On-target inhibition

of JAK2 was noted at all dose levels[7]. Unlike ruxolitinib, cytopenias were rare and limited to 9% grade 1-2 and 6% grade 3-4[7]. The primary toxicity was grade I-2 gastrointestinal symptoms, chiefly diarrhea at 32%. The MTD was not reached in this phase I study[7]. A phase II study of PAC in myelofibrosis enrolled 35 patients[5]. PAC was given at 400mg by mouth daily[5]. Grade I-2 diarrhea was the most common toxicity at 69%[5]. As reported at the 2015 ASCO Annual Meeting, the PERSIST-1 phase III trial of PAC versus best available therapy demonstrated that the JAK2 inhibitor was safe and effective reducing spleen size among 220 treated patients. In this large study, PAC was associated with <5% grade III diarrhea in keeping with earlier phase trials. Overall, PAC selectively inhibits JAK2 with reduced myelosuppression compared to other similar agents. These favorable clinical characteristics identify PAC as an ideal JAK2 inhibitor to pair with sirolimus and low-dose tacrolimus in the allogeneic HCT setting.

Trial Design: This is a single-arm, phase I/II, study of PAC/SIR/TAC for the prevention of acute GVHD after matched related and unrelated alloHCT. First, a standard 3+3 dose escalation method will be performed combining PAC with SIR/TAC. The MTD identified during phase I, as well as serial PK/PD data, will inform the recommended phase II dose. Therefore, the recommended phase II dose will be the lowest dose that decreases the amount of pSTAT3+ CD4+ T-cells <35% at day +21 and does not exceed the MTD (see Table 1). The phase II component is powered to determine if JAK2 inhibition with PAC significantly reduces CD4+ T-cell STAT3 activation early after allogeneic HCT compared to our historical benchmark. Our published study showed the mean CD4+ T-cell STAT3 phosphorylation at day +21 was 50% among all HCT recipients, 70% for those who later developed acute GVHD, versus 32% for those who did not[2]. Therefore, success will be defined as a reduction in CD4+ T-cell STAT3 phosphorylation to 35% or less at day +21. This phosphorylation value not only estimates biologic activity, but also falls within the standard deviation of what was observed among HCT recipients who never acquired acute GVHD[2]. The association of CD4+ T-cell STAT3 phosphorylation and acute GVHD risk is under prospective validation, but currently offers guidance in determining the degree of on-target pathway suppression for HCT recipients on this trial. As pacritinib is a direct inhibitor of JAK2/STAT3 signal transduction, greater reduction in CD4+ T-cell pSTAT3 phosphorylation is possible in this patient population. An initial 24 patients will be treated with PAC/SIR/TAC to investigate whether the biologic activity of STAT3 in CD4+ T-cells is reduced at day +21.

Secondarily, we will monitor enrolled patients for acute GVHD by day +100. To investigate the clinical efficacy of the PAC/SIR/TAC combination in GVHD prevention, the trial will be expanded by an additional 14 subjects if 9 or less of the first 24 patients develop grade II-IV acute GVHD. This expansion threshold is based on our data from the SIR/TAC trial where the cumulative incidence of acute GVHD incidence by day +100 was 43%. PAC/SIR/TAC will be considered favorable if the primary endpoint is met AND ≤12 of the total 38 (31.6%) patients develop acute GVHD by day +100.

Protocol design: A phase I dose escalation will first be performed to identify the maximum tolerated dose (MTD) of pacritinib when combined with SIR/TAC. SIR/TAC will be administered, dosed, and monitored per Moffitt BMT standard procedures. Additionally, the recommended phase II dose will also be informed by pharmacodynamic data generated from the phase I trial. As such, the dose escalation may stop once a tolerated dose is identified that suppresses CD4+ pSTAT3+ T-cells <35% at day +21. The pacritinib dose cohorts will include:

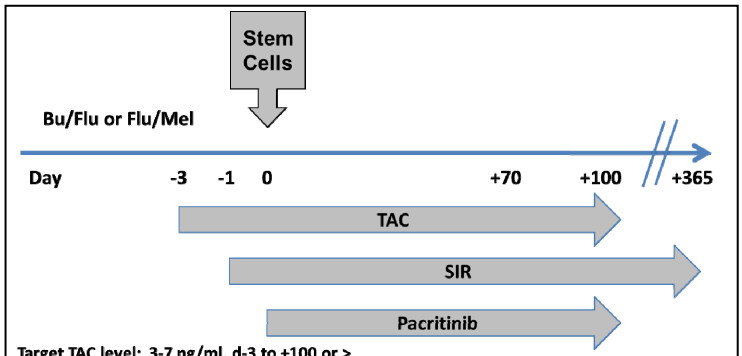

Figure 6: Protocol design schema.

(Dose level 1) PAC 100mg po daily, (Dose level 2) PAC 100mg po twice daily, and (Dose level 3) PAC 200mg po twice daily. Dose cohort 1 (PAC 100mg daily) represents the lowest dose level that would potentially inhibit CD4+ T-cell pSTAT3. This is based on PK/PD data from the completed phase I trial of pacritinib in lymphoma[7]. For all patients enrolled in the phase I portion of this study, pharmacokinetic sampling of plasma pacritinib levels will be drawn on days 0 and +21 at the following time points: pre-dose, +4 hours, +12 hours, and +24 hours (window of +/- 1 hour on each timed PK sample). Pharmacodynamic, CD4+ T-cell pSTAT3 values will be assessed at baseline and day +21 as well. EKGs (on days 0 and +21) will be performed pre-dose and at 4 hours (pacritinib Tmax)[7] – with +/- 1 hour window on the 4-hour post-dose EKG. The initial group of 3 patients will be enrolled in cohort 1. Dose escalation of pacritinib will proceed according to the following: If no DLT is observed among 3 patients in a cohort AND the average %pSTAT3+ CD4+ T-cells are >35% at day +21, the next group of 3 will be treated at the next highest dose level. If the current level (i.e., the level at which no DLTs are observed) is the highest dose level, three additional patients will be enrolled (total of six). If one DLT is observed among 3 patients in a cohort, the next group of 3 will be treated at this same dose level. If no more DLT is observed among the 3 additional patients (i.e. one DLT in total is observed among the 6 patients) AND the average %pSTAT3+ CD4+ T-cells are >35% at day +21, then the next 3 patients will be treatment at the next highest dose level. If at least 1 more DLT is observed among the 3 additional patients, then the MTD is exceeded. If 6 patients have been treated at the next lower dose level, it will be considered as the MTD. If not, three additional patients will be enrolled. This escalation approach will then be continued until MTD or a tolerated dose (≤1/6 DLT) is identified at which <35% of the CD4+ T-cells are pSTAT3+.on day +21 (see Table 1). That dose will be considered to be the recommended phase II dose. The total number treated on the phase I component will depend on the occurrence of dose limiting toxicity (DLT) or the average frequency of pSTAT3+ CD4+ T-cells at day +21 in a cohort, however the maximal number accrued on the phase I study would approximate 12 to 18 total evaluable subjects. Patients in the phase I component of the trial will be monitored throughout their 100 days of pacritinib therapy for DLT events, however the minimum DLT observation period required prior to escalation to higher dose levels in the phase I component is 30 days from the last patient's (within dose level) initiation of pacritinib therapy. In addition to informing the recommended phase II dose, the PK/PD data from days 0 and +21 will be used to identify any dose-dependent patterns among serum pacritinib concentrations and 1) CD4+ T-cell STAT3 phosphorylation, 2) Treg reconstitution, 3) QTcF prolongation, or 4) changes in plasma tacrolimus and sirolimus levels.

Table 1: Phase I: Identification of Recommended Phase II Dose

Dose Level	MTD exceeded	Day +21 pSTAT3+ CD4+ T-cells <35%
Dose cohort 1 (100 mg daily)	**Yes (Stop, do not proceed)**	**Yes (treat a total of 6, RP2D identified if ≤1/6 have DLT)**
	No (Proceed)	**No (Proceed to cohort 2)**
Dose cohort 2 (100 mg BID)	**Yes (Stop, return to complete 6 subjects in cohort 1)**	**Yes (treat a total of 6, RP2D identified if ≤1/6 have DLT)**
	No (Proceed)	**No (Proceed to cohort 3)**
Dose cohort 3 (200 mg BID)	**Yes (Stop, return to complete 6 subjects in cohort 2)**	
	No (RP2D identified, proceed to phase II component of trial))	

RP2D = recommended phase II dose

The definitions for DLTs on PAC/SIR/TAC are as follows (CTCAE v4):

1. Left ventricular systolic dysfunction grade 3 or above*
2. Myocardial infarction grade 3 or above
3. Atrial fibrillation or flutter grade 3 or above
4. Supraventricular tachycardia grade 3 or above
5. Ventricular arrhythmia grade 3 or above
6. Upper or lower gastrointestinal hemorrhage grade 3 or above
7. Intracranial hemorrhage grade 1 or above
8. Any grade 3 or above adverse event considered at least possibly related to pacritinib

** Patients demonstrating signs/symptoms of heart failure on PAC/SIR/TAC will undergo a 2-D echocardiogram to document the left ventricular ejection fraction. Subsequent echocardiograms will be performed per the discretion of the attending physician based on clinical need.*

The recommend phase II dose will be based on the lowest biologically active dose of pacritinib, that is safe and well tolerated. The patients treated at this dose during phase I will analyzed among those enrolled in the phase II component. The overall PAC/SIR/TAC treatment plan is illustrated in Figure 6. CTI BioPharma has given Moffitt Cancer Center authorization to cross reference the Investigational New Drug application 78,406 for Pacritinib (SB1518, Serial No. 357). A total daily dose of PAC 400mg (ie 200mg po twice a day) was investigated in a recently reported phase II study in myelofibrosis[5]. The primary toxicity was mild-moderate diarrhea occurring within the first week of therapy. Diarrhea from alkylators like busulfan and melphalan during transplant conditioning is a common toxicity after allogeneic HCT. Alkylator-related diarrhea typically occurs after the first week of the transplant and may continue into the third week of the procedure, overlapping with gastrointestinal side effects from PAC. Chemotherapy-induced diarrhea will be managed per Moffitt BMT standard practice (BMT-G-102.0), where loperamide is often effective as a first-line agent. Dose reductions of pacritinib will be made on a per patient basis, if stool volume exceeds 1500ml/day for 3 consecutive days in the absence of infectious etiology and if the diarrhea does not respond to antimotility medications (see Safety Assessment section). Importantly, phase I pharmacodynamic data demonstrated on-target inhibition of JAK2 activity at doses as low as 100mg daily[7], ensuring biologic activity in the setting of dose reductions for diarrhea. The administration of PAC with SIR/TAC, will continue until day +100. PAC will then be tapered to 50% of the total dose at day +70, then 25% of total dose at day +84, then stop at day +100 (+/- 7 days) (see Table 2 for dose reduction schedule). This will allow for close monitoring of patients for GVHD during the taper phase, as standard practice requires patients to be within 30 minutes of the transplant center until day +100. SIR/TAC will be given according to program standards. Conditioning regimens may include busulfan and fludarabine or melphalan and fludarabine as detailed in Section V. “Study Design”. Regimens that include total body irradiation or post-transplant cyclophosphamide are excluded to avoid compounding toxicities.

Table 2: Pacritinib taper schedule, based on day +69 dose

Pacritinib dose at day +69	**Day +70 reduction**	**Day +84 reduction**
200mg BID	200mg daily	100mg daily
200mg daily	100mg daily	100mg every other day (QOD)
100mg daily	100mg QOD	100mg on Sunday and

		Thursday

II. Trial objectives

a. Primary objectives:

i. Phase I: Determine the lowest biologically active dose of pacritinib that is safe and well tolerated when combined with SIR/TAC.

ii. Phase II: Determine if PAC/SIR/TAC suppresses STAT3 activity in circulating CD4+ T-cells at day +21[2], to at least 35% phosphorylation.

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b. Secondary objectives:

i. Investigate whether PAC/SIR/TAC reduces the cumulative incidence of acute GVHD by day +100, compared to our published rate of 43% from SIR/TAC alone. *This benchmark is bolstered by an ongoing retrospective analysis of 819 patients treated with SIR/TAC, MTX/TAC, or MMF/TAC, where the incidence of acute GVHD with SIR/TAC was similar to rates observed in our trial.*

ii. Study the effect of PAC/SIR/TAC on Treg, Th1, and Th17 differentiation among circulating T-cells at day +21 and day +100.

a) A subset of 15 patients treated with standard SIR/TAC alone who are receiving a matched related or unrelated alloHCT will also be enrolled for blood sample collections only on day +21 and day +100.

b) The effects of PAC/SIR/TAC versus SIR/TAC on Treg, Th1, and Th17 development will be investigated.

iii. Determine how PAC/SIR/TAC impacts CD28 and IL-2 receptor signal transduction by measuring S6 (mTOR), H3 ser10 (Aurora kinase), and STAT5 phosphorylation in CD4+ T-cells at days +21 and +100. Phosphoprotein values will be compared to SIR/TAC only patients (n=15) enrolled in the sample-only portion of the trial.

III. Trial endpoints

a. Primary endpoints:

i. Phase I: The lowest biologically active dose of PAC that is safe and well tolerated with SIR/TAC

ii. Phase II: STAT3 activity in circulating CD4+ T-cells at day +21[2].

b. Secondary endpoints (phase II only):

i. Cumulative incidence of acute GVHD by day +100

ii. Treg, Th1, and Th17 differentiation among circulating T-cells at day +21 and day +100.

iii. CD28 and IL-2 receptor signal transduction by measuring S6 (mTOR), H3 ser10 (Aurora kinase), and STAT5 phosphorylation in CD4+ T-cells at days +21 and +100.

IV. Patient population

a. Inclusion Criteria

1. Age ≥ 18.

2. Patients must have an available 8/8 HLA-A, -B, -C, and -DRB1 matched-related or unrelated donor allogeneic hematopoietic peripheral blood stem cell graft.

3. Signed informed consent.

4. Acute myeloid leukemia, myelodysplasia, acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia, myeloproliferative neoplasms, Hodgkin lymphoma, or non-Hodgkin lymphoma requiring a matched allogeneic HSCT.

   Acute Leukemia (AML or ALL) must be in complete remission defined as: <5% marrow blasts with no morphologic evidence of leukemia, no peripheral blasts, marrow >20% cellular, and peripheral absolute neutrophil count >1000/uL (platelet recovery is not required).

   Myelodysplasia (MDS) and chronic myeloid leukemia (CML): Must have <5% marrow blasts.

   Myeloproliferative neoplasms (MPN): Must have <5% peripheral / marrow blasts. Note: Prior use of a JAK2 inhibitor is allowed up to 4 weeks before day 0 of alloHCT.

   Hodgkin and non-Hodgkin lymphoma: Must have a complete or partial response with prior therapy.

5. Adequate vital organ function:

   a. LVEF ≥ 50% by MUGA scan or ECHO

   b. FEV1, FVC, and adjusted DLCO ≥ 50% of predicted values on pulmonary function tests

   c. Transaminases (AST, ALT) < 2 times upper limit of normal values

   d. Creatinine clearance ≥ 50 cc/min.

6. Performance status: Karnofsky Performance Status Score ≥ 80%.

b. Exclusion Criteria:

1. Active infection not controlled with appropriate antimicrobial therapy.

2. History of HIV, hepatitis B, or active hepatitis C infection.

3. Anti-thymocyte globulin, alemtuzumab, bortezomib, or post-transplant cyclophosphamide as part of GVHD prophylaxis.

4. Sorror's co-morbidity factors with total score >4 (Appendix B).

5. Any patient anticipating or scheduled to receive a tyrosine kinase inhibitor, FLT3 inhibitor, or JAK2 inhibitor (outside of this study) post-HCT.

6. QTc>450ms per Fridericia's correction

7. Thrombin time (TT), prothrombin time (PT), or partial thromboplastin time (PTT) >2x upper limit of normal

8. Grade 3 or higher recent (within the past 6 months) or ongoing cardiac dysrhythmias, family history of long QT syndrome, or serum potassium <3.0 mEq/L that is persistent and refractory to correction.

9. Grade 3 or higher recent (within the past 6 months) or ongoing bleeding events.

10. Symptomatic or uncontrolled cardiovascular disease, myocardial infarction or severe/unstable angina within the past 6 months, or New York Heart Association Class III or IV congestive heart failure

c. Donor eligibility

Eligible donors will include healthy sibling, relative or unrelated donors that are matched with the patient at HLA-A, B, C, and DRB1 by high resolution typing as defined by the Collaborative Trials Network[29].

V. Study Design

1. Sample size

a. A total of up to 12-18 evaluable patients will be enrolled in phase I. The 6 patients treated at the MTD (or lowest biologically active dose) in phase I will be analyzed among those treated in phase II. In phase II, 24 evaluable patients will be enrolled to study CD4+ T-cell STAT3 phosphorylation at the MTD. Additional 14 evaluable patients will be accrued to expansion stage if 9 or less of the initial patients treated at the MTD develop grade II-IV acute GVHD. Thus, a total of up to 50 evaluable patients could be enrolled to the combined phase I and II components of the trial. We anticipate up to 63 total could be **enrolled and treated** with PAC/SIR/TAC (which would account for a 10% replacement for non-evaluable subjects in phase II, and possible 10 subjects non-evaluable in the phase I component of the trial).

2. Withdrawal

a. Subject withdrawal from the study will take place under the following circumstances:

i. those patients who sign consent for the study, but fail to initiate treatment on account of:

1. denial of coverage from insurance provider
2. death prior to initiation of treatment
3. withdrawal of patient consent at any time after therapy

Subjects who withdraw consent for pacritinib therapy after day +21 but before day +100 will be asked to continue on study for follow-up of their condition and allow collection of research blood samples. These patients will be considered "off-therapy, on-study" reflecting **partial** withdrawal of consent. These patients will inform the secondary endpoint of GVHD prevention, and only be excluded from the analysis if they are off pacritinib for more than 4 weeks and do not resume the drug thereafter for causes other than acute GVHD (treatment failure). Subjects who withdraw consent for pacritinib therapy before day +21 will be excluded from any study analysis reflecting **full** withdrawal of consent.

Subjects who do not wish to permit follow-up of their condition and continued collection of their peripheral blood for research studies will also be called **full** withdrawal of consent.

Withdrawal of consent (partial or full) will be documented in OnCore and within the patient's medical record.

3. Intervention: Pacritinib

   a. Dose and Schedule: Up to 200mg BID orally (or MTD) from day 0 until day +100. PAC will be tapered to 50% of the total dose at day +70, then 25% of total dose at day +84, then stop at day +100 (+/- 7 day window is provided around timing of tapering events at day +70 and day +84, as well as last dose stop date around day +100).

4. Standard transplant care and practice

   a. Donor selection

   Donor must be a sibling, relative, or unrelated donor identical for HLA-A, -B, -C, and HLA-DRB1 by high resolution typing methods.

   b. Stem cell mobilization and collection

   1. Peripheral blood stem cell mobilization: Donors will be mobilized and collected per institutional or National Marrow Donor Program (NMDP) standard operating protocol. The desired target CD34+ cell dose is 5-10 x 10^6 cells/kg. Collection of <5 x 10^6 CD34/kg is not a deviation.

   2. Umbilical cord blood, HLA haploidentical, and bone marrow stem cells grafts are not included in this protocol.

   c. Conditioning regimen

   The conditioning regimen is per physician discretion but restricted to busulfan and fludarabine or melphalan and fludarabine. The specific details of these two regimens will adhere to BMT Program standards.

d. GVHD prophylaxis

In addition to the study drug, pacritinib, SIR/TAC will be administered and dosed according to Moffitt Cancer Center, Department of Blood and Marrow Transplantation standard practice. Attending physician discretion is permitted with regard to timing, rapidity, and completion of SIR/TAC taper. SIR/TAC levels will be monitored according to program standards. The therapeutic target for SIR is 5-14 ng/ml. The therapeutic target for tacrolimus is 3-7 ng/ml. It is expected that pharmacologic fluctuations of SIR and TAC levels may occur during this treatment, and such fluctuations in dose levels outside the target ranges will not be considered a deviation. Dose modifications of SIR/TAC for concurrent use of CYP3A4 inhibitors or inducers will be based on program standards.

Note: Pacritinib is neither an inhibitor nor an inducer of cytochrome P450 (CTI BioPharma Investigator Brochure).

e. Concurrent use of CYP3A inhibitors

While strong CYP3A inhibitors should be avoided when clinically feasible, it is understood that the use of azoles and other similar agents are necessary at times among allogeneic HCT recipients.

Tacrolimus / Sirolimus: Adherence to Moffitt BMT standard operating procedures (SOP BMT-G-105), including necessary dose reductions, is required when administering azoles with tacrolimus and sirolimus.

Dose reductions for tacrolimus and sirolimus when using concurrent voriconazole or posaconazole (per SOP BMT-G-105)	
Tacrolimus	**Reduce tacrolimus dose by 50-66%**
Sirolimus	**Reduce sirolimus dose by 66-90%**

Pacritinib: Data from phase I ADME studies using pacritinib in healthy participants (Al-Fayoumi S et al) shows that coadministration of strong CYP3A inhibitors, such as clarithromycin, increases the Cmax from 3.68 to 4.79, and AUC from 173 to 316 (n=20 per group), thus approximately increasing the systemic exposure by 50%. Therefore, patients requiring concurrent therapy with moderate to strong CYP3A inhibitors must reduce the dose of pacritinib by 50% as follows:

Pacritinib dose prior to CYP3A Inhibitor	**Pacritinib dose reduction on CYP3A Inhibitor**
100 mg daily	**100 mg every other day**
100 mg twice a day	**100 mg daily**
200 mg twice a day	**100 mg twice a day**

Patients may resume the pre-CYP3A inhibitor dose of pacritinib once they have been off the CYP3A inhibitor for at least 5 days.

The following medications are considered moderate to strong CYP3A inhibitors that are potentially used among allogeneic HCT recipients:

- Fluconazole
- Clotrimazole
- Voriconazole
- Posaconazole
- Isavuconazonium sulfate
- Amiodarone
- Diltiazem
- Verapamil
- Erythromycin
- Clarithromycin

Note: allogeneic HCT recipients are counseled to avoid herbal CYP3A inhibitors, such as grapefruit juice, Echinacea, wild cherry, chamomile, and natural licorice.

5. Safety assessment

a. Thrombotic microangiopathy (expected with SIR/TAC)

TMA definitions and grading system:

TMA will be defined and graded per the BMT Clinical Trial Network consensus statement. TMA is defined as: RBC fragmentation and > 2 schistocytes per high-power filed on peripheral blood smear; concurrent increased serum LDH above baseline; concurrent renal (defined as doubling of baseline serum creatinine or decrement of > 50% of baseline creatinine clearance) and/or neurologic dysfunction without an alternate explanation; and negative direct and indirect Coombs test results. The primary management strategy for should be the dose reduction or discontinuation of calcineurin-inhibitors upon the recognition of post-transplant TMA. Calcineurin inhibitors may be replaced by alternative immunosuppressive agent(s).[33] As per our completed trial, the expected incidence of TMA with SIR/TAC is 25%[25]. This is not statistically different when compared with MTX/TAC (20%, P=0.48) [25]. The grade of TMA between SIR/TAC and MTX/TAC did not differ as well[25].

Evaluation and management procedures:

Active surveillance for this condition will take place in all patients. In those who meet criteria (RBC fragmentation and >2 schistocytes per high-power field on peripheral smear; Concurrent increased serum LDH above institutional baseline; Concurrent renal and/or neurologic dysfunction without other explanations; Negative direct and indirect Coombs test results).

Changes to the immunosuppression regimen is recommended as follows for TMA:

For CTC grade 1-2, TAC dose reduction by 0-25%

For CTC grade 3, TAC dose reduction by 50%

For CTC grade 4, TAC will be discontinued

No indication for Plasma exchange upon recognition of TMA

b. Hepatic Veno-occlusive disease (VOD) (expected with SIR/TAC)

VOD definitions and risk factors:

The clinical syndrome of hepatic VOD is associated with weight gain, hepatomegaly with abdominal pain, cholestasis and transaminitis, as well as anasarca. Subsequent renal and pulmonary dysfunction may occur as well. Risk factors for VOD include pre-transplantation transaminitis, chronic hepatitis C and B, and exposure to cyclophosphamide-based transplant preparative regimens.[34]

Contemporary clinical trials have observed rates of VOD ranging from 8-9%.[35,36] Severe VOD is associated with a mortality rate in excess of 90%. The management of VOD primarily consists of eliminating exposure to offending agents and supportive care. Early phase clinical trials investigating the use of defibrotide in VOD have reported complete response rates up to 60%.[37] Patients will be monitored closely for any clinical signs of VOD. The specific management of any occurrence of VOD will be at the discretion of the physician of record.

c. Non-infectious diarrhea

Diarrhea is a known side effect of allogeneic HCT. Additionally, phase I-III studies of pacritinib in hematologic malignancies shows that grade 1-2 diarrhea is a common side effect of this medication. The pacritinib diarrhea typically occurs within 7 days of initiating the medication. Clinical trial data supports that the diarrhea does respond to anti-motility agents, and does not typically require dose reduction of pacritinib. Given the potential for compounding toxicities early after allogeneic HCT, dose reduction rules for diarrhea (1500ml/day x3 consecutive days, not responding to anti-motility agents) are provided in Table 1 of this protocol.

d. QTc prolongation

Life-threatening and sometimes fatal cardiac events have been observed in patients treated with pacritinib, including heart failure and arrhythmias, some of which may have been associated with sudden death. As such, EKGs will be performed at baseline as part of vital organ testing (VOT) and repeated at day +30 and day +60. Patients with a baseline QTc >450ms at time of VOT will be excluded from participation. Instructions for QTc >450ms that develop while taking pacritinib are provided in appendix D.

e. Coagulation parameters (TT, PT, and PTT)

Serious bleeding events, specifically bleeding of the brain (intracranial hemorrhage, subdural hematoma, cerebral hemorrhage), have been observed in association with pacritinib treatment and occasionally with life-threatening or

fatal outcomes. TT, PT, and PTT will be performed at baseline as part of vital organ testing (VOT) and repeated at day +30 and day +60. Patients with baseline TT, PT, and/or PTT >2x the upper limit of normal are excluded from participation. Instructions for a TT, PT, and/or PTT lab value >2x the upper limit of normal while on pacritinib are provided in appendix D.

6. Endpoints

Primary Endpoints

Phase I: The lowest biologically active dose of PAC that is safe and well tolerated with SIR/TAC

Phase II: STAT3 activity in circulating CD4+ T-cells at day +21[2]

8 green top tubes will be collected at baseline (day -30 to -5), day +21, and day +100. This is equivalent to 5.5 tablespoons of blood for each assessment. Peripheral blood mononuclear cells (PBMC) will be isolated by Ficoll density gradient. PBMCs will be stimulated with IL-6 for 20 minutes to activate STAT3. Phosphoproteins will be analyzed within T-cells by flow cytometry as described[2].

Secondary Endpoints

1. Cumulative incidence of acute GVHD[38] by day +100

Acute GVHD severity grading and monitoring:

Patients will be monitored for clinical signs of acute GVHD. Acute GVHD will be graded per the 1995 consensus guidelines.[27] (see Appendix A).

Therapy for established acute GVHD:

This protocol does not dictate any one specific therapy for acute GVHD. The primary physician may direct the management of acute GVHD per their discretion and in accordance with MCC standard practice.

2. Treg, Th1, and Th17 differentiation among circulating T-cells at day +21 and day +100

Treg and Th1 will be analyzed by flow cytometry. Treg will be identified by expression of CD4, bright CD25, lack of CD127, and intracellular expression of Foxp3. Th1 will be identified by expression of CD4+, and intracellular T-bet+ and IFN-gamma+ [post PMA/ionomycin stimulation]. Th17 differentiation will be assessed functionally by IL-17 Elispot using PBMCs on day +21 and day +100. IL-17 Elispot is preferred to analyze Th17 as the anticipated frequency of circulating Th17 in the periphery is low post allogeneic HCT. Treg and Th17 will be evaluated at baseline, day +21, and day +100. A subset of 15 patients treated with SIR/TAC alone per Moffitt standard practice will be enrolled for blood sample collection only on days +21 and +100 of their alloHCT. The effects of PAC/SIR/TAC versus SIR/TAC alone on Tregs, Th1, and Th17 will be compared among the two groups.

3. CD28 and IL-2 signal transduction assessment by S6, H3 ser10, and STAT5 phosphorylation in CD4+ T-cells at days +21 and +100

Steady-state mTOR and Aurora kinase activity (S6 and Histone 3 ser10, respectively) and IL-2 mediated STAT5 activity will be evaluated at baseline, day +21, and day +100. Phosphoproteins will be analyzed within T-cells by flow cytometry as described[2].

4. Pharmacokinetic (PK) parameters: For all patients enrolled in the phase I portion of this study, pharmacokinetic sampling of plasma pacritinib levels will be drawn on days 0 and +21 at the following time points: pre-dose, +4 hours, +12 hours, and +24 hours.

5. Analysis of tissue-infiltrating immune cell subsets: We will obtain available GVHD target organ (e.g. skin, liver, GI) biopsy samples to study tissue-infiltrating CD4 T cell subsets (Th1, Th2, Th17, Treg) through tissue IHC (immunohistochemistry). These biopsies were obtained as part of routine clinical practice for GVHD diagnosis, and these available resources only will be used for this purpose (i.e. no clinical trial-specific biopsies will be obtained). At time of this protocol amendment when trial accrual is now complete, we expect approximately 25 total representative samples (respecting biopsy samples from skin, and GI sites including gastric, duodenal, and rectum) available to be included in this analysis. We will also enlist 25 representative historical control subject GVHD biopsy samples for comparison, arising from patients previously treated with standard of care sirolimus/tacrolimus GVHD prevention off-protocol. These will also be obtained from existing historical biopsy samples (no new biopsies to be performed). From these available biopsy samples, slides will be prepared, tissue stained for Th-subset markers (CD4/Tbet, CD4/GATA3, CD4/RORy, and CD4/FoxP3), and data acquired in collaboration with the Moffitt Pathology department, Tissue/Histology Core, and Image Analysis Core. Statistical comparisons will be made across pacritinib trial-treated subjects vs. standard of care off-protocol subjects to discern any differences. Other variables considered in this analysis will include clinical GVHD stage, time from transplant, and pathologic GVHD grade.

7. Statistical considerations

Clinical trial design, power calculations, and analysis

Phase I consists of a standard 3+3 design with three PAC dose cohorts. The DLT definitions are described on page 9 of this protocol. The MTD is defined as the highest dose level at which one or none of 6 subjects develop a DLT, and the combination of safety and biologic activity of pacritinib will dictate selection of recommended phase II dose. A total of 12-18 patients will be enrolled to determine the PAC MTD. Patients treated at the MTD (or lowest biologically active dose) in phase I will be analyzed among those treated in phase II.

An evaluable patient for the phase I portion of the trial must have either met one of the defined clinical events in the DLT definition, or if not, must have been receiving pacritinib within 72 hours of the planned day +21 blood sample for determination of pSTAT3 level. Those patients that have not had a clinical DLT event and are not actively taking pacritinib for any reason within 72 hours of day +21 will be considered not evaluable, and will be replaced.

The phase II component of this trial is designed to detect a significant change in the biologic endpoint of STAT3 activity at day +21. The preliminary data revealed that the

mean and standard deviation (SD) of CD4+ T-cell pSTAT3 activity among 18 HCT recipients at day +21 were ~50% and ~25%, respectively, regardless of acute GVHD severity. We conservatively anticipate that mean of pSTAT3 level at day +21 will decrease to 35% or less by combining PAC to SIR/TAC. With a two-sided significance level of 0.05, a total sample size of 24 will achieve 80% power to detect a decrease of 15% when the anticipated SD is 25%, which is equivalent to an effect size of 0.6. We anticipate that the actual SD will be lower than 25%. If the actual SD is 20%, the study will have 94% power to detect a difference of 15% (an effect size of 0.75).

We also aim to understand whether the observed incidence of grade II-IV acute GVHD is sufficiently promising to warrant further study in a trial adequately powered to address this clinical endpoint. Based on published and unpublished experience of over 300 treated-patients, the incidence of GVHD with SIR/TAC is 43%. We hypothesize that the cumulative incidence rate of GVHD with PAC/SIR/TAC by day +100 will reduce to 23% (a decrease of 20%) by day +100. The study expansion will be considered if 9 or less of 24 patients (37.5%) initially treated with PAC/SIR/TAC develop grade II-IV acute GVHD by day +100. The expansion will permit an additional 14 patients will be accrued.

The expanded phase II trial (second stage of phase II) will be considered favorable if 12 or less of the total 38 (31.6%) patients develop acute GVHD by day +100. This sample size was computed using Simon's two-stage design with 10% one-sided significance level and 90% power. If this regimen is actually not effective, there is 9.8% probability of concluding that it is. If the regimen is actually effective, there is 8.6% probability of concluding that it is not. If the second stage is open (n=38), the study will achieve 95% power to detect a decrease of 15% when the SD is 25%. The Blood and Marrow Transplant Program at MCC performs approximately 190 allogeneic procedures annually. A conservative estimated accrual rate of 20% would provide at least 38 patients each year allowing for full accrual by 24 months.

An evaluable patient for primary endpoint is defined as patients who received PAC/SIR/TAC until at least day +21 of their alloHCT and who has neither relapsed nor died of any reasons without experiencing grade II-IV aGVHD until day +100. A non-evaluable PAC/SIR/TAC patient will be replaced and we anticipate 41 patients will be accrued to the interventional study in order to account for 10% of competing risks (relapse or death without GVHD) at day +100.

Additional 15 patients who receive the standard GVHD prevention regimen of SIR/TAC, will be enrolled for blood sample collection only (8 green top tubes of blood) on days +21 and +100 of the alloHCT. The amount of circulating Treg, Th1, and Th17 cells will be analyzed. The exact same eligibility criteria will apply for these subjects.

A total of up to 68 (up to 18 patients in phase I [with the 6 treated at MTD included among those analyzed in phase II] + 32 [PAC/SIR/TAC] + 3 [non-evaluable replacement] +15 [blood sample collection only]) patients will be enrolled.

Statistical analysis methods

Patient baseline characteristics will be summarized using descriptive statistics including mean, median, standard deviation and range for continuous measures and proportions and frequencies for categorical measures. As the primary endpoint of the study, one sample t-test will be employed to examine if pSTAT3 at day +21 is decreased by 15% from historic mean of 50%. A two-sided p-value of <0.05 is considered statistically

significant. If data is not normally distributed, an appropriate transformation (i.e., log-transformation) or nonparametric test will be considered. As the key secondary endpoint, the cumulative incidence of grade II-IV acute GVHD will be estimated using the competing risk approach, where relapse and non-relapse mortality are considered the competing risks. The one-sided 90% confidence interval for grade II-IV acute GVHD will be computed using log-log transformation.

Overall and relapse-free survival (OS and RFS) will be analyzed using the Kaplan-Meier method. The association of time-to-event data with/without competing risks with other potential predictors will be explored by the Cox proportional hazards regression model and the Fine and Gray method[39], respectively. Fifteen patients treated with SIR/TAC alone will be enrolled for blood sample collection only on days +21 and +100 of their alloHCT. The effects of PAC/SIR/TAC versus SIR/TAC alone on Tregs, Th1, and Th17 at day +21 and +100 will be compared using the generalized linear model with identity link function, adjusting for the effect of acute GVHD (grade 0-I vs. grade II-IV). In addition, the mixed effect model will be considered to explore the difference in trends between two groups. With a two-sided significance level of 0.05, the sample size of 39 (24 in PAC/SIR/TAC and 15 in SIR/TAC) will have 84% power to detect an effect size (=difference/pooled SD) of 1 between two groups. If the second stage is open, the power will increase to 90% as the sample size of PAC/SIR/TAC increases to 38. No multiplicity adjustment will be considered for secondary and exploratory endpoints.

VI. Data collection and safety monitoring

The Data & Safety Monitoring Plan (DSMP) adopted by the H. Lee Moffitt Cancer Center & Research Institute, Inc. (MCC) will ensure that all clinical research conducted or coordinated by the Cancer Center is scientifically well designed, responsibly managed, appropriately reported and protects the rights and welfare of human participants. The methods and amount of monitoring required are dictated by the degree of risk involved to the individual participants and the complexity of the clinical research. Other entities that will assume responsibility for data and safety monitoring include:

- Principal Investigators (PI)
- The Scientific Review Committee (SRC);
- The Protocol Monitoring Committee (PMC);
- The Research Compliance Division (RCD) of the Cancer Center's Corporate Compliance Office;
- Institutional Review Board (IRB)
- U.S. Food and Drug Administration (FDA)
- National Heart, Lung, and Blood Institute (NHLBI)
- CTI BioPharma

Please see **SAFETY AND REPORTING REQUIREMENTS** for specifics regarding the collection and reporting mechanisms to the SRC, PMC, RCD, IRB, FDA, and CTI BioPharma.

Any amendments to this protocol or accompanying consent form will be sent for IRB review. Approved changes to either of the documents will be disclosed with the NHLBI and the FDA.

1. Data collection

The Principal Investigator and the Clinical Trial Coordinator(s) assigned to the case will be primarily responsible for maintaining all study related documents including clinical research forms, as applicable. ONCORE is the database of record for all CRF entries and will be verified with source documentation. The review of medical records within PowerChart will be done in a manner to assure that patient confidentiality is maintained.

Data collected will be stored in Moffitt Cancer Center's database system, ONCORE. Identifying patient information will be kept confidential. Representatives of the IRB and the FDA will have access to patient information as it pertains to the study. Privacy and confidentiality of the information will be protected to the extent provided by law.

2. Safety monitoring

a. Safety monitoring

i. The principal investigator will have the primary responsibility for data safety and monitoring. Input will be sought from sub-investigators and other members of the BMT Program concerning data and safety issues.

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the SRC and IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to a DSMB and/or to the PMC and IRB as required.

ii. The investigators and members of the BMT Research Staff will meet at least monthly. The following data will be reviewed:

1. rate of accrual
2. adverse events and unanticipated problems
3. protocol deviations and/or violations.

iii. If necessary, corrective action and/or educational programs will occur to ensure subject safety and data integrity. Reports to the SRC, PMC, and IRB will be submitted as required.

iv. Stopping criteria for observed toxicity:

The PI of this study will have responsibility for continuous monitoring of adverse events on this trial. Additionally, these events will be reviewed formally in monthly research meetings of the BMT program. As described in appendix C of this protocol, there are numerous anticipated adverse consequences of transplantation, which include, but are not limited to conditioning regimen related toxicity such as severe mucositis, idiopathic pneumonia syndrome, hepatic veno-occlusive disease and death, early and late infectious complications, potentially severe or fatal acute or chronic graft vs. host disease, and relapse of primary disease and its complications. As these are anticipated complications, they will not inform specific stopping criteria for toxicity in this study. If four related grade 4-5 unexpected adverse events occur in the conduct of this trial, an analysis will be performed, and further accrual to the trial will be temporarily halted. Data

will be analyzed, and a report will be submitted to the protocol monitoring committee (PMC). Further decisions regarding resuming accrual will be determined by the PMC with input from the PI of this study. If the study requires suspension or termination, an NHLBI Program Official will be notified in writing.

Scientific Review Committee (SRC)

The Cancer Center's internal SRC provides the review for scientific merit and prioritization and monitors scientific progress for all protocols at the Cancer Center. The SRC has a defined membership representing all of the major research divisions of the cancer center, including biostatisticians. The SRC reviews newly proposed clinical research studies based on the following categories:

a. study significance to evaluate its potential for contribution to medical science.
b. the adequacy of study objectives, design, specific aims and hypotheses.
c. the methods to be employed to conduct the study. Ensuring that the study is adequately described, including inclusion/exclusion criteria, sample size, procedures and instruments to be used.
d. Feasibility of the study. Ensuring the investigator has adequate staffing and facilities to conduct the study. Ensuring that the timeframe for the study and projected annual accrual are adequately addressed.
e. Review of all data and safety monitoring plans.

The SRC will also evaluate the risk/benefit assessment and corresponding Data & Safety Monitoring Plan (DSMP), evaluate and may recommend frequency of monitoring. The SRC will identify any potential conflicts of interest related to the proposed research. The SRC may also evaluate and recommend the monitoring frequency of clinical trials they approve.

The Protocol Monitoring Committee (PMC)

The Protocol Monitoring Committee (PMC) is a multidisciplinary, peer review, standing committee established to oversee clinical research conducted at Moffitt Cancer Center to monitor scientific progress and data quality. It also serves as a component of the Data Safety Monitoring Plan (DSMP) for oncology trials at MCC. The membership of the PMC includes physicians and scientists from various program areas.

The PMC provides ongoing monitoring of all clinical research studies for safety, validity and integrity of data, adverse events, conflicts of interest, and overall compliance with GCP or other applicable clinical research guidelines or regulations. In addition to the protocol stopping rules, the PMC is authorized to suspend a trial for non-compliance with a DSMP or as a result of audit findings deemed unacceptable.

Research Compliance Division (RCD)

The Cancer Center has established the RCD of the Corporate Compliance Office as the coordinating center for internal audits of clinical trials conducted at the Cancer Center and its affiliates. These audits will provide for a systematic and independent examination of trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data was recorded, analyzed and accurately reported, according to the Institutional Review Board (IRB) approved protocol, Center's policies, Good Clinical Practices (GCP): Consolidated Guidance, and applicable regulatory requirements.

Audits are conducted by the RCD in accordance with applicable regulatory standards. The RCD will conduct and report the findings of audits to the PMC. The PMC will determine the findings to be: *acceptable, acceptable with corrective action, or unacceptable.* The RCD will notify the IRB of the audit determinations made by the PMC. The RCD will follow up to ascertain whether corrective actions, which have been agreed to, are achieving the desired results. The PMC will be informed of all significant open follow-up items. For those observations where no action has been taken, the RCD will inform the PMC who may take action as appropriate.

Internal Monitoring

Monitoring will be performed regularly by the MCC Clinical Monitoring Core for accuracy, completeness, and source verification of data entry, validation of appropriate informed consent process, reporting of SAEs, and adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

Institutional Review Board (IRB)

The trial will not be initiated without approval of the appropriate Institutional Review Board (IRB). All administrative requirements of the governing body of the institution will be fully complied with. This protocol, consent procedures, and any amendments will be approved by the IRB in compliance with current regulations of the Food and Drug Administration prior to initiation unless necessary to protect the safety and welfare of subjects; in which case, the IRB will be notified within 24 hours of implementing the change. A letter of approval will be sent to the institution(s) funding the study prior to initiation of the study and when any subsequent modifications are made. The IRB will be kept informed by the investigator as to the progress of the study as well as to any serious or unusual adverse events.

Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained. The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval.

Reporting of changes or amendments to the protocol or consent form

All changes or amendments to the protocol or consent form will be reviewed by the SRC and/or IRB. Once the revised documents are approved, they will be distributed to the FDA, NHLBI, and CTI BioPharma.

3. Safety: Monitoring of adverse events and pacritinib dose modifications for toxicity

Dose Adjustments for Pacritinib

Specific dose adjustment rules are provided here for diarrhea, QTc prolongation, and left ventricular dysfunction (see below sections and tables). For all other pacritinib-related (probably or definitely) ≥ Grade 3 non-hematologic toxicity, the following rules apply:

- Discontinue pacritinib administration until toxicity resolves to Grade 2 or better
- Once toxicity has returned to grade 2 or better, patients can resume pacritinib at 100mg less per day than the previously administered dose at which they were discontinued. Only two, 100mg, dose reductions are allowed before treatment with pacritinib is discontinued. Patients cannot reduce the dose of pacritinib <100mg daily. Patients that do not tolerate 100mg pacritinib daily must discontinue pacritinib. Following dose reductions, pacritinib dose re-escalation is not permitted.
- Patients who do not recover (≤ Grade 2 or baseline) within 14 days of discontinuation of study drug will not be permitted to re-start study drug.

Pacritinib Dose Management Guidelines for QTcF Interval Prolongation and Left Ventricular Dysfunction

Dose management for QTcF interval prolongation is summarized below.

QTc prolongation: CTCAEv4 Toxicity Grade	Management/Action
G1 (QTcF > 450ms)	No change
G2 (first occurrence) (QTcF > 480ms)	Hold treatment: - hold minimum of one day, e.g. two doses on the 100mg BID dose level - then repeat EKG and consider resuming dosing if toxicity resolves to ≤ grade 1-If the toxicity resolves to ≤ grade 1 within 7 days, treatment may be resumed at next lowest dose level Toxicity that does not resolve to ≤ grade 1 within 7 days requires treatment discontinuation
G2 (second occurrence)	Discontinue treatment
G3 and G4 (QTcF > 500ms)	Discontinue treatment

After dose reduction, no dose re-escalation is allowed.

All patients are required to have an ECG at baseline, and then at month 1 (day 30 post-transplant) and month 2 (day 60 post-transplant). No further monitoring is required beyond this time, as pacritinib will be tapered off within the subsequent month on this trial. In the event of grade 1 or greater QTcF prolongation, EKG must be checked within 7 days of holding treatment to determine if treatment may be resumed by the above rules.

All patients will have a pre-transplant ECHO or MUGA performed to evaluate left ventricular ejection fraction. Patients will be monitored closely as standard care for allogeneic HCT (daily until engraftment and then at least once a week if not more until day +100). A full cardiopulmonary exam will be performed at each scheduled visit to clinically monitor for any signs or symptoms of heart failure as recommended by the National Institute for Health and Care Excellence (NICE)[40]. Repeat ECHO will be performed at the 1 month and 3 month mark from initiation of pacritinib therapy (i.e. +30 and +90 days

from day 0) in keeping with monitoring recommended in the pacritinib Investigator Brochure. Dose management for left ventricular dysfunction is as follows:

LV dysfunction: CTCAEv4 Toxicity Grade*	Management/Action
G1	No change
G2 (first occurrence) *LVEF < 40%	Hold treatment Repeat echocardiogram 14 days. If the treatment resolved to ≤ grade 1, treatment may be resumed at next lowest dose level.
G2 (second occurrence)	Discontinue treatment
G3 and G4**	Discontinue treatment

*The CTCAE v4 term 'left ventricular systolic dysfunction' and its grading are based on the degree of clinical manifestations and therapy needed to control this issue. In contrast, specific cut-off values for abnormal ejection fraction are not provided, and grades 1 and 2 AE have no other descriptors. Detail is only provided for grade 3 ("*symptomatic due to drop in ejection fraction responsive to intervention"*). To be included on this trial, patients must have at least an ejection fraction of 45%. Adhering to accepted definitions of LV systolic dysfunction, we will consider a decline in LV ejection fraction to less than 40% as an event worthy of holding pacritinib therapy (and treat this as the equivalent of grade 2 per above). To resume therapy, a repeat echocardiogram within 14 days of stopping pacritinib must show return to > 45% ejection fraction.

**As per above, symptomatic heart failure (grades 3 and 4 per CTCAE ver4) require complete discontinuation of pacritinib.

After dose reduction, no dose re-escalation is allowed.

Management of Gastrointestinal Toxicity
The need for managing GI effects of pacritinib, particularly diarrhea, should be anticipated. A careful baseline evaluation of bowel habits (frequency and consistency of bowel movements) should be obtained at baseline for all patients.

Dose reduction rules for pacritinib (combined with SIR/TAC)		
Diarrhea	**Current Dose (pacritinib)**	**Dose Reduction (pacritinib)**
None / responds to antimotility agents	200mg twice a day	N/A
≥1500ml/day x3 days*	200mg twice a day	200mg qAM and 100mg qPM
	200mg qAM and 100mg qPM	200mg daily
	100mg twice a day	Hold pacritinib until stools are formed or volume is <500ml/day, then resume at 100mg twice a day and continue until day +70 taper. If diarrhea (≥1500ml/day x3 days) returns after holding pacritinib and does not respond to antimotility agents, patient comes "off-drug" but remains "on-study" for data collection and research labs.
	100mg daily	Hold pacritinib until stools are formed or volume is <500ml/day for at least 5 days, then resume at

		100mg a day and continue until day +70 taper. If diarrhea (≥1500ml/day x3 days) returns after holding pacritinib and does not respond to antimotility agents, patient comes “off-drug” but remains “on-study” for data collection and research labs.
Note diarrhea must be non-infectious and unresponsive to anti-motility agents		

*****This total measured stool volume of > 1500mL/day has been approximated to > 7 episodes of diarrhea per day (*Harris, et al. BBMT, 2016*) based on review of diarrhea volume and stool frequency of over 300 patients in this analysis. Thus, we will treat either this measured volume, or > 7 episodes of diarrhea per day (when volume is not measured) as the threshold requiring dose modification. This rule also harmonizes with CTCAE ver4, as this same frequency of stools constitutes grade 3 diarrhea.

General Administration Guidelines

Missed or Vomited Doses

Missed doses will not be replaced. All missed doses should be documented in the patient diary. If a dose is vomited within one hour of ingestion, it will be considered a missed dose and recorded as such on the patient diary. The dose will not be repeated that same day but the patient will follow regular schedule starting the next study dosing day. If vomiting occurs more than 1 hour after dosing, it will still be considered a complete dose.

SAFETY AND REPORTING REQUIREMENTS

ASSESSMENT OF SAFETY

Safety assessments will consist of monitoring and recording adverse events and serious adverse events and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s) and should be consistent with institutional standards and Good Clinical Practice.

DEFINITIONS

Unanticipated Problems (UP)

A UP includes any incident, experience, or outcome that involves risks to patients that are 1) unexpected with relation to the planned study or intervention, 2) related or possibly related to the research study or intervention, and 3) places patients at more risk than anticipated. For the purposes of this clinical study, UPs include only treatment-emergent problems which are either new or represent detectable exacerbations of pre-existing conditions.

Adverse Events (AE)

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution. For the purposes of this clinical study, adverse events include only treatment-emergent events which are either new or represent detectable exacerbations of pre-existing conditions.

This includes the following:

• Subjective or objective symptoms spontaneously offered by the subject and/or observed by the investigator or study staff including laboratory abnormalities of clinical significance.

• Any adverse events experienced by the subject through the completion of final study procedures.

• AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with MCL that were not present before the AE reporting period

• Complications that occur as a result of protocol-mandated interventions (eg, invasive procedures such as biopsies)

The following are NOT considered an adverse event or unanticipated problem:

• **Pre-existing condition:** A pre-existing condition (documented on the medical history CRF) is not considered an AE unless the severity, frequency, or character of the event worsens during the study period.

• **Preplanned hospitalization:** A hospitalization planned before signing the informed consent form is not considered an SAE, but rather a therapeutic intervention. However, if during the pre-planned hospitalization an event occurs, which prolongs the hospitalization or meets any other SAE criteria, the event will be considered an SAE. Surgeries or interventions that were under consideration but not performed before enrollment in the study will not be considered serious if they are performed after enrollment in the study for a condition that has not changed from its baseline level. Hospitalizations for social reasons or due to long travel distances are also not SAEs.

• **Diagnostic Testing and Procedures:** Testing and procedures should not be reported as adverse events or serious adverse events, but rather the cause for the test or procedure should be reported.

Serious Adverse Event

The terms “severe” and “serious” are not synonymous. Severity (or intensity) refers to the grade of an AE (see below). “Serious” is a regulatory definition and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient’s life or functioning. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations to applicable regulatory authorities.

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

• It results in death (ie, the AE actually causes or leads to death).

• It is life-threatening (with regards to determining if an AE is serious, “life-threatening” is defined as an AE in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe. If either the investigator or the Sponsor believes that an AE meets the definition of life-threatening, it will be considered life-threatening.).

• It requires or prolongs in-patient hospitalization.

• It results in persistent or significant disability/incapacity (ie, the AE results in substantial disruption of the patient’s ability to conduct normal life functions).

• It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product.

• It is considered a significant medical event by the investigator based on medical judgment (eg, may jeopardize the patient or may require medical/surgical intervention to prevent 1 of the outcomes listed above).

Severity

Definitions found in the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) will be used for grading the severity (intensity) of AEs. The CTCAE v4.0 displays Grades 1 through 5 with unique clinical descriptions of severity for each referenced AE. Should a patient experience any AE not listed in the CTCAE v4.0, the following grading system should be used to assess severity:

• Grade 1 (Mild AE) – experiences which are usually transient, requiring no special treatment, and not interfering with the patient's daily activities

• Grade 2 (Moderate AE) – experiences which introduce some level of inconvenience or concern to the patient, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures

• Grade 3 (Severe AE) – experiences which are unacceptable or intolerable, significantly interrupt the patient's usual daily activity, and require systemic drug therapy or other treatment

• Grade 4 (Life-threatening or disabling AE) – experiences which cause the patient to be in imminent danger of death

• Grade 5 (Death related to AE) – experiences which result in patient death

Suspected Adverse Reaction

• A Suspected Adverse Reaction is any adverse event for which there is a "reasonable possibility" that the drug caused the adverse event.

• "Reasonable Possibility", for the purposes of safety reporting, means there is evidence to suggest a causal relationship between the drug and the adverse event. Examples of evidence that would suggest a causal relationship between the drug and the adverse event are:

• A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure.

• One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug. If the event occurs in association with other factors strongly suggesting causation (eg, strong temporal association, event recurs on rechallenge), a single case may be sufficiently persuasive; but often, more than one occurrence would be needed before the sponsor could make a determination of whether the drug caused the event.

Unexpected

An "unexpected" AE is an AE that is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed. "Unexpected" also refers to AEs that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the study drug under investigation.

DOCUMENTING AND REPORTING OF ADVERSE OR SERIOUS ADVERSE EVENTS AND UNANTICIPATED PROBLEMS

The Sponsor-investigator is responsible for ensuring that AEs, SAEs, and UPs that are observed or reported during the study, as outlined in the prior sections, are recorded on the study adverse event log

with AE collection timelines adhering to the standards of the Institution. All SAEs also must be reported according to the local IRB guidelines as well as to the CTI PVG

Adverse Event and Unanticipated Problem Reporting Period
The AE and UP reporting period for this study begins after first dose of study drug administration until 30 days after discontinuation of the study drug. Grade 3 or greater AEs, SAEs, or UPs that are encountered during the protocol specified AE reporting period should be collected, and followed to resolution or until the patient is lost to follow up or withdrawals consent. Resolution/stable means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.

Assessment of Adverse Events and Unanticipated Problems
Investigators will assess the occurrence of AEs, SAEs, and UPs at all patient evaluation time points during the study. Grade 3 or greater AEs, SAEs, and UPs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded in the patient's adverse event log.

Each recorded AE, SAE, or UP will be described by its duration (ie, start and end dates), severity, regulatory seriousness criteria, if applicable, suspected relationship to the investigational product (see following guidance), and any actions taken.

All deaths should be reported with the primary cause of death as the AE term, as death is typically the outcome of the event, not the event itself. The primary cause of death on the autopsy report (if done) should be the term reported.

An SAE will qualify for expedited reporting to regulatory authorities if the SAE is considered a Suspected Adverse Reaction and is not listed in the current Investigator's Brochure (ie, an unexpected event). To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Related to study drug: Any AE or UP (serious or not) that appears to have a reasonable possibility of causal relationship to the use of the study drug. Guidelines to determine whether an event might be considered related include (but are not limited to) the following:

- The event occurred in close temporal relationship to study drug administration.
- The event abated (diminished) or disappeared when treatment with the study drug was down-titrated, interrupted, or discontinued.
- The event re-occurred when treatment was re-introduced.

Unrelated to study drug: Any AE or UP (serious or not) that does not appear to have a reasonable relationship to the use of study drug.

Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

Rules for contraception:

If fertile, both males and females must agree to use effective birth control. Women of childbearing potential must use highly effective methods (defined as those resulting in a failure rate of <1% per year when used consistently and correctly) for the duration of study treatment and for 12 months after last dose of study drug. The contraceptive methods considered highly effective are intrauterine devices and hormonal contraceptives (contraceptive pills, implants, transdermal patches, hormonal vaginal devices, or injections with prolonged release).

Before study enrollment, subjects must agree to take appropriate measures to avoid pregnancy. Report any pregnancy that occurs in a patient or patient's partner from the time of consent to 365 days after the last dose of study drug. Record any occurrence of pregnancy in the patient's medical records and notify the treating physician within 24 hours of learning of the event. Abortion, whether therapeutic, elective or spontaneous, will be reported as an SAE.

A patient must immediately inform the investigator if the patient or patient's partner becomes pregnant from the time of consent to 365 days after the last dose of study drug. Any female patients receiving pacritinib PO who become pregnant must immediately discontinue the drug. The investigator should counsel the patient, discussing any risks of continuing the pregnancy and any possible effects on the fetus. Although pregnancy itself is not regarded as an AE, the outcome will need to be documented. Report any pregnancy that occurs in a subject or subject's partner from the time of consent to 365 days after the last dose of study drug. Record any occurrence of pregnancy on appropriate case report form and fax it to Cell Therapeutics, or designee, within 24 hours of learning of the event. The pregnant female will be followed for outcome, which is defined as elective termination of the pregnancy, miscarriage, or delivery of the fetus. For pregnancies with an outcome of live birth, the newborn infant will be followed until 30 days old by completing the Pregnancy Report Form Part II. Any congenital anomaly/birth defect noted in the infant must be reported as an SAE.

Expedited Reporting Requirements for Serious Adverse Events

Serious, unexpected, and suspected adverse events must be reported to FDA, local IRB, and Cell Therapeutics within 24 hours of the principal investigator's knowledge of the event.
Such IND safety reports should be submitted in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review and archive.

CTI Pharmacovigilance
FAX #: + 1 866 660 8967
E-mail: pv@ctiseattle.com

The study number should be referenced on the fax or subject line of the notification. Additional follow-up information should be completed on a SAE follow-up form as soon as it becomes available and should cross reference the initial SAE report form.

Routine Adverse Event Reporting
Adverse events which are not serious, unexpected and suspected do not require expedited reporting, but rather will be submitted per local IRB standards on an annual basis or more frequently as required.

Type and Duration of Follow-up of Patients after Adverse Events
AEs and SAEs that are encountered during the protocol-specified AE reporting period should be followed to resolution, or until the investigator assesses the patient as stable, or the patient is lost to follow-up or withdraws consent.

4. Suspension/Termination

   The PMC and/or the IRB may vote to suspend or terminate approval of a research study not being conducted in accordance with the IRB, the Cancer Center and/or regulatory requirements or that has been associated with unexpected problems or serious harm to subjects. The PMC/IRB will notify the PI in writing of such suspension or terminations. It is the responsibility of the PMC/IRB Chairperson to ensure prompt written notification of

any suspensions or terminations of PMC/IRB approval to the relevant Federal Agencies, including OHRP, FDA, and if applicable, the Affiliate Program.

5. Trial Discontinuation

For reasonable cause the Investigator and/or Moffitt Cancer Center may terminate this study prematurely. Conditions that may warrant termination include, but are not limited to: the discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study or if the accrual goals are met. A written notification of termination will be issued.

External study site compliance

Compliance to the Protocol and Adherence to Moffitt External Site Coordination Handbook

Moffitt is responsible for monitoring each sites compliance to adherence to applicable Moffitt External Site Coordination Handbook. In coordination with the Monitoring office, the ESC Office assists to monitor compliance to the protocol.

External Site Access to Clinical Trial Database

To obtain access to OnCore, the External Site Coordinator will supply forms required to be completed by the site staff. Once the completed forms are received, the site coordinator will receive VPN access, logon/password, and information on how to access OnCore. The ESC office will provide OnCore training to the site once initial access is granted and on an ongoing basis, as needed.

Registration Procedures for External Sites

All external enrolled subjects must be registered with the External Site Coordination (ESC) office to be able to participate in a trial. The participating site must email the completed current eligibility checklist, registration form, all supporting documents, and signed, unredacted informed consent to the Coordinating Center. Unsigned or incomplete forms will be returned to the site. Once documents are received, the ESC Coordinator will review them to confirm eligibility and complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

Upon completion of registration, the ESC Coordinator will provide the participating site with the study sequence number and, when applicable, randomization information. Within 48 hours after registration, it is the site's responsibility to:

Enter the on-study patient information into the Oncore database

Order investigational agent(s) if indicated per protocol

It is the responsibility of the participating Investigator or designee to inform the subject of the research treatment plan and to conduct the study in compliance with the protocol as agreed upon with Moffitt Cancer Center and approved by the site's IRB.

To register a patient, send the completed signed eligibility checklist along with the patient registration form and supporting documentation to the ESC via email at ESC_Partnerships@Moffitt.org, Monday through Friday between 8:00AM and 5:00PM (EST). If a short turnaround time is required between registration and first treatment, please consider discussing this with you ESC Coordinator and, if possible, submit a partial submission.

Required Documentation for External Sites

Before the study can be initiated at any site, the site will be required to provide regulatory documentation to the External Site Coordination (ESC) office at Moffitt Cancer Center. Sites must provide a copy of their informed consent to the ESC office for review and approval prior to submission of any documents to the site's IRB. Any changes requested by the site's IRB must be provided to the ESC staff for review and approval prior to resubmission to the IRB.

The ESC office must receive the following trial specific documents either by hardcopy or email before a site can be activated for any trial. All corresponding updates to these documents are required to be submitted to the ESC office throughout the trial:

- IRB Approval Letter that includes the protocol version and date
- FDA Form 1572 Protocol Signature Page
- Investigator Brochure (or Package Insert) Signature Page(s)
- IRB Approved Consent Form
- Site Delegation of Authority Log
- Signed Financial Interest Disclosure Forms (For all individuals listed on the 1572)
- Investigator/Personnel documents (CVs, licenses, GCP and HSP training certificates, etc.) as needed
- Laboratory Documents (certifications, normal ranges, etc.) as needed
- Signed Clinical Trial Agreement
- Protocol specific documentation as needed

A study initiation teleconference will be held prior to the start of any study related activity at the site. Attendance is required for:

The site PI and appropriate research staff

Moffitt PI and ESC Coordinator

The requirements of the protocol and all associated procedures and processes will be reviewed and agreed upon prior to the activation of the study. The ESC utilizes the EDC system, OnCore. OnCore training will be scheduled, if indicated, with the appropriate staff from the site.

External sites are required to send updated documentation to the ESC Office at ESC_Partnerships@Moffitt.org within 10 business days of updating.

SAE Reporting Special Language for External Sites

Information about all serious adverse events will be collected and recorded. To ensure patient safety, each serious adverse event must be reported to the PI and to the sponsor expeditiously. Moffitt Cancer Center and all participating sites will report SAEs by completing an SAE report in OnCore, the electronic data capture system. The SAE Report from OnCore must be signed and reported by email (ESC_Partnerships@moffitt.org) to the External Site Coordination (ESC) office within 2 working days. If applicable, the site should also follow protocol guidelines for additional reporting to Financial Sponsors and government agencies.

External Site Monitoring and Reporting

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management. All monitoring efforts will occur based on the protocol specific monitoring plan. Following each monitoring visit, a monitoring follow-up report will be provided to the Participating Site (i.e. Site PI and Coordinator). The monitoring report will summarize any issued queries or data clarification requests, identify any reportable events or required follow-up on prior events and will specify details of any non-compliance. Participating Sites are requested to respond to all queries and data clarifications requests within 20 business days. The Moffitt Cancer Center Protocol Monitoring Committee will review all monitoring reports and issue resolution. This Committee reserves the right to close accrual for non-compliance to monitoring.

VII. Ethics

This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines, including parts 50 and 56 concerning informed consent and IRB regulations) applicable government regulations and Institutional research policies and procedures.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject undergoes any study procedure.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. IRB approval will be secured prior to commencement of this study.

VIII. Publication policy

a. The primary investigator is responsible for analyzing the data and drafting a manuscript for publication.

b. Secondary investigators will offer critical review of this manuscript and therefore will be cited as secondary authors.

IX. Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities, including the National Heart, Lung, and Blood Institute (NHLBI), are notified.

X. References:

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XI. APPENDICES

Appendix A: Acute GVHD grading system

Table 1 Recommended staging and grading of acute GVHD

	Extent of organ involvement		
	Skin	*Liver*	*Gut*
Stage			
1	Rash on <25% of skin[a]	Bilirubin 2–3 mg/dl[b]	Diarrhea >500 ml/day[c] or persistent nausea[d]
2	Rash on 25–50% of skin	Bilirubin 3–6 mg/dl	Diarrhea >1000 ml/day
3	Rash on >50% of skin	Bilirubin 6–15 mg/dl	Diarrhea >1500 ml/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe abdominal pain with or without ileus
Grade[e]			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	–	Stage 2–3 or	Stage 2–4
IV[f]	Stage 4 or	Stage 4	–

[a]Use 'Rule of Nines' or burn chart to determine extent of rash
[b]Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented
[c]Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Gut staging criteria for pediatric patients was not discussed at the Consensus Conference. Downgrade one stage if an additional cause of diarrhea has been documented.
[d]Persistent nausea with histologic evidence of GVHD in the stomach or duodenum
[e]Criteria for grading given as minimum degree of organ involvement required to confer that grade
[f]Grade IV may also include lesser organ involvement but with extreme decrease in performance status

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1994 Consensus Conference on Acute GVHD Grading.

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Appendix B: comorbid conditions

i. Comorbidity score per Sorror, et al.

Comorbidity	Definition of comorbidities included in the new HCT-CI	HCT-CI weighted scores
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or venricular arrhythmias	1
Cardiac	Coronary artery disease, congestive heart failure, myocardial infarction, or EF <50%	1
Inflammatory bowel diseases	Crohn disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Depression or anxiety requiring psychiatric consult or treatment	1
Hepatic, mild	Chronic hepatitis, bilirubin > ULN to 1.5 X ULN, or AST/ALT > ULN to 2.5 X ULN	1
Obesity	Patients with a body mass index >35 kg/m2	1
Infection	Requiring continuation of antimicrobial treatment after day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Moderate/severe renal	Serum creatinine >2 mg/dL, on dialysis, or prior renal trnasplant	2
Moderate pulmonary	Adjusted DLCO and/or FEV1 66%=80% or dyspnea on slight activity	2
Prior solid tumor	Treated at any time point in the patient's past medical history, excluding nonmelanoma skin cancer	3
Heart valve disease	Except mitral valve prolapse	3
Severe pulmonary	Adjusted DLCO and/or FEV1 <65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 X ULN, or AST/ALT > 2.5 X ULN	3

*composite score obtained by summing total points
*psychiatric disturbance: only those with psychiatric disturbance (such as depression and/or anxiety) requiring therapy or treatment will be assigned 1 point on this measure.
*modification made to "pulmonary" co-morbidity: given marked discrepancy in frequency distribution of reduced DLCO in analysis of 59 contemporary patients from our center compared to published standards, decrement in DLCO alone will not factor into calculation of "pulmonary" comorbidity. Only those with DLCO < 50% will be considered to have "severe pulmonary" comorbidity and be excluded from the trial.

Appendix C: anticipated transplant related toxicities

TABLE 4. EXPECTED TRANSPLANT/GVHD-RELATED TOXICITIES IN HSC TRANSPLANT PATIENTS.

Category	Symptoms/Event	Category	Symptom/Event
Dermatological	• Pruritus • Rash/dermatitis associated with high-dose chemotherapy or GVHD	**Renal/Genitourinary**	• Bladder spasms • Creatinine elevation • Dysuria • frequency-urgency • Urinary retention
Constitutional	• Fever, Rigors, Chills	**Reproductive**	• Persistent amenorrhea
Gastrointestinal	• Gastritis • Colitis • Dehydration Diarrhea associated with GVHD • Nausea/vomiting • Dysphagia • Stomatitis/pharyngitis • Hematocheza/melena • Abdominal pain or cramping	**Metabolic/Laboratory**	• Acidosis/alkalosis • Amylase/lipase elevation • Hypo-/Hyperglycemia • Hypo-/Hyperkalemia • Hypo-/Hypermagnesemia, Hypophosphatemia • Jypo-/Hypernatremia • Hypertriglyceridemia • Hyperuricemia
Hepatic	• Lft elevation • Bilirubin elevation • Hypoalbuminemia • Hepatomegaly	**BMTComplex/ Multicomponent Events**	• GVHD • Stem-cell infusion complications • Venoocclusive Disease
Cardiovascular	• Edema • Hypertension/Hypotension • Sinus tachycardia	**Hematologic**	• Cytopenias • Catheter associated venous thrombosis
Infections	• Bacterial, fungal and/or viral infections • Sepsis		

BMT = Bone marrow transplant; LFT = Liver function test.

Appendix D: Study Calendar

Assessment	Day -45 to -5 (Screening period)*	Day 0	Day +7	Day +14	Day +21	Day +30	Day +60	Day +69	Day +83	Day +100	Day +130
2-D Echocardiogram	X					X				X	
History & Physical, CBC with diff, liver function tests, and comprehensive metabolic panel	X	X	X	X	X	X	X			X	X
Disease status	X					X				X	X
PFT, hepatitis B, C, HIV	X										
EKG	X	X				X	X				
TT, PT, PTT	X					X	X				
Acute GVHD scoring[38] (GVHD will be assessed and scored at least weekly per standard practice, and data will be captured at the defined intervals: days 0-+30, +31-60, +61-+100, and +101-+130)						X	X			X	X
Research phone call to instruct patient to taper study drug**								X	X		
8 green top tubes (~5.5 tablespoons) of peripheral blood for T cell subset and STAT3/STAT5/S6/H3ser10 studies[1]	X				X					X	
IDP-Treg panel (flow cytometry)	X				X					X	
Phase I trial only assessments:***											
PK monitoring		X			X						
EKG		X			X						

PAC/SIR/TAC trial monitoring and interval assessments

A 3-day grace period is allowed for each study item from day-45 to +30, and +14 days for all points thereafter.

***Procedures (routine pre-HCT vital organ testing) done prior to informed consent signing can be used for eligibility determination within the stated 45 day window**

**** for research phone call to instruct patient on taper, a +/- 3 day window is provided.**

*****for phase I assessments of timed PK samples and post-dose EKG, a +/- 1 hour window is provided.**

[1]**Day +21 and Day +100 samples will be collected on patients who partially withdraw consent**

As detailed in the protocol text, patients in the phase I component of the trial will also complete day 0 and day 21 serial blood samples for PK monitoring of pacritinib, and will also have EKG monitoring. Patients in the phase I component will otherwise also complete all the other assessments outlined in this study calendar.