A Phase II Trial Evaluating the use of a Histone Deacetylase Inhibitor Panobinostat for graft versus host disease (GVHD) prevention

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List of abbreviations

AE	adverse event
ALT	alanine aminotransferase/glutamic pyruvic transaminase/GPT
ANC	absolute neutrophil count
AST	aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
AUC	area under the curve
BUN	blood urea nitrogen
Cmax	maximum concentration of drug
CNS	central nervous system
CR	complete response/remission
CS&E	clinical safety and epidemiology
CTCAE	NCI common terminology criteria for adverse events (version 4.0)
CV	coefficient of variation
DLT	dose-limiting toxicity
DNA	deoxyribose nucleic acid
EKG	12 lead electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eRT	eResearchTechnology
FDA	food and drug administration
G-CSF	granulocyte colony-stimulating factor (e.g. filgrastim)
GM-CSF	granulocyte-macrophage colony-stimulating factor (e.g. sargramostim)
H3, H4	histones H3, H4
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
i.v.	intravenous(ly)
IEC	independent ethics committee
IRB	institutional review board
LLN	lower limit of normal
LVEF	left ventricular ejection fraction
mg/m2	milligrams per square meter
MTD	maximum tolerated dose
MUGA	multiple uptake gated acquisition scan
MWF	monday, wednesday, friday
NIH	national institutes of health
PANO	panobinostat
PD	pharmacodynamic
P-gp	p-glycoprotein
PK	pharmacokinetic
PLT	Platelet
PR	partial response
REB	research ethics board

SAE	serious adverse event
SAHA	suberoylanilide hydroxamic acid
SOP	standard operating procedure
T4	Thyroxine
TSH	thyroid stimulating hormone
ULN	upper limit of normal
WBC	white blood cell
WNL	within normal limits
WOCBP	women of childbearing potential
a-GVHD	Acute Graft versus Host Disease
SR/D-GVHD	steroid-refractory/dependent GVHD

1. Background and Rationale

1.1. Overview of Allogeneic hematopoietic cell transplantation (HCT) and Graft Versus Host Disease (GVHD) prevention

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment modality for many patients with hematological malignancies, solid tumors and acquired or congenital non-malignant disorders. Despite recent advances in the understanding of transplantation immune tolerance, graft versus host disease (GVHD) remains a principal obstacle to achieve successful outcomes. Serious infections and impairment of generalized immune dysfunction are responsible for GVHD-associated mortality. GVHD incidence and severity depends primarily on donor and recipient matching for human leukocyte antigens (HLA) and the regimen used for post-grafting immune suppression. The NIH consensus development project working group recognized 2 main categories of GVHD, each with 2 subcategories. The acute GVHD (aGVHD) category is defined in the absence of diagnostic or distinctive features of chronic GVHD and includes (1) classic acute GVHD occurring within 100 days after transplantation and (2) persistent, recurrent, or late acute GVHD (features of acute GVHD occurring beyond 100 days, often during withdrawal of immune suppression). The broad category of chronic GVHD (c-GVHD) includes (1) classic chronic GVHD (without features or characteristics of acute GVHD) and (2) an overlap syndrome in which diagnostic or distinctive features of chronic GVHD and acute GVHD appear together.[1]

Identification of novel agents that can prevent GVHD without compromising graft versus tumor (GVT) effect is essential. Ideal GVHD prevention requires a strategy that will generate donor immune tolerance to recipient allo-antigens while preserving host immune reconstitution and reactivity against tumor and infectious agents. GVHD prevention has largely been based on the use of pharmacological agents, and to a lesser degree on depletion of T cells from the bone marrow (BM) or blood (PBSC) hematopoietic stem cell graft. Randomized phase III clinical studies have shown that the combination of tacrolimus (TAC) and methotrexate (MTX) is more effective in preventing GVHD compared to cyclosporine (CsA) and MTX following matched related donor (MRD) transplant [2]. A Phase III, multicenter, controlled trial comparing MTX and TAC with MTX and CsA for acute GVHD prophylaxis after marrow transplantation from matched unrelated donors (MUD), showed that there was a significant trend toward decreased severity of acute GVHD with TAC/MTX and established this regimen as state-of the-art for GVHD prophylaxis for MUD transplants [3].

Sirolimus (SIR), or rapamycin, is an immunosuppressive medication with potential for improving outcomes in allogeneic HCT with dual benefit of both immune suppression and anti-malignancy effect. SIR produces at least partial blockade of CD28 mediated co-stimulatory signaling and is permissive for CD4+CD25+ regulatory T cell (Treg) expansion, proliferation, and survival. SIR has also been shown to inhibit differentiation

of naïve CD4 T cells to Th17 cells, cells involved in GVHD pathophysiology creating an immune tolerant environment. We completed a randomized Phase II trial of TAC and SIR vs. TAC and MTX as GVHD prophylaxis after allogeneic transplantation.[4] The 100-day cumulative incidence of grade 2-4 acute GVHD for SIR was 43% (95% CI 30-63%), and 89% (95% CI 80-100%) for MTX, p<0.0001. Grade 3-4 acute GVHD for SIR was 16% (95% CI 7-36%) and 13% (95% CI 5-33%) for MTX, p=0.16. The incidence of any grade chronic GVHD for SIR was 51% (95% CI 34-78%) and 67% (95% CI 52-85%) for MTX, p=0.56. The cumulative incidence of moderate to severe chronic GVHD was 20% (95% CI 9-43%) following SIR, and 63% (95% CI 47-83%) for MTX, p=0.013. Median percent Tregs among blood CD4 T cells at day 30 was 16.3 (range 12.5-17.9) for SIR versus 9.9 (8.6-13.5) for MTX, p < 0.0001, and 14.6 (10.8-18.1) for SIR and 9.7 (7.5-11.6) for MTX at day 90 post-HCT, p = 0.0009. SIR-treated patients had increased absolute numbers of Treg, and decreased absolute numbers of non-Treg CD4+ cells on days 30 and 90 after HCT supporting the hypothesis [4].

A Phase III, randomized, multicenter, open label clinical trial recently published compared TAC/SIR versus TAC/MTX as GVHD prophylaxis using peripheral blood stem cells (PBSC) in MRD transplants only. The primary objective was comparison of day+114 grade II-IV acute GVHD free survival between patients in the two arms from the time of randomization using intent-to treat analysis (NCT00406393)**.** The cumulative incidence of grade II-IV acute GVHD at 114 days was 34% in the TAC/MTX group and 26 % in the TAC/SIR group. Grade III-IV acute GVHD was 15% in TAC/MTX group, and 8 % in TAC/SIR group with a rate of chronic GVHD of 43% (TAC/MTX) compared to 54% (TAC/SIR). Overall survival did not differ (60% vs. 61%, *p NS*).[5]

New immunosuppressive agents and strategies are required for GVHD prevention as it might improve survival after allogeneic HCT. In this protocol we will build upon prior experience using full intensity conditioning regimen by adding panobinostat (PANO) to TAC/SIR GVHD prevention backbone in order to investigate whether we can achieve further reductions in the incidence and severity of acute GVHD while maintaining a graft versus tumor effect.

1.2. Overview of Histone deacetylase inhibitors immunomodulation and their role in a-GVHD

Histones are part of the core proteins of nucleosomes, and acetylation and deacetylation of these proteins play a role in the regulation of gene expression. Highly charged deacetylated histones bind tightly to the phosphate backbone of DNA, inhibiting transcription, presumably, by limiting access of transcription factors and RNA polymerases to DNA. Acetylation neutralizes the charge of histones and generates a more open DNA conformation. This conformation allows transcription factors and associated transcription apparatuses access to the DNA, promoting expression of the corresponding genes. The opposing activities of two groups of enzymes, histone acetyltransferases (HATs) and Histone protein deacetylases (HDACs) control the amount of acetylation. HDACs regulate chromatin remodeling and gene expression as well as the functions of

more than 50 transcriptions factors and non-histone proteins such as P53. HATs acetylate and HDACs deacetylate, ε-acetylcystein residues of the histone tails. HATs generally increase accessibility and gene transcription, whereas HDACs dampen histone-DNA and histone/non-histone interactions. In normal cells a balance exists between HAT and HDAC activity that leads to cell specific patterns of gene expression. Interruption of this balance produces changes in gene expression. Several lines of evidence suggest that aberrant recruitment of HDAC and the resulting modification of chromatin structure may play a role in changes in gene expression seen in cancer cells suggesting that HDAC inhibitors (HDACi) may provide a novel approach to treat malignancies. [6] Silencing of tumor suppressor genes at the level of chromatin is common in human tumors[7,8 9 10 11 12] and HDAC complexes have been shown to be crucial to the activity of the AML-specific fusion proteins PLZF-RAR-α, PML-RAR-α, and AML1/ETO.[13 14 15 16] HDACi have been shown to induce differentiation, cell cycle arrest or apoptosis in cultured tumor cells, and to inhibit the growth of tumors in animal models.[17,18 19 20 21 22 23]Tumor growth inhibition and apoptosis in response to HDACi treatment may also be mediated through changes in acetylation of non-histone proteins (e.g. HSP90, p53, HIF-1α, α-tubulin).[24 25 26] Antitumor activity has been reported in preclinical studies with valproic acid, PXD101 and MGCD0103 and in clinical studies with other HDACi, including intravenous (IV) depsipeptide and oral MS-275.[27] HDACi suberoylanilide hydroxamic acid (SAHA) has been shown to have activity in cutaneous T cell lymphoma (CTCL), diffuse large cell lymphoma, and head and neck cancer.

HDACi are very attractive molecules in the allogeneic transplant setting since they have a dual effect, anti-tumor activity and immune modulatory effects. PANO is one of most potent HDACi known to date in clinical development by Novartis. PANO effects are nonselective, targeting HDACs classes I (HDAC1, 2, 3, 8), class IIA (HDAC4,5,7,9), class IIb (HDAC6,10), class III (SIRT1-7), and class IV (HDAC11). The clinical development program for PANO includes Phase III clinical trial in multiple myeloma[28], early-stage trials in acute myeloid leukemia and myelodysplastic syndromes and for advanced Hodgkin lymphoma or as maintenance therapy following autologous stem cell transplant in this disease.

1.3. HDACi immune-modulatory properties and GVHD role:

A preclinical study demonstrates that PANO significantly impairs the phenotype and function of dendritic cells (DCs) to stimulate antigen-specific immune responses and represses the production of pro-inflamatory cytokines (IL-6, IL-10, IL-12p70, IL-23 and TNF-α).[6,29-31] Deacetylase inhibition promotes the generation and function of regulatory T cells [32]. Preclinical studies in mice have shown that vorinonstat resulted in protection of GVHD with moderate efficacy without impairing GVL activity [29,30]. Furthermore, Choi et al studied the safety and activity of vorinostat, in combination with standard immunoprophylaxis, for prevention of GVHD in patients undergoing related-donor reduced-intensity conditioning HCT (NCT00810602). All patients (n=50) received a conditioning regimen of fludarabine (40 mg/m2 daily for 4 days) and busulfan (3·2 mg/kg daily for 2 days) and GVHD immunoprophylaxis of mycophenolate mofetil (1 g three

times a day, days 0—28) and tacrolimus (0·03 mg/kg a day, titrated to a goal level of 8—12 ng/mL, starting day −3 until day 180). Vorinostat (either100 mg or 200 mg, twice a day) was initiated 10 days before HCT until day 100. The cumulative incidence of grade 2-4 acute GVHD by day 100 was 22% (95% CI 13—36). The most common non-hematological adverse events included electrolyte disturbances (n=15), hyperglycemia (n=11), infections (n=6), mucositis (n=4), and increased activity of liver enzymes (n=3). Non-symptomatic thrombocytopenia after engraftment was the most common hematological grade 3-4 adverse event (n=9) but was transient and all cases resolved swiftly. Acetylation of H3 and H4 was increased in PBMC in association with decreased TNF-alpha by intracellular staining. Total number of T cells, CD4+ and CD8+ cells were not increased although the number of regulatory T cells was increased in association to enhanced FOX-P3 and IDO expression by RT-PCR. In summary, administration of vorinostat proved safe and was associated with a lower than expected incidence of severe acute GVHD establishing HDACi role in GVHD prevention in patients undergoing related-donor reduced-intensity conditioning HCT [33] which is a less stringent setting compared to the one that we proposed in this study (full intensity conditioning and matched unrelated donors).

1.4. Panobinostat use in allogeneic HCT setting:

Pan-HDACi in allogeneic BMT may have different effects and encourages investigations to study the function of individual HDAC members which are not elucidated to date. PANO tested in murine models accelerated GVHD compared to vorinostat which was unexpected but has not translated in humans in 2 ongoing clinical trials discussed herein. Pathological changes in mice showed increased liver damage without significant changes in colon or small intestine. Changes were associated with increased systemic Th1 cytokines, enhanced CXCR3 expression on donor $CD4^+$ and $CD8^+$ T cells, and T cell infiltration in the liver [34].

In parallel to the development of murine models, we have tested the safety of PANO administered to patients with acute GVHD within 72 hours of initiation of glucocorticoid therapy (methylprednisolone 0.8 mg/Kg/day IV or equivalent for at least 14 days) as first line therapy. As of November 2014, We have enrolled 20 subjects, median age 53 years (range, 34-76), male (n=13)/female (n=7), white(n=15)/hispanic(n=5); with diagnosis of CLL (n=2), MDS (n=2), Myeloma (n=1), Follicular NHL (n=1), CML(n=1), Myelofibrois (n=3), AML (n=6), MDS/CMML (n=3) or ALL(n=1). Conditioning regimens included Busulfan(BU)/fludarabine(FLU) AUC 5300 (n=10) or AUC 3500 (n=3), FLU/Melphalan (n=5) or Pentostatin/BU (n=2); and GVHD prophylaxis for MUD 8/8 (n=11) or MRD (n=5) HCT with TAC/MTX (n=7), TAC/rapamycin(n=7)(sirolimus-SIR), TAC/MMF(n=3) and for mismatched transplants with either TAC/SIR/ATG (n=2) or TAC/MTX/ATG (n=1). Median day of acute GVHD (n=19) onset was day + 37 post HCT (26 -100 days) with overall grade GVHD II (n=) or III (n=6); and median day of acute symptoms in overlap GVHD patients (n=5) was day + 528 (109-981). All Patients were treated with voriconazole (n=14) or micafungin (n=5) for fungal prophylaxis. For the first four patients Panobinostat was administered intravenously (IV) weekly x 4 at 2.5MG/M2 (n=3) or 5MG/M2 IV (n=1) with all 4 achieving either CR (n=3) or PR (n=1) GVHD responses by day +15 of Panobinostat. Due to manufacturer discontinuation of IV

formulation, the protocol was amended to use PO Panobinostat. Using 10mg PO TIW 3 doses q week x 4 weeks, we treated 2 subjects which were both discontinued from study drug due to presumed GHVD progression within 7 days of Panobinostat (after 3-4 doses). First subject had grade II GVHD (skin stage 3, gut stage 1 and liver stage 0) that progress in gut and skin; second subject with grade II GVHD (skin stage 3, gut stage 1, liver stage 1) with LFTs worsening ultimately evolving into VOD. Due to safety concerns next subjects were treated with 5 mg PO TIW 3 doses q week x 4 weeks, dose that was determined to be the maximal tolerated dose (MTD) after 6 patients completed therapy in phase I. Currently we are enrolling in phase II portion (n=8). GVHD response rate among MTD treated was complete in 86% (n=12), partial in 7.6% (n=1) or progressive in 7.6% (n=1) by day +36 after Panobinostat with majority achieving responses by day +21. Chronic GVHD at day +365 in evaluable patients (n=6) was none (n=3) or mild (n=3) and steroid was discontinued at a median of 3 months (3-6). Hematological toxicities in evaluable patients (n=14) were mild with worsening of prior thrombocytopenia (n=8/10), anemia (n=3/10) and leukopenia (n=3/10) and returned to baseline within 1-2 weeks; LFTs deterioration (n=1) within 1 week of Panobinostat in a GVHD stage 3 liver/3 skin patient; pericarditis/cardiogenic shock CTCAE(v4) 5 of unclear etiology (n=1); worsening thyroid function (n=1) and hypercholesterolemia (n=1). Preliminary correlative studies in MTD treated patients showed that CD4 and CD8 numbers remained stable during treatment. T regulatory cells numbers decreased at day +8 after Panobinostat and recovered by days +15 and +29 of treatment. Level of T regs inducing cytokines (TGFB and IL-10) increased, possibly contributing to an immune-modulatory environment. There is evidence of an increased in acetylation of histone 3 in CD4, CD8 and monocytes subsets over time. We are encouraged with tolerability of level -1 Panobinostat dose and the high GVHD response rate of 86% which may compare favorably to the historical GVHD response rate. These results suggest a potential role for Panobinostat as a tool to improve success of glucocorticoids for acute GVHD treatment[35].

Phase I/II Study with oral PANO maintenance therapy following allogeneic HCT in patients with MDS or AML (PANOBEST) (NCT01451268) has been reported by Dr. Bug et al [36]. PANO was started at a median of 73 days (range, 60-126) after HCT, for patients with active disease (n=11) or in CR2 (n=1) in 12 patients (11 AML, 1 MDS), median age of 52 years (range, 21-62). The MTD was determined to be 20 mg TIW based on one DLT (fatigue grade 3) at 20 mg and two DLTs (nausea/emesis and colitis grade 3 each) at 30 mg. Grade 2-4 adverse events (AEs) were reported in 83%. Grade 3/4 AEs included hematologic toxicity (50%), laboratory alterations (33%), gastrointestinal symptoms (25%), fatigue, pulmonary infection (17% each), sepsis, herpes stomatitis, diabetes, syncope, deep vein thrombosis and pulmonary embolism (8% each). Toxicity was reversible and required at least one PANO dose reduction in 3 patients. As of ASH 2013, acute GVHD was reported in few patients (grade 2 (n=1) and 3 (n=2) responsive to steroids (n=2) or salvage therapy (n=1). Four patients developed mild (n=3) or moderate (n=1) chronic GVHD. With a median follow up of 579 days (range, 129-911), 11/12 patients are alive and 10/12 in continuous CR after HCT. Immunophenotyping revealed no impact of PANO on absolute T reg numbers (n=9), but a significantly reduced proportion of $CD4^{+}CD25^{++}CD127^{dim/-}$ T reg to $CD3^{+}CD4^{+}$ T helper (Th) cells by day 8 after 3 doses of PANO (mean±SEM: 14.6±2.6 vs. 9.6±1.2%, p value of t test =0.03). PANO was well tolerated with low relapse rate in this high risk population in association with modulation of the T reg/Th proportion. A Phase II Study using PANO as Second-Line Therapy in Patients with Chronic GVHD is reported at clinicaltrial.gov

(NCT01028313). To date, two clinical protocols reporting the use of PANO in the allogeneic HCT patients have proven to be safe and may provide GVHD control with additive function to target MRD.

Summary of trial rationale: The burden of acute GVHD that remains despite current preventive strategies makes clear the rationale for novel approaches. Our clinical results demonstrate reduction in acute GVHD, suppression of non-Treg CD4 cells, and significantly improved Treg reconstitution with SIR/TAC over MTX/TAC acute GVHD prophylaxis. However, prevention is not complete. PANO is a potent and versatile inhibitor that targets various proteins in the cells and has been shown to be active in vorinostat resistant cancers which is an advantage. This protocol will test the hypothesis that addition of PANO to TAC/SIR may abolish GVHD in matched related or unrelated HCT while sparing GVL effect. Accordingly, the combination of SIR-based immune suppression and PANO may more effectively facilitate Treg differentiation, may inhibit the differentiation of pathogenic Th1 and Th17 cells, and more effectively prevent acute GVHD based on known biological activity and role in GVHD treatment while potentially targeting minimal residual disease.

We will test this hypothesis in a single arm phase II clinical trial testing potential reduction of acute GVHD incidence, from 50% to 30%, with screening intent to discern biologic and potential clinical activity of PANO worthy of a larger randomized trial. HDACs are a diverse family of enzymes acting on various substrates and HDACi mechanism in GVHD remains unknown to date. We hypothesize that PANO has a higher efficacy in preventing GVHD while sparing GVL effect. The findings of this study should indicate whether the effect noted with vorinostat is shared by additional non-selective HDACi and/or if have a superior efficacy or fewer toxic effects and/or adverse effects in patients receiving a full-intensity conditioning regimen using matched unrelated/related donors.[37]

1.5.Panobinostat Clinical experience

Clinical development of PANO focuses on the oral formulation. The clinical program for the i.v. formulation is completed with no further company-sponsored studies currently planned. As of 31 December 2013, 36 clinical studies, including clinical pharmacology (CP), Phase I and Phase II trials, as well as two randomized Phase III studies have either been completed or are ongoing. A total of 2428 patients were enrolled, 235 for i.v. and 2193 for oral, who received at least one dose of PANO either as a single agent or in combination with other agents.

Patients were treated with PANO either TIW QW (666 patients) or TIW QOW (96 patients) in single agent oral PANO clinical trials. These patients comprise the pooled safety population experiencing AEs during study treatment. The most frequent non-hematologic toxicity included GI events (diarrhea, nausea, vomiting), mostly of Grade 1-2, in both groups. Blood and lymphatic system disorders were the second most often reported specific system organ class, with dose-related thrombocytopenia being the most frequent AE. Fatigue, mostly Grade 1-2, was also common among patients treated for TIW QW and TIW QOW.

Thyroid function, as monitored by the measurement of TSH and free T4, did not reveal overt hyper- or hypo- thyroidism, with fluctuations in TSH values being within normal limits.

Table 1 All grade adverse events regardless of causality in patients receiving oral panobinostat three-times-a-week every-week (TIW QW)

Primary system organ class Preferred term	**20 mg (N=309 n %**	**30 mg (N=81) n %**	**40 mg (N=163) n %**	**60 mg (N=113) n %**	**TOTAL (N=666) n %**
-Any primary system organ class					
-Total	307 (99.4)	81 (100.0)	163(100.0)	113(100.0)	664 (99.7)
Blood and lymphatic system disorders					
-Total	193 (62.5)	50 (61.7)	145 (89.0)	87 (77.0)	475 (71.3)
Anaemia	63 (20.4)	32 (39.5)	65 (39.9)	35 (31.0)	195 (29.3)
Febrile neutropenia	9 (2.9)	0 (0.0)	7 (4.3)	33 (29.2)	49 (7.4)
Leukopenia	19 (6.1)	1(1.2)	18 (11.0)	4 (3.5)	42 (6.3)
Neutropenia	53 (17.2)	4 (4.9)	48 (29.4)	29 (25.7)	134 (20.1)
Thrombocytopenia	133 (43.0)	31(38.3)	137(84.0)	58 (51.3)	359 (53.9)
Endocrine disorders					
-Total	21 (6.8)	7 (8.6)	27 (16.6)	9 (8.0)	64 (9.6)
Hypothyroidism	15 (4.9)	3 (3.7)	21 (12.9)	4 (3.5)	43 (6.5)
Gastrointestinal disorders					
-Total	246 (79.6)	76 (93.8)	152 (93.3)	107(94.7)	581(87.2)
Abdominal pain	33 (10.7)	11 (13.6)	30 (18.4)	23 (20.4)	97 (14.6)
Abdominal pain upper	26 (8.4)	5 (6.2)	24 (14.7)	9(8.0)	64(9.6)
Constipation	46 (14.9)	15 (18.5)	32 (19.6)	24 (21.2)	117(17.6)
Diarrhoea	158 (51.1)	46 (56.8)	117 (71.8)	85 (75.2)	406 (61.0)
Dry mouth	17 (5.5)	6 (7.4)	22 (13.5)	9 (8.0)	54 (8.1)
Nausea	142(46.0)	51(63.0)	112(68.7)	67(59.3)	372(55.9)
Vomiting	68(22.0)	33(40.7)	73(44.8)	47(41.6)	221(33.2)
General disorders and administration site conditions					
-Total	229(74.1)	70(86.4)	137(84.0)	98(86.7)	534(80.2)
Asthenia	52(16.8)	6(7.4)	29(17.8)	21(18.6)	108(16.2)
Chills	19(6.1)	4(4.9)	13(8.0)	17(15.0)	53(8.0)
Fatigue	126(40.8)	56(69.1)	85(52.1)	59(52.2)	326(48.9)
Oedema peripheral	62(20.1)	21(25.9)	30(18.4)	24(21.2)	137(20.6)
Pyrexia	61(19.7)	18(22.2)	67(41.1)	47(41.6)	193(29.0)

Primary system organ class **Preferred term**	**20 mg (N=309)** **n %**	**30 mg (N=81)** **n %**	**40 mg (N=163)** **n %**	**60 mg (N=113)** **n %**	**TOTAL (N=666)** **n %**
Infections and infestations					
-Total	152(49.2)	40(49.4)	101(62.0)	76(67.3)	369(55.4)
Sepsis	6(1.9)	3(3.7)	4(2.5)	13(11.5)	26(3.9)
Upper respiratory tract infection	12(3.9)	5(6.2)	24(14.7)	4(3.5)	45(6.8)
Urinary tract infection	23(7.4)	11(13.6)	10(6.1)	7(6.2)	51(7.7)
Investigations					
-Total	143(46.3)	40(49.4)	71(43.6)	57(50.4)	311(46.7)
Blood creatinine increased	37(12.0)	7(8.6)	13(8.0)	13(11.5)	70(10.5)
Platelet count decreased	13(4.2)	14(17.3)	4(2.5)	7(6.2)	38(5.7)
Weight decreased	44(14.2)	12(14.8)	30(18.4)	22(19.5)	108(16.2)
Metabolism and nutrition disorders					
-Total	179(57.9)	56(69.1)	92(56.4)	83(73.5)	410(61.6)
Anorexia	45(14.6)	10(12.3)	16(9.8)	24(21.2)	95(14.3)
Decreased appetite	49(15.9)	30(37.0)	49(30.1)	31(27.4)	159(23.9)
Dehydration	12(3.9)	12(14.8)	13(8.0)	19(16.8)	56(8.4)
Hypocalcaemia	22(7.1)	3(3.7)	8(4.9)	21(18.6)	54(8.1)
Hypokalaemia	33(10.7)	11(13.6)	28(17.2)	35(31.0)	107(16.1)
Hypophosphataemia	25(8.1)	1(1.2)	9(5.5)	14(12.4)	49(7.4)
Musculoskeletal and connective tissue disorders					
-Total	114(36.9)	29(35.8)	90(55.2)	43(38.1)	276(41.4)
Back pain	35(11.3)	8(9.9)	30(18.4)	13(11.5)	86(12.9)
Muscle spasms	19(6.1)	6(7.4)	32(19.6)	2(1.8)	59(8.9)
Myalgia	16(5.2)	1(1.2)	17(10.4)	2(1.8)	36(5.4)
Nervous system disorders					
-Total	147(47.6)	36(44.4)	86(52.8)	52(46.0)	321(48.2)
Dizziness	42(13.6)	14(17.3)	15(9.2)	10(8.8)	81(12.2)
Dysgeusia	44(14.2)	13(16.0)	28(17.2)	22(19.5)	107(16.1)
Headache	49(15.9)	14(17.3)	34(20.9)	16(14.2)	113(17.0)
Psychiatric disorders					
-Total	65(21.0)	17(21.0)	43(26.4)	38(33.6)	163(24.5)
Anxiety	12(3.9)	3(3.7)	18(11.0)	9(8.0)	42(6.3)
Insomnia	20(6.5)	10(12.3)	14(8.6)	19(16.8)	63(9.5)
Respiratory, thoracic and mediastinal disorders					
-Total	117(37.9)	42(51.9)	97(59.5)	53(46.9)	309(46.4)

Primary system organ class **Preferred term**	**20 mg (N=309** **n %**	**30 mg (N=81)** **n %**	**40 mg (N=163)** **n %**	**60 mg (N=113)** **n %**	**TOTAL (N=666)** **n %**
Cough	30(9.7)	11(13.6)	48(29.4)	19(16.8)	108(16.2)
Dyspnoea	49(15.9)	28(34.6)	32(19.6)	20(17.7)	129(19.4)
Epistaxis	16(5.2)	3(3.7)	22(13.5)	20(17.7)	61(9.2)
Skin and subcutaneous tissue disorders					
-Total	147(47.6)	21(25.9)	73(44.8)	38(33.6)	279(41.9)
Pruritus	56(18.1)	3(3.7)	22(13.5)	3(2.7)	84(12.6)
Vascular disorders					
-Total	61(19.7)	17(21.0)	26(16.0)	33(29.2)	137(20.6)
Hypotension	18(5.8)	4(4.9)	10(6.1)	18(15.9)	50(7.5)

Includes only events occurring in ≥10% of patients
A patient with multiple occurrences of an AE is counted only once in that AE category.
A patient with multiple adverse events within a primary system organ class is counted only once in the total row.
If an AE frequency matches the criteria in one dose category, the frequency of that event is shown for all doses.
Primary system organ classes are presented alphabetically; preferred terms are sorted within primary system organ class alphabetically.

As shown in Table 1 above, AEs regardless of causality for TIW QW dosing were reported in 664 patients, 99.7% of the safety population for this dosing schedule. The most commonly reported AEs across doses were gastrointestinal (GI), i.e., diarrhea in 406 patients (61.0%) and nausea in 372 patients (55.9%). Thrombocytopenia was the third most frequent AE in 359 patients (53.9%) with the highest frequency in the 40 mg dose level (137 patients; 84%). Fatigue also was commonly seen across dose levels in 326 patients (48.9%) overall. Of note hypothyroidism was reported in 12.9% of patients treated at the dose level of 40 mg, mostly deriving from study [CLBH589E2214] in HL patients who are known to have an increased risk for hypothyroidism.

Table 2 Grade 3-4 adverse events regardless of causality in patients receiving oral panobinostat three-times-a-week every-week (TIW QW)

Primary system organ class	20 mg	30 mg	40 mg	60 mg	Total
Preferred term	N=309	N=81	N=163	N=113	N=666
	n (%)	n (%)	n (%)	n (%)	n (%)
-Any primary system organ class					
-Total	**209(67.6)**	**70(86.4)**	**150(92.0)**	**105(92.9)**	**534(80.2)**
Blood and lymphatic system disorders					
-Total	**120(38.8)**	**26(32.1)**	**138(84.7)**	**84(74.3)**	**368(55.3)**
Anaemia	**29(9.4)**	**8(9.9)**	**35(21.5)**	**31(27.4)**	**103(15.5)**
Febrile neutropenia	**9(2.9)**	**0(0.0)**	**6(3.7)**	**31(27.4)**	**46(6.9)**
Leukocytosis	**4(1.3)**	**2(2.5)**	**0(0.0)**	**3(2.7)**	**9(1.4)**
Leukopenia	**2(0.6)**	**0(0.0)**	**11(6.7)**	**4(3.5)**	**17(2.6)**
Neutropenia	**42(13.6)**	**4(4.9)**	**39(23.9)**	**26(23.0)**	**111(16.7)**
Thrombocytopenia	**66(21.4)**	**21(25.9)**	**129(79.1)**	**56(49.6)**	**272(40.8)**
Gastrointestinal disorders					
-Total	**24(7.8)**	**20(24.7)**	**24(14.7)**	**31(27.4)**	**99(14.9)**
Abdominal pain	**5(1.6)**	**3(3.7)**	**7(4.3)**	**2(1.8)**	**17(2.6)**
Ascites	**0(0.0)**	**4(4.9)**	**0(0.0)**	**0(0.0)**	**4(0.6)**
Diarrhoea	**10(3.2)**	**6(7.4)**	**6(3.7)**	**18(15.9)**	**40(6.0)**
Nausea	**6(1.9)**	**6(7.4)**	**2(1.2)**	**7(6.2)**	**21(3.2)**
Vomiting	**4(1.3)**	**3(3.7)**	**4(2.5)**	**3(2.7)**	**14(2.1)**
General disorders and administration site conditions					
-Total	**35(11.3)**	**25(30.9)**	**38(23.3)**	**41(36.3)**	**139(20.9)**
Asthenia	**8(2.6)**	**2(2.5)**	**7(4.3)**	**10(8.8)**	**27(4.1)**
Fatigue	**13(4.2)**	**19(23.5)**	**26(16.0)**	**24(21.2)**	**82(12.3)**
General physical health deterioration	**6(1.9)**	**2(2.5)**	**0(0.0)**	**1(0.9)**	**9(1.4)**
Pain	**3(1.0)**	**0(0.0)**	**0(0.0)**	**3(2.7)**	**6(0.9)**
Pyrexia	**4(1.3)**	**1(1.2)**	**4(2.5)**	**2(1.8)**	**11(1.7)**
Hepatobiliary disorders					
-Total	**3(1.0)**	**3(3.7)**	**6(3.7)**	**3(2.7)**	**15(2.3)**
Hyperbilirubinaemia	**2(0.6)**	**0(0.0)**	**4(2.5)**	**2(1.8)**	**8(1.2)**
Infections and infestations					
-Total	**37(12.0)**	**12(14.8)**	**27(16.6)**	**49(43.4)**	**125(18.8)**

Bacterial infection	**0(0.0)**	**0(0.0)**	**1(0.6)**	**5(4.4)**	**6(0.9)**
Infection	**1(0.3)**	**2(2.5)**	**1(0.6)**	**1(0.9)**	**5(0.8)**
Pneumonia	**7(2.3)**	**2(2.5)**	**6(3.7)**	**6 (5.3)**	**21(3.2)**
Sepsis	**5(1.6)**	**3(3.7)**	**4(2.5)**	**9(8.0)**	**21(3.2)**
Septic shock	**2(0.6)**	**1(1.2)**	**1(0.6)**	**3(2.7)**	**7(1.1)**
Urinary tract infection	**1(0.3)**	**3(3.7)**	**0(0.0)**	**3(2.7)**	**7(1.1)**
Investigations					
-Total	**39(12.6)**	**14(17.3)**	**22(13.5)**	**26(23.0)**	**101(15.2)**
Alanine aminotransferase increased	**1(0.3)**	**1(1.2)**	**4(2.5)**	**1(0.9)**	**7(1.1)**
Aspartate aminotransferase increased	**1(0.3)**	**0(0.0)**	**5(3.1)**	**1(0.9)**	**7(1.1)**
Electrocardiogram qt prolonged	**6(1.9)**	**1(1.2)**	**0(0.0)**	**4(3.5)**	**11(1.7)**
Platelet count decreased	**8(2.6)**	**7(8.6)**	**3(1.8)**	**6(5.3)**	**24(3.6)**
Weight decreased	**2(0.6)**	**1(1.2)**	**4(2.5)**	**1(0.9)**	**8(1.2)**
Metabolism and nutrition disorders					
-Total	**42(13.6)**	**16(19.8)**	**27(16.6)**	**38(33.6)**	**123(18.5)**
Anorexia	**3(1.0)**	**1(1.2)**	**2(1.2)**	**5(4.4)**	**11(1.7)**
Decreased appetite	**2(0.6)**	**0(0.0)**	**5(3.1)**	**3(2.7)**	**10(1.5)**
Dehydration	**2(0.6)**	**4(4.9)**	**2(1.2)**	**5(4.4)**	**13(2.0)**
Failure to thrive	**1(0.3)**	**2(2.5)**	**0(0.0)**	**0(0.0)**	**3(0.5)**
Hyperglycaemia	**6(1.9)**	**1(1.2)**	**3(1.8)**	**5(4.4)**	**15(2.3)**
Hypoalbuminaemia	**1(0.3)**	**2(2.5)**	**1(0.6)**	**0(0.0)**	**4(0.6)**
Hypocalcaemia	**4(1.3)**	**0(0.0)**	**1(0.6)**	**5(4.4)**	**10(1.5)**
Hypokalaemia	**10(3.2)**	**3(3.7)**	**9(5.5)**	**15(13.3)**	**37(5.6)**
Hyponatraemia	**7(2.3)**	**3(3.7)**	**2(1.2)**	**2(1.8)**	**14(2.1)**
Hypophosphataemia	**7(2.3)**	**1(1.2)**	**5(3.1)**	**10(8.8)**	**23(3.5)**

Musculoskeletal and connective tissue disorders					
-Total	18(5.8)	2(2.5)	12(7.4)	7(6.2)	39(5.9)
Back pain	5(1.6)	1(1.2)	4(2.5)	2(1.8)	12(1.8)
Nervous system disorders					
-Total	24(7.8)	4(4.9)	16(9.8)	14(12.4)	58(8.7)
Lethargy	1(0.3)	0(0.0)	5(3.1)	4(3.5)	10(1.5)
Respiratory, thoracic and mediastinal disorders					
-Total	17(5.5)	14(17.3)	18(11.0)	10(8.8)	59(8.9)
Dyspnoea	10(3.2)	8(9.9)	8(4.9)	1(0.9)	27(4.1)
Hypoxia	0(0.0)	2(2.5)	2(1.2)	3(2.7)	7(1.1)
Pleural effusion	3(1.0)	2(2.5)	1(0.6)	0(0.0)	6(0.9)
Pulmonary embolism	1(0.3)	2(2.5)	3(1.8)	0(0.0)	6(0.9)
Skin and subcutaneous tissue disorders					
-Total	20(6.5)	1(1.2)	6(3.7)	4(3.5)	31(4.7)
Pruritus	9(2.9)	0(0.0)	3(1.8)	1(0.9)	13(2.0)
Vascular disorders					
-Total	8(2.6)	5(6.2)	9(5.5)	8(7.1)	30(4.5)
Hypertension	1(0.3)	2(2.5)	1(0.6)	1(0.9)	5(0.8)
Hypotension	3(1.0)	1(1.2)	4(2.5)	5(4.4)	13(2.0)

Includes only events occurring ≥ 2% of patients in any dose group.
A patient with multiple occurrences of an AE is counted only once in that AE category.
A patient with multiple adverse events within a primary system organ class is counted only once in the total row.
Primary system organ classes are presented alphabetically; preferred terms are sorted within primary system organ class alphabetically.
If an AE frequency matches the criteria in one dose category, the frequency of that event is shown for all doses.

As shown in Table 2 above, Grade 3-4 AEs regardless of causality were reported in 534 patients, 80.2% of the safety population for the QW schedule. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia in 272 patients (40.8%), neutropenia in111 patients (16.7%), anemia in 103 patients (15.5%), fatigue in 82 patients (12.3%) and febrile neutropenia in 46 patients (6.9%). There were more Grade 3-4 hematologic AEs at higher dose levels, with the highest incidences at 40 and 60 mg. The highest incidence of febrile neutropenia was seen at the dose level of 60 mg (27.4%) compared to the other dose levels where the incidence was ≤ 3.7%. This could be because this dose level was only tested in leukemia patients, in whom febrile neutropenia is a common AE. Grade 3-4 thrombocytopenia, grade 3-4 neutropenia and grade 3-4 anemia accounted for 75.7% (272/359), 82.8% (111/134) and 52.8% (103/195) of their respective all grade events. Grade3-4 diarrhea, Grade 3-4 vomiting and Grade 3-4 nausea accounted for less than 10% of their respective all grades events (see also Table

1).For the TIW QOW dosing schedule, AEs regardless of causality were reported in 96 patients, which is 100% of the safety population. The most commonly reported AEs (all grades) across doses were diarrhea in 65 patients (67.7%), nausea in 60 patients (62.5%), fatigue in 54 patients (56.3%), vomiting in 42 patients (43.8%), thrombocytopenia in 41 patients (42.7%), pyrexia in 35 patients (36.5%) and anorexia in 33 patients (34.4%).

In the TIWQOW schedule, Grade 3-4 AEs regardless of causality were reported in 81 patients, 84.4% of the safety population. The most commonly reported Grade 3-4 AEs across doses were thrombocytopenia in 35 patients (36.5%), neutropenia in 25 patients (26.0%), fatigue in 14 patients (14.6%), diarrhea in 11 patients (11.5%), anemia in 10 patients (10.4%), and febrile neutropenia in 8 patients each (8.3%).

Overall, the most frequent Grade 3-4 AEs regardless of causality for both schedules (TIWQW and TIWQOW) were ascribed to the same SOC, namely blood and lymphatic system disorders.

1.6. Cardiac Safety

As of 31 December 2013, cardiac safety data for 666 patients treated with oral PANO TIW QW are presented in Table 3. All patients underwent intensive pre- and post-dose ECG monitoring intended to measure the occurrence of QTcF (Fridericia Correction Formula) and to capture other ECG abnormalities as well. The most common finding continues to be a post-baseline QTcF increase of >30 and ≤60 msec with both dosing schedules (approximately 22%). No cases of torsades de pointes have been observed with either oral dosing schedule.

QTcF abnormalities are shown in Table 1-3, for the QW schedule.

Table 3. QTcF changes in patients receiving oral panobinostat three-times-a-week every-week (TIW QW)

QTcF variable	20 mg (N=309) Total n %	30 mg (N=81) Total n %	40 mg (N=163) Total n %	60 mg (N=113) Total n %	TOTAL (N=666) Total n %
QTcF increase from baseline > 30 and <= 60 ms	309 47 (15.2)	81 12 (14.8)	163 46 (28.2)	113 38 (33.6)	666 143 (21.5)
QTcF increase from baseline > 60 ms	309 7 (2.3)	81 1 (1.2)	163 6 (3.7)	113 13 (11.5)	666 27 (4.1)

Absolute QTcF > 450 and <= 480 ms	**305 38 (12.5)**	**81 10 (12.3)**	**162 13 (8.0)**	**110 27 (24.5)**	**658 88 (13.4)**
Absolute QTcF > 480 ms and <= 500 ms	**309 5 (1.6)**	**81 1 (1.2)**	**163 4 (2.5)**	**113 7 (6.2)**	**666 17 (2.6)**
Absolute QTcF > 500 ms	**309 1 (0.3)**	**81 0 (0.0)**	**163 0 (0.0)**	**113 5 (4.4)**	**666 6 (0.9)**

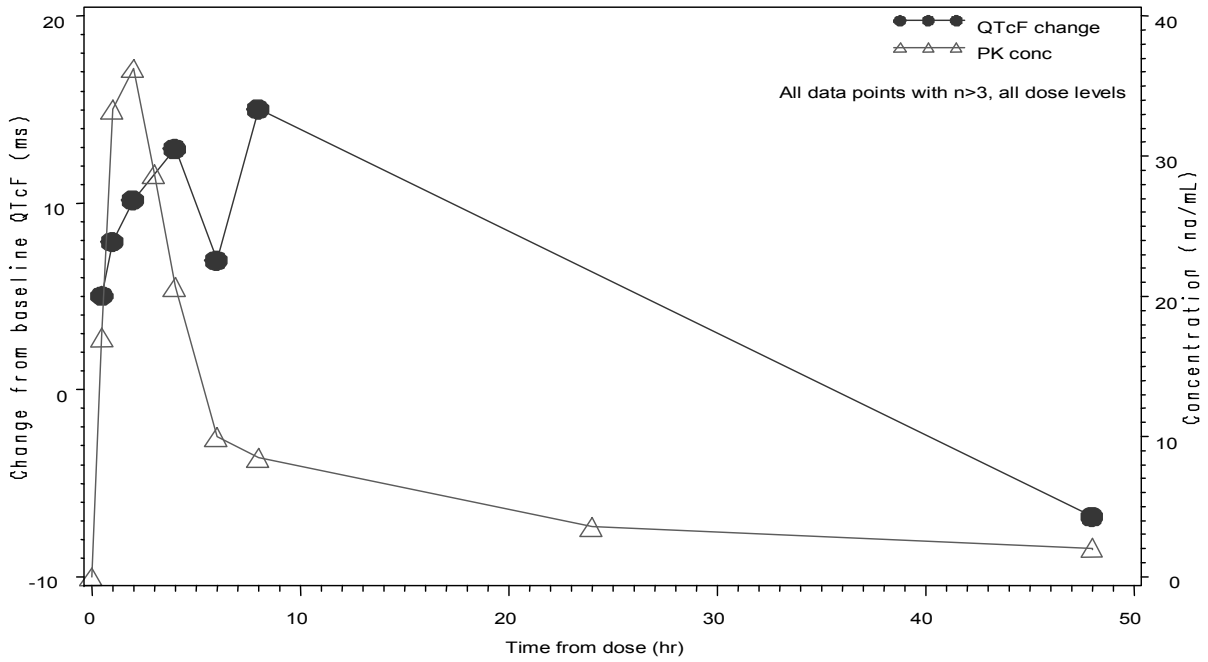
N= Number of patients in the group.
n= Number of patients at risk for a designated change with both non-missing baseline and post-baseline values.
Patients are counted only for the worst grade observed post-baseline.

As shown in Table 3 above, post-baseline increase of >30 and ≤60 msec (Grade 1) was frequently reported (143 patients, 21.5%). A post-baseline increase of >60 msec was less frequent (27 patients, 4.1%). QTcF prolongation translating into an absolute value of 450 to 480 msec and of > 480 - 500 msec was measured in 88 patients (13.4%) and in 17 patients (2.6%), respectively. Absolute QTcF prolongation > 500 msec was uncommon (6 patients, 0.9%), mostly referred to 5 patients treated at 60 mg weekly dose level. For the QOW schedule, post-baseline increased values of >30 and ≤60 msec were observed in 16 (16.7%) patients. Post-baseline increase of >60 msec was less frequent (4 patients, 4.2%). Absolute QTcF prolongation values of 450 msec to 480 and of >480 to 500 msec were reported in 9 patients (9.6%) and in 1 patient (1.0%), respectively. Absolute QTcF prolongation above 500 msec was not observed.

1.7. Relationship between panobinostat plasma concentrations and QTcF

As presented in Figure 2 (po) below, the maximum change of QTcF from baseline does not coincide with the peak plasma concentration-time course of PANO suggesting a possible delayed effect.

Figure 2 QTcF change from baseline over time vs. PANO conc-time course following the first oral PANO doses of a MWF schedule



1.8. Human pharmacokinetics

After oral administration, PANO is rapidly absorbed with no observed lag phase. Maximum plasma concentrations were generally reached within 1 hour after oral dosing. The absolute bioavailability was 30% (data on file) and the mean (SD) half-life of PANO was comparable following i.v. and oral dosing ~15.0 (5) hours (Figure 3). Moderate drug accumulation was observed with oral three-times-a-week schedule but not with the weekly i.v. schedule (1.4-fold drug accumulation with oral three-times-a-week dosing), consistent with the terminal half-life of 15 hours and dosing interval. Different degrees of renal impairment (mild, moderate and severe) did not alter PANO plasma exposure, whereas, mild hepatic dysfunction increased PANO plasma exposure by 43% and moderate hepatic impairment marginally increased PANO plasma exposure by 105% in cancer patients.

Figure 3 Mean panobinostat plasma concentration versus time profiles following single oral or intravenous administration

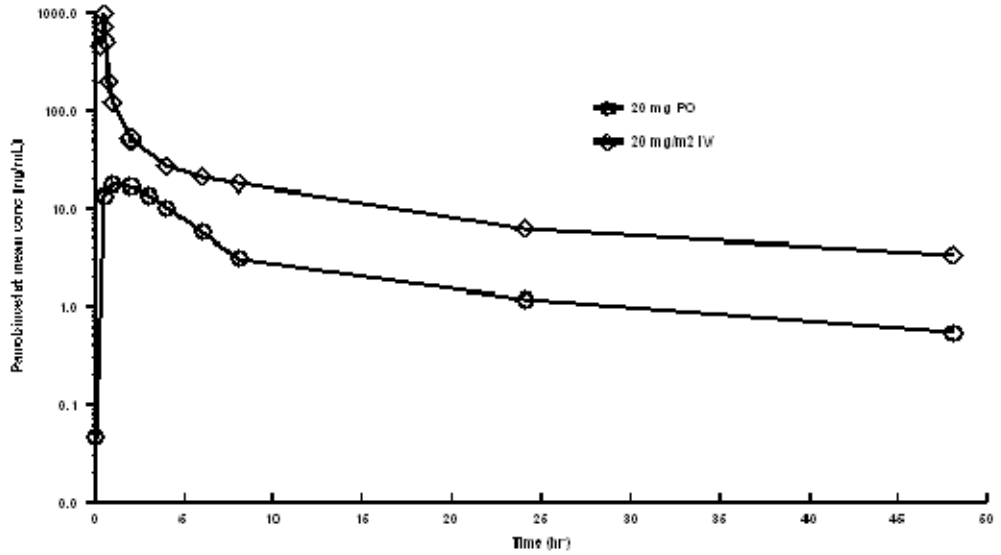


Table 4 Pharmacokinetic parameters of panobinostat in three phase I studies

Route of administration & Dose (No. of patients following single dose)	**Mean (CV%) Single dose C_{max}(ng/mL)**	**Mean (CV%) Single dose $AUC_{0\text{-}inf}$ (ng*hr/mL)**	**Mean (CV%) Multiple dose $AUC_{0\text{-}48}$ (ng*hr/mL)**
i.v. 20 mg/m^2 (n=31)	784 (45)	1041 (38)	n/a
p.o. 20 mg (n=45)	22.8 (58)	194 (58)	258 (65)
p.o. 30 mg (n=49)	36.2 (62)	267 (54)	279 (54)
p.o. 40 mg (n=24)	58.0 (59)	329 (77)	270 (59)
p.o. 60 mg (n=57)	66.1 (68)	362 (62)	306 (50)
p.o. 80 mg (n=18)	63.5 (58)	397 (49)	369 (52)
n/a: not applicable with weekly i.v. administration			

In vitro experiments suggested that the hepatic oxidative metabolism of PANO is mediated primarily by cytochrome P450 (CYP)3A4, and to a lesser extent by CYP2D6 and CYP2C19. In addition to monooxygenation, hydrolysis of the hydroxamic sid chain

(M43.5) were also found to be mediated (at least in-part) by the CYPs. These same metabolic pathways, were also observed in the recent human ADME and mass balance study [CLBH589B218].

1.9. Dose proportionality

A positive and linear dose-exposure relationship was found following single i.v. administration (1.2 to 20 mg/m^2, Rs = 0.83; p<0.0001). After oral dosing with 15 mg to 80 mg of PANO, dose-proportionality analysis indicated that systemic exposure increased nearly dose-proportionally at doses below 60 mg and there is less than proportional increase in AUC after 60 mg and 80 mg doses of panobinostat. It appears that absorption may become limiting at doses ≥ 60 mg of PANO.

1.10. Food Effect

Influence of food on PANO PK was evaluated in patients with advanced cancer who received 20 mg panobinostat twice a week and were randomized to receive PANO under fasting, high fat, and normal breakfast conditions [CLBH589B2111]. The overall exposure and inter-patient variability (CV 59%) in 34 patients remained unchanged with or without food, whereas C_{max} was transiently reduced by <45% and T_{max} prolonged by food (i.e., both normal and high fat breakfast). Since food did not alter the overall extent of absorption, food is unlikely to significantly impact PANO's systemic exposure in cancer patients. The findings from this formal food effect are consistent with the results from an earlier pilot food effect [CLBH589B2101] arm 1. Therefore, panobinostat can be administered without regard to food in future studies.

1.11. Study rationale/purpose

For patients with hematological malignancies that cannot be cured by standard chemotherapy/immune-modulators/radiation alone, allogeneic HCT represents the only curative option. Treatment failure occurs most commonly due to GVHD, toxicity from the preparative regimen and/or disease relapse. GVHD continues to be a major cause of morbidity and mortality to date. The reported rate of acute GVHD by day 100 in a Phase III, randomized, multicenter, trial comparing the use of PBSC versus bone marrow in MUD transplants showed a rate of acute GVHD II-IV by day 100 was 47% (40%-53%) with grade III-IV 16% (12%-21%) using PBSC (16). In our randomized Phase II trial of TAC and SIR vs. TAC and MTX as GVHD prophylaxis after allogeneic transplantation we observed an acute GVHD incidence of 50% with TAC/SIR [4].

Allogeneic HCT patients could benefit from PANO given its putative immunosuppressive activity in order to improve the success rate of allogeneic HCT. HDACi have several unique properties such as pro-inflammatory cytokines reduction[38,39] that translate into decreased GHVD mortality without affecting GVL in murine transplantation models.[29,30] HDACi increases production and suppressive function of regulatory T-Regs,[32] exhibit immunomodulatory properties in human dendritic cells,[40] and have antitumor activity.[41] These properties make them an attractive class of agents to explore for GVHD prevention and potentially to target minimal residual disease to reduce relapse rate after allogeneic HCT.

In this Phase II trial we will test the preliminary clinical activity of a PANO in addition to TAC/SIR for GVHD prevention. Patients will be evaluated for parameters that measure the safety, efficacy and pharmacodynamics of these agents combination.

2. Study objective

2.1. **Primary Objective**

To prospectively determine the cumulative incidence of acute GVHD grades II-IV by day 100 when combining PANO with standard combination of TAC/SIR for GVHD prevention. It is anticipated that the combination will decrease of acute GVHD risk to 30% compared to our historical control of 50% with TAC/SIR alone.

2.2. **Secondary Objectives**

2.2.1. Clinical objectives:
- 2.2.1.1. Cumulative incidence of chronic GVHD
- 2.2.1.2. Engraftment, relapse, non-relapse mortality, overall and relapse-free survival at one year.

2.2.2. Biological studies to test the pharmacodynamics of TAC/SIR/PANO combination for GVHD prevention:
- 2.2.2.1. Immune Reconstitution determined by flow cytometry
- 2.2.2.2. Histone acetylation of T cells and DC subsets by Western Blot and flow cytometry
- 2.2.2.3. T reg immune-reconstitution, Foxp3+ expression and acetylation by flow cytometry
- 2.2.2.4. Inflammatory cytokine and Biomarker expression profiling
- 2.2.2.5. Stat-3 activation and acetylation
- 2.2.2.6. Panobinostat pharmacokinetics

	Objective	**Endpoint**
Primary Clinical Objectives	Prospectively determine the cumulative incidence of acute GVHD grades 2-4 using TAC and SIR in addition to PANO for GVHD prevention. Study parameters: We will consider ≥43% incidence of grade II-IV aGVHD not acceptable. We will use 23% incidence rate of GVHD as target.	GVHD severity stage and grading and distribution will be measured weekly from day of transplant to day 90 +/- 14using standard scoring system. Stage of GVHD will be given for each site of involvement (e.g. skin, liver, and gut), as well as a composite score for overall acute GVHD grade. Pathologic confirmation of aGVHD will be dictated by usual clinical practice, and not

		mandated by this protocol. Treatment of GVHD will not be mandated.
Secondary Clinical Objectives	Cumulative incidence of chronic GVHD	GVHD with onset after 100 days post-HCT with presence of at least one diagnostic manifestation of chronic c-GVHD or distinct manifestation confirmed by biopsy or other relevant tests (eg PFT). Classified as: 1-Classic chronic GVHD - meets criteria for chronic GVHD and has no features consistent with aGVHD or 2-Overlap syndrome - features of acute and chronic GVHD exist together. C-GVHD will be measured prospectively in all patients on days 90+/-14 , 120 +/- 14, 150 +/- 14, 180+/-14, 270+/- 30, and 365 +/- 30 as per standardized scoring system.
	Engraftment	Stable engraftment for WBC is defined as a sustained absolute neutrophil count > 500 over 3 days without cytokine support. Stable platelet engraftments is defined as count of > 20,000 over 7 days without transfusion support. Time to engraftment is defined as time from day 0 to day of sustained engraftment per above criteria for both platelets and WBC.
	Cumulative incidence of relapse/progression and non-relapse mortality at one year. Overall Survival and	Incidence of primary disease relapse and non-relapse related death will be reported per standard definitions. These will be treated as competing risk events. Non-

	Relapse-free Survival at one year.	relapse death is defined as death in continuous remission from primary disease requiring transplantation. Survival outcomes: Overall survival: Time from transplant date to death from any cause. Relapse-free survival: Time from transplant date to death or primary disease relapse.
Secondary Exploratory Objectives	Biologic studies (appendix F)to test thepharmacodynamics of TAC/SIR/PANO combination for GVHD prevention. Results will be compared with compared to data from similar external control patients treated with an identical protocol except for PANO	T Cells, especially T regs, immune-reconstitution, will be measured to assess whether PANO increased amounts of Treg cells post HCT. Histone acetylation of T cells, T regs and DC subsets will be measured to test the activity of PANO as an inhibitor of histone deacetylases on targeted specific cell subsets. Inflammatory cytokine will be measure to assess whether PANO contribute to generate an immune-modulatory cytokine micro-enviroment decreasing the production of pro-inflammatory cytokines . Biomarker expression profiling will be measured to assess correlation with GVHD clinical outcome. Stat-3 activation and acetylation will be measured in cells subsets to test the activity of PANO as an inhibitor of histone deacetylases

3. Overall study design

3.1. **This study will test PANO in combination with TAC/SIR for acute GVHD prevention.** This is a Phase II, single arm, non-randomized study and does not contain a treatment control group. This Phase II study will assess whether TAC/SIR/PANO provides potential efficacy for GVHD prevention, and whether results hold sufficient promise for definitive testing of PANO in subsequent randomized clinical trials and/or TAC de-escalation studies. Correlative studies results ONLY will be compared to an "external control" patient subset that will be treated with TAC/SIR with same inclusion and exclusion criteria as this protocol except that they will not be treated with PANO.

3.2. **Site(s):** Protocol will be performed at Moffitt Cancer Center.

3.3. **Sample Size and accrual rate**. It is estimated that 2 patients will be entered into the treatment study each month, therefore, we anticipate 24 months for completion of the study that will require **n=44** total patients for the treatment arm. We are planning to enroll **n=20** patients for "external control patients". We anticipate 0.5 patients/month for "external control" subjects and accrual will happen concomitant to treatment arm patients. Therefore a total of **64** subjects will be accrued in total including treatment arm and external control.

3.4. **Patient Follow up**. Patients will be evaluated weekly from day of transplant until day 100 to measure acute GVHD using standard scoring system (defined in Appendix A). Stage of GVHD will be assessed for each site of involvement (e.g. skin, liver, and gut), as well as a composite score for overall acute GVHD grade determined. Pathologic confirmation of aGVHD will be dictated by usual clinical practice. Patient will be followed on days +90 +/-14, 120 +/- 14, 150 +/- 14, 180+/- 14, 270+/-30, and 360 +/- 30 for chronic-GVHD assessment and adverse events toxicity assessment. Taper of immunosuppression is suggested- not mandatory and treatment of GVHD is not mandated (section 6.2-6.3).

3.5. **Withdrawal.** from the study will take place under the following circumstances: those patients who sign consent for the study, but fail to initiate treatment due to:

3.5.1. denial of coverage from insurance provider
3.5.2. disease progression prior to initiation of treatment
3.5.3. death prior to initiation of treatment
3.5.4. withdrawal of patient consent at any time after therapy

4. Patient Population: Inclusion and Exclusion criteria

4.1. **Inclusion Criteria**:

4.1.1. Male or female

4.1.2. Age ≥ 18 years or older at time of enrollment
4.1.3. Signed informed consent
4.1.4. Hematologic disorder requiring allogeneic hematopoietic cell transplantation
4.1.5. LVEF ≥ 45% by MUGA scan or echocardiogram
4.1.6. FEV1, FVC, and DLCO adjusted ≥ 50% of predicted values on pulmonary function tests
4.1.7. Transaminases (AST, ALT) < 3 times upper limit of normal values
4.1.8. Creatinine clearance calculated ≥ 50mL/min
4.1.9. Karnofsky Performance Status Score ≥ 60%.
4.1.10. HLA matched 8/8 (A, B, C, DRB1) related or unrelated donor

4.2. **Exclusion Criteria**:

4.2.1. Active infection not controlled with appropriate antimicrobial therapy
4.2.2. HIV, hepatitis B (HBcAb positive but HBsAg negative with undetectable viral load are eligible), or hepatitis C infection
4.2.3. Sorror's co-morbidity factors with total score ≥ 4
4.2.3.1. Important modification to co-morbidity index calculation: DLCO adjusted will not be included in assessment of pulmonary risk, except those patients with DLCO adjusted < 50% who are excluded from the trial.
4.2.4. Anti-thymocyte globulin (ATG) as part of the conditioning regimen
4.2.5. Cyclophosphamide as part of the conditioning regimen or for GVHD prophylaxis.
4.2.6. Pregnancy
4.2.7. HDAC, DAC, HSP90 inhibitors or valproic acid for the treatment of cancer within 30 days. Only FDA approved drug are Vorinostat and Romidepsin the rest are considered investigational and are not allowed.
4.2.8. Patients who will need valproic acid for any medical condition during the study or within 5 days prior to first PANO treatment
4.2.9. Impaired cardiac function or clinically significant cardiac diseases, including any one of the following:
4.2.9.1. Any history of ventricular fibrillation or torsade de pointes
4.2.9.2. Bradycardia defined as HR< 45 bpm. Patients with pacemakers are eligible if HR ≥ 45 bpm.
4.2.9.3. Screening ECG with a QTcF ≥ 480 msec
4.2.9.4. Right bundle branch block + left anterior hemiblock (bifascicular block)
4.2.9.5. Patients with myocardial infarction or unstable angina ≤ 12 months prior to starting study drug
4.2.9.6. Other clinically significant heart disease (e.g., CHF NY Heart Association class III or IV , uncontrolled hypertension) as per discretion of principal investigator and/or treating physician

4.2.9.7. Patients using medications that have a relative risk of prolonging the QT interval or inducing torsade de pointes if treatment cannot be discontinued or switched to a different medication prior to starting study drug with the exception of drugs listed on Appendix B that are required for HCT patients.

5. Intervention/Treatments

5.1. **Stem cell mobilization and collection of Donor:**

5.1.1. Peripheral blood stem cells: the stem cell product will be mobilized and collected as per institutional (Moffitt) or NMDP standards for related and unrelated donors respectively. The target graft will have a CD34 count of 5-10 x 10^6 cells/kg; however, a cell dose $\geq$ 2 x 10^6 CD34/kg is the minimal dose permitted.

5.1.2. Umbilical cord blood and bone marrow stem cells are not an accepted stem cell source per this protocol.

5.2. **Conditioning regimen:** The conditioning regimen is per physician discretion and can include either 1) myeloablative (MAC) busulfan targeted at an AUC of 4800 μM/L*min/day and fludarabine; 2) reduced-intensity (RIC) busulfan (6.4mg/kg total over 2 days or targeted at an AUC or 3500 μM/L*min/day) and Fludarabine; or 3) RIC fludarabine and melphalan (140mg/m2). Busulfan targeted to an AUC of greater than 5,300μM/L*min/day will not be allowed, given the concurrent administration of SIR, as this combination (escalated AUC of busulfan and sirolimus) was associated with increased risk for veno-occlusive disease (VOD) of the liver and non-relapse mortality in prior Moffitt trial (MCC 15372). Conditioning regimens/GVHD prophylaxis utilizing cyclophosphamide and/or anti-thymocyte globulin (ATG) as part of the conditioning regimen are not eligible.

5.3. **Supportive Care**: Transfusion as per institutional practice. Anti-infective prophylaxis or preemptive therapy as per institutional guidelines.

5.4. **Antifungal Prophylactic Medications:**

5.4.1. Micafungin is the preferred primary antifungal prophylaxis treatment given its minimal interaction with TAC and SIR metabolism.

5.4.2. Voriconazole or Posaconazole is allowed as per treating physician discretion.

5.4.3. Patients with presumed or proven pre-transplant infection treated with Voriconazole or Posaconazole will be allowed to continue with this treatment as it has been shown that for primary treatment of invasive aspergillosis, azoles like voriconazole have shown better responses and improved survival when compared to amphotericin B.[42]

5.4.4. If patients develop GVHD (see section 6) on trial it is our standard practice to use voriconazole or posaconazole in patients receiving prednisone at a dose higher than 0.5mg/kg/day. In a randomized trial for prophylaxis against fungal infections among patients with GVHD, posaconazole was similar to fluconazole for prophylaxis against fungal infections among patients with GVHD; and posaconazole was superior in preventing invasive aspergillosis and reducing the rate of deaths related to fungal infections. [43] .

5.5. **Other medications**: In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (e.g. antiemetics) with the following precautions:

5.5.1. Any medications listed in Appendix B:

5.5.1.1. Which may cause QTcF prolongation or inducing torsades de pointes should not be used. Concomitant use of CYP3A4 inhibitors with PANO should be avoided to prevent potential increase in PANO exposure during concomitant treatment with these drugs.

5.5.1.2. Medications known to be substrates of the isoenzyme CYP2D6 should be avoided in use with PANO as this drug can inhibit isoenzyme CYP2D6 at low micromolar ranges. Please refer to Appendix B for the list of CYP2D6 substrates.

5.5.2. Patients receiving any medications that have the potential to alter serum electrolytes (e.g., diuretics) should be monitored very closely for electrolyte abnormalities as these can contribute to the risk of QT prolongation and ventricular arrhythmias.

5.5.3. No other investigational therapy should be given to patients during PANO administration or until toxicity related to PANO has not resolved.

5.5.4. Leukocyte growth factors (e.g.G-CSF and GM-CSF) are not to be administered systematically but may be prescribed for ANC < 750 per microliter if this is thought to be appropriate.

5.5.5. Ursodiol is allowed as per institutional guidelines at any time during this trial.

5.5.6. Budesonide and beclomethasone. For patients with gastrointestinal GVHD, budesonide and/or beclomethasone, with or without systemic corticosteroids are allowed.

5.6. **EKG monitoring:**

5.6.1. **Screening.** One 12-lead EKGs will be performed to assess study eligibility at time of enrollment to accurately estimate Fridericia correction for QTC (QTcF) calculation as listed in Table 4 which is available in MUSE system. If EKG QTcF is above 480msec a minimum of 3 sequential 12 lead EKGs, separated by at least 2 minutes, must be performed. The QTcF , or average of 3, (at baseline must be ≤ 480 msec for the patient to be **eligible** for participation in the trial). The EKG will be analyzed by the treating physician or principal investigator or designee to assess eligibility of the patient.

5.6.2. Additional 12-lead EKGs will be performed at a minimum at scheduled time points as indicated in Table 4 as follows:
5.6.2.1. With 1st dose: Pre-dose EKG and post dose EKG
5.6.2.2. With 3rd dose: Pre-dose EKG
5.6.2.3. If EKG QTcF prior to 1st dose, or any EKG performed then after, is above 480msec a minimum of 3 sequential 12 lead EKGs, separated by at least 2 minutes, must be performed.

5.6.3. For the multiple EKGs which are obtained, the average of the QTcF intervals must be ≤ 480 msec before the patient is dosed. Dosing management based on QTc interval it is described in table 5.

5.6.4. If EKG average is ≥ 480 msec patient must have an assessment of serum potassium, magnesium, and calcium (total corrected for albumin, or ionized calcium) in order to rule out correctable electrolytes abnormalities that could potentially correct prolonged QTcF. On any day and time in which electrolytes are assessed, if the value is < LLN, then the patient's potassium, calcium, phosphorous or magnesium should be supplemented as per standard Moffitt procedure. After electrolytes replacement 3 sequential 12 lead EKGs, separated by at least 2 minutes, must be performed to ensure it is ≤ 480 msec to proceed.

5.6.5. Treatment decision to administered PANO will be made by the treating provider at Moffitt Cancer Center and will be based on QTcF as determined by the automated machine reading at the investigational site which is available in the MUSE system. All cardiac events should be treated as per the local standard of care and referred to a cardiologist if clinically indicated. Any final decisions concerning dose modifications or permanently

discontinuing the patient from study drug due to QTcF prolongation will be based on the Investigator's clinical assessment.

If ANY EKG performed while patient is on trial shows a QTcF ≥ 480, a minimum of 3 sequential 12 lead EKGs separated by at least 2 minutes, should be performed. QTcF from single EKG or average of 3 EKG must be ≤ 480 msec for the patient to be treated with PANO. Electrolyte replacement and/or avoiding drug(s) that could potentially prolonged QTcF should be attempted when indicated. PANO could be resumed once QTcF is ≤ 480 as per clinical judgement of the treating physician at Moffitt Cancer Center or PI or designee. If Muse system is malfunctioning, EKG machine should provide EKG printout which provides QT correction based on Bazett's formula. In these circumstances, QTcB is allowed to be used to decide eligibility and/or PANO dosing if heart rate is equal or above 60. QTcF can also be calculated with QT and heart rate provided in printout. QTcF is obtained based on the following formula: QT/RR^0.33. RR= 60000/Heart rate. Can use web based link http://www.thecalculator.co/health/QTc-Calculator-385.html. or similar to avoid calculation errors.

Table 4 Cardiac assessment monitoring schedule

Day of Treatment	**EKG monitoring**
Screening	*1 EKG* If QtcF ≥ 480 msec a total of 3 sequential EKGs separated by at least 2-5 minutes will be performed and the average QTcF should be less than 480 msec to be eligible.
First dose of PANO	*PRE DOSE: 1 EKG.* If QtcF ≥ 480 msec a total of 3 sequential EKGs separated by at least 2-5 minutes will be performed and the average QTcF should be less than 480 msec to proceed. Check correctable electrolytes abnormalities (section 5.6.4), replace and repeat 3EKG to ensure Qtc≤ 479 to proceed. *POST DOSE*: should be performed at 3 hours ± 0.5 hour. Perform 1 EKG. If QtcF ≥ 480 msec then performed 3 EKGs at least 2-5 minutes. If average QTcF ≥ 480 msec or 60 msec above baseline, dosing should be held including follow-up triplicate ECGs and correction of electrolyte abnormalities should be performed to proceed with dose number 2 of PANO.

Day of Treatment	EKG monitoring
Third dose of PANO	*PRE DOSE: 1 EKG.* If QtcF ≥ 480 msec a total of 3 sequential EKGs separated by at least 2-5 minutes will be performed and the average QTcF should be less than 480 msec to proceed. Check correctable electrolytes abnormalities (section 5.6.4), replace and repeat 3EKG to ensure bellow QtcF ≤ 479 to proceed.

Table 5: Treatment decision based on EKGs findings:

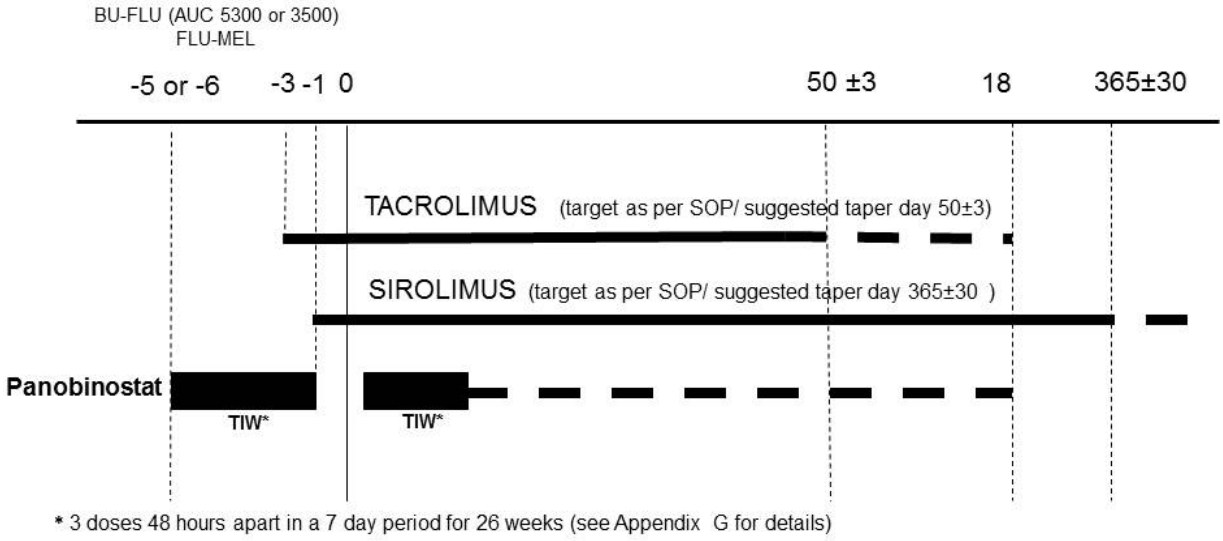
Time Point	Average QTcF*	Action
Pre-dose before first dose	≥ 480 msec (if 1 EKG more than 480 obtain a total of 3 sequential EKGs separated by at least 2-5 minutes to calculate the average)	Delay treatment andcorrect any electrolyte abnormal values ** and repeat 3 EKG. If after electrolyte replacement QTcF remains ≥ 480 msec, do not dose and patient is not eligible
	Above 500 msec	Patient is not eligible
Pre-dose before third dose	≥ 480 msec or above 60 msec from baseline (last EKG average at time of first dose)	Delay treatment Correct any electrolyte abnormal values ** and repeat 3 EKG. If after electrolyte replacement QTcF remains ≥ 480 msec, do not dose patient. PANO could be resume if QTcF is ≤480 as per clinical judgement of the treating physician at Moffitt Cancer Center or PI or designee before subsequent doses.
	Above 500 msec	Do NOT dose patient. Correct any electrolyte abnormal values** and repeat 3 EKGs. PANO could be resume if QTcF is ≤480 as per clinical judgement of the treating physician at Moffitt Cancer Center or PI or designee before subsequent doses.

*QTcF: Heart rate corrected QT interval using the Fredericia formula:
QTc=QT/RR0.33 as per automated machine available in MUSE system or calculated manually (see 5.6.7).
**: serum potassium, magnesium, calcium

5.7. **GVHD prophylaxis**

We will test the combination of TAC and SIR, a combination that has proven to be safe and effective for GVHD prevention in matched allogeneic transplants (related or unrelated) in addition to PANO. We propose to test the hypothesis that this combination not only enhances T regulatory development but may modulate host-donor antigen presentation to allow an immune-tolerant microenvironment to prevent GVHD. Therefore, PANO will be initiated prior to stem cell infusion (see Appendix G)and continued for 26 weeks as shown in Figure 4:

Figure 4. GVHD prophylaxis plan.



5.7.1. **Standard GVHD prophylaxis regimen:**

5.7.1.1 Tacrolimus(TAC)

5.7.1.1.1. Tacrolimus will be administered starting on day -3 and following our institutional guidelines for dosing.

5.7.1.1.2. Micafungin will be used for primary fungal prophylaxis, given its minimal interaction with TAC and SIR metabolism; there will be no dose adjustment made of TAC or SIR with Micafungin as fungal prophylaxis. In those patients with active fungal infection, anti-fungal treatment will be changed to Voriconazole or Posaconazole. Additionally, those patients on corticosteroid treatment (cumulative dose of greater than or equal to 0.5 mg/kg/day) will be treated with Voriconazole or Posaconazole. In those patients on Voriconazole or Posaconazole, TAC dose will be decreased by 50-67% of its original dose and further adjusted based on serum levels.

5.7.1.1.3. Thrombotic microangiopathy (TMA) will be graded for severity according to CTC version 4. No changes in TAC dose will be made for TMA of grades 1 or 2. For grade 3 TMA, TAC dose will be reduced by 50%. For grade 4 TMA, TAC will be discontinued.

5.7.1.2. Sirolimus (SIR)

SIR will be administered starting on day -1 and thereafter, dosing will be adjusted to maintain therapeutic targets per our institutional standards.
SIR levels should be monitored on days 0, 2, and at least once weekly thereafter until on stable oral dose and schedule. Dose adjustments should be made according to drug levels per standard practice guidelines.

5.7.1.3 Investigational therapy: Panobinostat (PANO)

5.7.3.1 Patients will be treated with PANO 5 MG PO TIW (3 doses about 48 hours apart in a 7 day period) QW for 26 weeks starting prior to stem cell infusion (see Appendix G) Patient follow up will be for a total of 365 ± 30 days from initiation of the drug. During the study, PANO will be administered orally as once daily dose. Patients should receive their once-a-day oral dose of PANO approximately at the same time each day in AM or PM

(preferable PM). Each dose of PANO should be taken with about 8 oz / 240 ml glass of water. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit or grapefruit juice and seville (sour) oranges during the entire study.

5.7.3.2 PANO will be provided by Novartis. Oral PANO will be supplied as 5-mg pink gelatin capsules. Medication labels will comply with US legal requirements and be printed in English. They will supply no information about the patient. The storage conditions for study drug will be described on the medication label.

5.7.3.3 If the patient cannot take Panobinostat due to mucositis and/or HCT related GI toxicities (nausea/vomit/diarrhea), then he/she should take PANO on that same day within 16 hours after the missed dose if possible. After more than 16 hours, that day's dose should be withheld, and the patient should wait to take PANO until the next scheduled treatment day (i.e., patients should be instructed not to try to make-up the missed dose after 16 hours). (In our prior experience majority of patients with mucositis and/or GI toxicitieswere able to complete SIR PO as specified in protocol[4]).

5.7.3.4 The investigator should instruct the patient to take the study drug exactly as prescribed (promote compliance). Pill diary to help adhere with compliance is highly recommended to record actual drug compliance and will be provided to patients. All dosages prescribed and dispensed to the patient and all dose changes during the study should be recorded in pharmacy chart.

5.7.3.5 Patients will receive the planned treatment with PO PANO until they experience unacceptable toxicity that precludes further treatment (as specified in section 9).

5.7.3.6 If they develop GVHD and requires treatment with high dose corticosteroids (1-2MG/Kg) as defined in section 6 they should stop PANO as determined in section 6.3.

6. GVHD

Acute GVHD manifests clinically as involvement of the skin, gastrointestinal tract, and liver, either as an isolated site of involvement or in combination. The traditional grading scheme for GVHD was developed by Glucksberg, *et al*, in 1974. This grading

scheme was updated in 1995 with the publication of new consensus guidelines.[44] A major addition was the incorporation of persistent nausea with pathologic evidence of acute GVHD as grade 1 gastrointestinal GVHD. This established grading scheme will be utilized for the grading of this endpoint in the trial. (see Appendix A)

6.1. Evaluation procedures: Incidence of acute GVHD will be characterized by weekly clinical evaluations from day of transplant to day 90±10 using the above described objective standard scoring system. Organ stage of GVHD will be given for each site of involvement (e.g. skin, liver, and gut), as well as a composite grade for overall acute GVHD (see Appendix A). Pathologic confirmation of aGVHD will be dictated by usual clinical practice and it is highly encouraged, but not mandated by this protocol. If patients develop diarrhea while receiving PANO see recommended management in section 7.2. If patients develop isolated upper GI symptoms, pathology confirmation of GVHD is needed to score as GVHD.

6.2. Taper of immunosuppression is suggested- not mandatory- on days 50±3 for TAC or 365±30 for SIR in the absence of GVHD.

6.3. Therapy for established acute GVHD: Upon recognition of acute GVHD, standard treatment may involve high dose (1-2mg/kg) corticosteroids. The primary therapy of acute GVHD is not mandated by this protocol. Interval progress will be assessed on a routine basis by the treating physician. Without anticipated resolution, additional immunosuppression may be added per the treating physicians' discretion. The agents used, time of initiation, duration of exposure, interval response, and any specific toxicities will be recorded.

If GVHD develops and requires treatment with high dose corticosteroids (1-2mg/kg) while on planned PANO dosing schedule PANO should be stopped as failed primary endpoint.

7. Safety assessment:

Safety assessments will consist of monitoring and recording focused toxicities/adverse events (AE) and serious adverse events (SAE) unexpected to HCT (table 8), the regular monitoring of hematology, blood chemistry and urine values, vital signs, ECOG/Karnosfsky performance status, and the regular physical examinations and EKG assessments as specified in the protocol.

7.1. Anticipated adverse events of the transplant protocol and recommended management

7.1.1. Mucositis: The advent of mucositis will be anticipated, objectively characterized and scored per CTCAE (v 4.0) criteria, and recorded. It is suggested that patients with inadequate oral nutrition will have Nutrition evaluation and consideration for TPN. Pain control is at the discretion of the treating physician

7.1.2. Cytopenias: Transfusion support will be provided as needed per standard of care. Hematopoietic growth factors will not be routinely employed to hasten recovery.

7.1.3. Thrombotic microangiopathy (TMA): The BMT Clinical Trial Network has developed a consensus statement regarding both a uniform diagnostic scheme for post-transplant thrombotic microangiopathy, as well as recommendations for its management. The authors provide a definition for TMA which includes the following: 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 should be the dose reduction or discontinuation of calcineurin-inhibitors upon the recognition of post-transplant TMA. Consideration of calcineurin inhibitor replacement by non-calcineurin inhibitor immune suppressant agents is at the discretion of the treating physician.[45] In those who meet criteria, changes to the immunosuppression regimen will be dictated by the following strategy: No change for CTCAE(v.4) grade 1-2; For CTCAE(v.4) grade 3 dose reduction by 50% of original dose of TAC; For CTCAE(v.4) grade 4 TAC will be discontinued. Plasma exchange upon recognition of TMA is not indicated.

7.1.4. Hepatic veno-occlusive disease (VOD)/ sinusoidal obstruction syndrome (SOS): Hepatic VOD/SOS, is clinically characterized by painful hepatomegaly, jaundice, abnormal liver function tests, and volume overload. This syndrome is seen in association with several precipitants, notably including high dose therapy with hematopoietic cell transplantation. Several risk factors have been identified by multivariable analysis including pre-transplantation abnormalities of liver transaminases, chronic hepatitis C and B, certain chemotherapeutic agents used in conditioning regimens primarily cyclophosphamide, mismatched or unrelated donor graft source, prior irradiation therapy to the abdomen, other medications including acyclovir and vancomycin administered at the time of pre-transplant

conditioning, and prior exposure to gemtuzumab ozogamycin. McDonald, et al. examined liver toxicity in the setting of cyclophosphamide/TBI conditioning prior to HCT. These authors monitored plasma levels of cyclophosphamide metabolites and demonstrated that its metabolism widely varied among subjects. Exposure to elevated levels of a metabolite, o-carboxyethyl-phosphoramide mustard, was significantly related to development of hepaticVOD/SOS, non-relapse mortality, and survival. Those in the highest quartile for exposure to this metabolite had a 5.9-fold increased risk of non-relapse mortality[46]. Accordingly, we will exclude those conditioning regimens employing cyclophosphamide.

The reported rate of VOD/SOS complicating HCT varies from 0 to 70% due to differences in diagnostic criteria, recognition, sample populations, and distribution of risk factors. More current reports, including a large multicenter prospective trial from the EBMT, describe hepatic VOD/SOS rates of 8-9%[47]. While mild hepatic VOD/SOS produces minimal morbidity and mortality, severe hepatic VOD/SOS related mortality reaches 90% which is rare in our experience [4].

Evaluation and management: Surveillance for development of this condition will take place in all patients. Criteria for diagnosis include painful hepatomegaly, abnormal liver function tests, fluid overload, and the absence of alternate etiology to explain these abnormalities. Management of VOD/SOS consists of supportive care, and is not mandated by this protocol.

7.2. **Anticipated events for PANO and recommended management**:

To date the main adverse events reported are similar among all the studies performed with oral PANO formulations. Information about common side effects already known about the investigational drug can be found in the Investigators' Brochure (IB) or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

7.2.1. The most common non-hematological events (Table 1) reported were:
  7.2.1.1. GI events (nausea/vomiting/ diarrhea);
  7.2.1.2. Fatigue and electrolytes abnormalities are less common.
7.2.2. The most common hematological toxicity reported was thrombocytopenia and to a lesser extent mild anemia and leukopenia.

These potential adverse effects will be monitored, and their occurrence recorded as specified in section 9.2. No specific diagnostic or therapeutic measures are prescribed by this protocol for majority of toxicities unless specified. GI toxicity is a common side effect of HCT especially in the immediate transplant period (see expected HCT complications section 9). Etiology of GI symptoms could be multifactorial including conditioning

regimen, medications, GVHD and/or infections. For patients with diarrhea, evaluations for other etiologies including infection are highly recommended. Endoscopy with biopsies should be considered for evaluation of GVHD if the patient is beyond day +22, but is left to the discretion of the treating physician.

8. Reasons to hold/discontinue treatment:

8.1. Investigator(s) decision to withdraw patient from the study in the event of inter-recurrent illness, adverse events, or other reason that he deems necessary to discontinue study drug. Patients who discontinue from the study for AE or SAE will all be evaluable. Date of and reason for study discontinuation will be noted. Patients whose treatment is interrupted or permanently discontinued due to an AE must be followed until resolution or stabilization of the event, whichever comes first.

8.2. Abnormal laboratory result unable to be corrected to levels acceptable for treatment with the study drug.

8.3. Patient non-compliance

8.4. Subject withdrew consent from study at any time for any reason.

8.5. Death.

8.6. Prolongation of QTcF (see section 5.6).

8.7. For patients that PANO is discontinued due to GVHD they are allowed to continue to be ON study. All assessment, procedures, CRF forms, correlative studies (appendix F) will be performed identically to patients that continue to take PANO and we will not deviate from what is stipulated in the protocol for patients that are receiving study drug on study.

8.8. Special considerations for toxicity observed up to day +7 post transplant: Due to unknown interactions between conditioning regimen and PANO, guidance is provided between PANO 1st dose to day +7 HCT as follows:

8.8.1. PANO will be stopped for any non-hematological CTCAE (v4) grade 3 or higher toxicities unexpected for HCT (see table 8) seen between first dose of study drug and day +7 post-HCT. If there is complete reversal of toxicity, PANO can be resumed after 7 days and a minimum of one week off study drug and continued until completion of protocol-specified dosing schedule. In the absence of complete reversal of toxicity within 7 days, the study drug will be discontinued and the patient removed from the study. All such patients will remain evaluable for analysis and not replaced.

8.9. For gastrointestinal (GI) symptoms (diarrhea or nausea/vomiting) thought to be related to PANO from day -5/-6 to day +7 and/or after recovery from acute GI toxicity due to chemotherapy: The PANO dose may be modified according to the severity of the GI side effects as suggested and described in Table 6 and 7. Depending on the patients' clinical status, dose adjustments may be made within 1 week from when the lab work is completed. Patients who require PANO adjustment will have their dose HOLD for 3 doses and may resume 100% dosing thereafter at physician discretion. A trial of anti-emetics and anti-motility agents should be considered (institutional Clinical Practice Guidelines) before modifying the dose of PANO. In case patient regurgitate PANO pill will be allowed to re-dose after anti-emetics trial. PANO should continue for patients who develop GI symptoms related to infection.

Table 6. Management guidance for Diarrhea.

	Diarrhea Grade	**PANO dose reduction**
Mild	Grade 1: <4 stools/day over baseline	0%
Moderate	Grade 2: Increase 4-6 stools/day over baseline	0%
Severe	Grade 3: Increase >7 stools/day over baseline	No GI GVHD: hold only after failure of antimotility agents. Resume once the GI toxicity has resolved to grade 0-2 and continued until completion of protocol-specified dosing schedule If GI GVHD*
Life Threatening	Grade 4: Life-threatening consequences	No GI GVHD: Discontinue permanently. If GI GVHD*
*If acute GI GVHD develops and requires treatment with high dose corticosteroids (1-2MG/Kg) PANO should be discontinued if still taking protocol specified dosing as failed primary endpoint		

Table 7. Management guidance for Nausea/Vomiting*

	Nausea/vomiting Grade	PANO dose reduction
Mild	Grade 1: Loss of appetite without alteration in eating habits; 1 episode of vomiting w/i 24 hrs.	0%
Moderate	Grade 2: Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs; 2-5 episodes of vomiting in 24 hrs.	0%
Severe	Grade 3: Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ≥24 hrs; ≥6 episodes of vomiting in 24 hrs.	No GI GVHD: HOLD ONLY after failure antiemetics. Resume once the GI toxicity has resolved to grade 0-2.
Life Threatening	Grade 4: vomiting with life-threatening consequences	No GI GVHD: Discontinue permanently. If GI GVHD*
* If acute GI GVHD develops and requires treatment with high dose corticosteroids (1-2MG/Kg) PANO should be discontinued if still taking protocol specified dosing as failed primary endpoint		

* Patients receiving high dose chemotherapy may develop mucositis which inhibits oral intake and may require total parental nutrition (TPN). Use of TPN does not necessarily constitute Grade 3 nausea/vomiting and patient must be assessed for the indication of TPN.

8.10. Definition of engraftment and delayed engraftment:

8.10.1. Engraftment: Engraftment for neutrophils is defined as the first of three consecutive days in which the absolute neutrophil count (ANC) is > 500/uL. Engraftment for platelets is defined as the first of three

consecutive days in which the platelet count is > 20,000/uL, without platelet transfusion in the prior 7 days.

8.10.2. Delayed engraftment: In preceding HCT trials we observed engraftment of WBC (defined as ANC >500 for 3 days) by median day 16 (range 11-30), while engraftment of platelets occurred by median day 14 (range 0-71) after HCT. In the current trial, delayed engraftment will be defined as either ongoing ANC <500/uL on or after day 23; or ongoing dependence of platelet transfusions on or after day 21. Patients who engraft prior to day 21 or 23, for platelets or ANC respectively, and later have low ANC or platelet transfusion requirements, as occasionally occurs in HCT patients due to infection and other complications will NOT be considered to have delayed engraftment.

8.11. Dosing modifications during pre-engraftment period: If ANC <500/uL on day 23: **HOLD** PANO until ANC > 1000, then resume at 100% dose. If platelets <20,000/uL on day 21: **HOLD** PANO until platelet count > 30,000/ul (without platelet transfusion support x 3 consecutive days), then resume at 100% dosing.

8.12. Dosing modifications after primary engraftment:

8.12.1. If ANC decreases to 500–1000/uL, **CONTINUE** PANO at 100% dosing. Begin growth factor support.

8.12.2. If the ANC decreases to <500/uL, **HOLD** PANO until ANC recovers to >1000/uL, then **RESTART** PANO at 100% dosing.

8.12.3. If ANC decreases to < 500/uL again, **HOLD** PANO until the ANC recovers to >1000/uL, then **RESTART** PANO at every other week regime(TIW QOW). May resume 100% dosing of PANO (weekly dosing) when G-CSF is discontinued, provided the ANC remains >1000/uL.

8.12.4. If the platelet count decreases to < 20,000/uL, **HOLD** PANO until platelet count recovers to >20,000/uL (without platelet transfusion support x 3 consecutive days) then **RESTART** at 100% dosing.

8.12.5. If platelet count decreases to <20,000 /uL again, **HOLD** the PANO until the platelet count recovers to >20,000/uL (without platelet transfusion support x 3 consecutive days), then **RESTART** PANO every other week. May resume 100% dosing provided the platelet count remains >20,000/uL (without platelet transfusion support).

9. Adverse events (AE):

9.1. AE definition: An adverse event (AE) is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting PANO that is unexpected with HCT. Table 8 describes criteria for "expected" toxicities for HCT/GVHD-related symptoms that are commonly observed in transplant patients. Certain Grade 4 toxicities under particular categories in the NCI CTCAE are common in the HCT transplant setting, and patients with Grade III or IV GVHD are likely to experience additional serious symptoms. These listed toxicities are likely related either to the HCT or preparatory and treatment regimens, however, the investigator must use best medical judgment in making an assessment as whether or not the study drug (PANO) may have had a contributory role.

Table 8. 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/cystitis Creatinine elevation/renal failure Dysuria frequency-urgency Urinary retention
Constitutional	Fever, Rigors, Chills Headache Tremors Weight lost and gain Confusion due to medications Peripheral edema	Reproductive	Persistent amenorrhea
Gastrointestinal	Gastritis GERD Colitis Dehydration Diarrhea Nausea/vomiting Dysphagia Stomatitis/pharyngitis Hematocheza/melena Abdominal pain or cramping	Metabolic/Laboratory	Acidosis/alkalosis Amylase/lipase elevation Hypo-/Hyperglycemia Hypo-/Hyperkalemia Hypo-/hypercalcemia Hypo-/Hypermagnesemia, Hypophosphatemia Hypo-/Hypernatremia Hypertriglyceridemia/Hyperlipidemia/hypercholesterolemia Hyperuricemia Increased LDH

Category	Symptoms/Event	Category	Symptom/Event
Hepatic	LFT elevation Bilirubin elevation Hypoalbuminemia Hepatomegaly	BMTComplex/ Multicomponent Events	GVHD* Stem-cell infusion complications Venoocclusive Disease/ascites
Cardio-vascular	Edema Hypertension/Hypotension Sinus tachycardia Arrythmia (Atrial fibrillation/flutter; Sinus ventricular tachycardia and others) Vascular access complication- deep vein thrombosis requiring anticoagulation Thomboembolic event	Hematologic	Cytopenias Catheter associated venous thrombosis
Infections*	Bacterial, fungal and/or viral infections Sepsis/confusion		

*Infection and GVHD: These toxicities will not be considered AEs but data will be captured on eCRFs. Other toxicities will NOT be captured as expected HCT complications.

Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version(CTCAE) version 4.0 (v.4) (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).

9.2. AE reporting:

9.2.1. All non-hematological toxicities unexpected with HCT will be reported from the first day PANO is administered until day 180±7 days unless specified otherwise in the protocol. After day 180±7 post HCT subjects will only be followed only for GVHD, relapse and survival until day 365±30.

9.2.2. Information about relevant AE unexpected to HCT, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded. AE should be followed as

appropriate for at least 14 days following the last dose of PANO. All AE should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention.

9.2.3. As far as "possible or definitive" related AE unexpected to HCT, each adverse event should be evaluated to determine:

9.2.3.1. the severity grade (mild, moderate, severe) or (grade 1-4)

9.2.3.2. its relationship to the study drug(s) (suspected/not suspected)

9.2.3.3. its duration (start and end dates or if continuing at final exam)

9.2.3.4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)

9.2.3.5. whether it constitutes a serious adverse event (SAE).

9.3. SAE is an undesirable sign, symptom or medical condition which:

9.3.1. Is fatal or life-threatening

9.3.2. Results in persistent or significant disability/incapacity

9.3.3. Constitutes a congenital anomaly/birth defect

9.3.4. Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

9.3.5. Requires inpatient hospitalization or prolongation of existing hospitalization

9.3.6. Note that hospitalizations for the following reasons should not be reported as serious adverse events:

9.3.6.1. Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition

9.3.6.2. Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent

9.3.6.3. Social reasons and respite care in the absence of any deterioration in the patient's general condition

9.3.6.4. Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

9.3.7. Reporting: See section 11.7.

9.4. Special considerations for AE attribution:

9.4.1. Diarrhea:

9.4.1.1. From PANO first dose to day +21 post-HCT attribution to study drug is difficult as majority of patients develop diarrhea as part of the conditioning regimen and/or rarely due to GVHD.

9.4.1.2. After day + 22 post HCT, if a patient develops diarrhea grade 3 (increase more than 7 stools above baseline) or grade 4 (life threatening), excluding infection and/or GVHD, the AE is considered "probable" or "definitive" related to PANO. Protocol specific stopping rules have been formulated for excess GI toxicity (section 12.2).

9.4.2. Vomiting:

9.4.2.1. From PANO first dose to day +21 post-HCT attribution to study drug is difficult as vomiting is a possible complication of the conditioning regimen and/or rarely due to GVHD.

9.4.2.2. After day + 22 post HCT if a patient develops vomiting grade 3 (increase more than 6 episodes in 24 hours) or grade 4 (life threatening), excluding infection and/or GVHD, it is considered "probable" or "definitive" related to PANO. Protocol specific stopping rules have been formulated for excess GI toxicity (section 12.2).

10. Required Evaluations: Evaluations and study visits are listed in Appendix C.

10.1. **Demographics**: Ethnic origin, date of birth, gender, primary diagnosis necessitating HCT, HLA-A, B, C, DRB1 matching of donor and recipient, stem cell product (peripheral blood stem cells are only allowed), Date of HCT.

10.2. **Physical examination**: A directed examination will be performed as per treating physician discretion and based upon patient symptoms. Performance status will be specified by Karnofsky (Appendix D). Comorbidity score (Appendix E).

10.3. **Vital signs**: body surface area of recipient (at enrollment), height (at enrollment), pulse, blood pressure, respiration rate, temperature and weight.

10.4. **Laboratory evaluations**: should be done at baseline (within ≤ 72 hours prior to to the first administration of oral PANO), during the course of the study and at the time of the study treatment completion visit as specified in calendar (Appendix C). Results must be reviewed prior to administering PANO. More frequent examinations may be performed if medically indicated.

10.5. **Hematology**: include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. The coagulation profile includes a

prothrombin time or International Normalized Ratio (INR), activated partial thromboplastin time and fibrinogen.

10.6. **Biochemistry**: includes the following parameters: BUN, creatinine, sodium, potassium, chloride, CO_2 (HCO_3), glucose, calcium, albumin, total protein, total bilirubin, alkaline phosphatase, LDH, AST/SGOT, ALT/SGPT, phosphorous and magnesium,. If total bilirubin is greater than the upper limit of normal, direct and indirect bilirubin should be performed. Biochemistry tests should be obtained after patient has fasted, if possible. Cholesterol, LDL, HDL, triglycerides, and uric acid should be performed at baseline only.

10.7. **Viral studies**: Plasma CMV PCR as per institutional guidelines.

10.8. **Standard urinalysis**: dipstick assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed. This must be supplemented with laboratory quantification of any potentially relevant abnormalities.

10.9. **Thyroid function test**: Thyroid Stimulating Hormone (TSH) and free T4 (thyroxine) will be measured at screening, and at study treatment completion.

10.10. **Serum pregnancy test**: All females of childbearing potential should complete a pregnancy test (serum or urine) prior to transplant. Postmenopausal women must have been amenorrheic for ≥ 12 months in order to be considered "of non-childbearing potential".

10.11. **Electrocardiogram (ECG)**: as specified in section 5.6

10.12. **Follow up**:

10.12.1. GVHD: Date of onset of acute GVHD, names of immunosuppressive drugs received and dates, Date of onset of chronic GVHD, Organ staging will be assessed prior to therapeutic intervention, according to the consensus grading[44] (Appendix A). The diagnosis of Grades II-IV GVHD should be confirmed by histo-pathological examination whenever possible but is not mandatory. Pathology will be reviewed by H. Lee Moffitt Cancer Center pathologists.

10.12.2. Study medications: Records of study medication used, dosages administered, and intervals between visits will be recorded during the study. Drug accountability will be noted by research pharmacy.

10.12.3. AE: Significant findings made after the start of study drug which meet the definition of an AE and SAE must be recorded.

10.12.4. Infections and relapse will be captured but they are not considered AE

10.13. **Correlative studies.** See appendix F

11. Data and Safety monitoring:

The Data Safety & Monitoring Plan (DSMP) will ensure that this trial is well designed, responsibly managed, appropriately reported, and that it protects the rights and welfare of patients. The following internal and external review and monitoring processes provide oversight and active monitoring of this trial:

- The Principal Investigators (PI)
- The Scientific Review Committee (SRC)
- The Protocol Review and Monitoring Committee (PRMC);
- The Research Compliance Division (RCD) of the Cancer Center's Compliance Office;
- The Institutional Review Board.
- We will follow the Moffitt Cancer Center policy for monitoring of Investigator Initiated Trials (MRI-P.PSO.03)

The protocol includes a section that specifies the following with respect to Adverse Event (AE) reporting: what constitutes an AE (versus what is a serious adverse event), the entities to which AE should be reported, the timing of this reporting, and the person or persons responsible for reporting. This includes prompt (within one day of knowledge of the event) reporting to the IRB for unanticipated risks to subjects and reporting in writing within five working days to the IRB. It is required reporting of SAE to Novartis but not AEs as indicated in section 9. 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.

11.1. Initial and Ongoing Monitoring and Review: 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, that all adverse events are reported according to protocol guidelines, and that any adverse actions reflecting patient safety concerns are appropriately reported. The investigators and members of the BMT Research Staff will meet at least monthly. The following data will be reviewed: rate of accrual, adverse events and protocol deviations and/or violations.

11.2. The Scientific Review Committee (SRC): The Cancer Center's internal Scientific Review Committee (SRC) provides for a formal internal peer review of all protocols and general scientific oversight of interventional clinical research. The Committee has a defined membership representing all of the

major research divisions of the Cancer Center, including biostatisticians. All new protocol submissions must contain the required elements of the protocol, and must include a DSMP prior to approval by the Committee. The plan has to be appropriate for the phase and risk of the proposed study.

11.3. The Protocol Review and Monitoring Committee (PRMC): The Protocol Review and Monitoring Committee (PRMC) will monitor this trial for safety, progress, protocol compliance, accrual, adverse event reporting, and data integrity. The membership of the PRMC includes physician representation from each program area and a biostatistician. In addition to the existing stopping rules, the PRMC is authorized to suspend a trial for non-compliance with a DSMP or as a result of audit findings deemed unacceptable. The PRMC will report significant findings to the IRB and the applicable regulatory body. Interim meetings are scheduled to address specific issues that require immediate attention to ensure safety of research participants.

11.4. The Cancer Center's Compliance Office: Corporate Compliance conducts internal audits of selected clinical trials conducted at the Cancer Center and its affiliates. The purpose of the internal audit program is to:

11.4.1. Assure patient safety by monitoring compliance

11.4.2. Assure regulatory compliance by reviewing consent and adverse event reporting

11.4.3. Assure scientific value by monitoring accuracy and completeness of data collection

11.4.4. Monitor and coordinate research compliance activities associated with institutional and individual conflict of interest

11.4.5. Make recommendations for modification of research practices as necessary and provide education on issues that are critical to good research practices

Audits are conducted by Corporate Compliance in accordance with applicable regulatory standards. Investigator initiated trials, such as the one proposed here, receive the highest priority for audit. Corporate Compliance will conduct and report the findings of audits to the PMC in accordance with a protocol's annual review. The PRMC will determine the findings to be acceptable with minor deviations, acceptable with corrective action, or unacceptable with suspension or closure. The PRMC Chairperson will notify the IRB of the audit findings. The PRMC will be informed of all significant open follow-up items. For those observations where no action has been taken, Corporate Compliance will inform the PRMC and may conduct a focused audit.

11.5. Protocol amendments, or changes in study conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed by Novartis and the investigator before implementation and submission for IRB review. A copy of the written approval of the IRB must be provided to Novartis. Examples of amendments requiring such approval are:

11.5.1. increases in drug dose or duration of exposure of subjects,

11.5.2. significant changes in the study design (e.g. addition or deletion of a control group),

11.5.3. increases in the number of invasive procedures,

11.5.4. addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include either changes in the staff used to monitor trials or minor changes in the packaging or labeling of study drug.

11.6. Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation. Any amendments to the protocol, other than administrative ones, must be reviewed by Novartis approved by this committee.

11.7. Instructions for data reporting

11.7.1. To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 14 days after the patient has stopped study treatment must be reported to Novartis within 24 hours of learning of its occurrence.

Any SAEs experienced after this 14 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be

reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form as outlined per contractual agreement; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and send the completed, signed form by fax within 24 hours to the oncology Novartis Drug Safety and Epidemiology (DS&E) department.

The telephone and telefax number of the contact persons in the local department of Drug Safety and Epidemiology (DS&E) are included in the contractual agreement. The original copy of the SAE Report Form and the fax confirmation sheet should be kept with the case report form documentation at the study site.

Follow-up information is sent to the same contact(s) to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology Novartis Drug Safety and Epidemiology (DS&E) department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in

accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

11.7.2. Pregnancies: To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the oncology Novartis Drug Safety and Epidemiology Department (DS&E). Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the panobinostat any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

11.8. Data review and management: 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. The Principal Investigator has the ultimate responsibility. Investigators must enter the information required by the protocol onto Case Report Forms (CRFs). 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 a secure BMT program database and Moffitt Cancer Center's database system, ONCORE. Identifying patient information will be kept confidential. Representatives of the USF 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.

12. Statistical methods

12.1. Sample Size Determination

In our randomized Phase II trial of TAC and SIR vs. TAC and MTX as GVHD prophylaxis after allogeneic transplantation we observed an acute GVHD incidence of 43% (95% CI 30-63%) with TAC/SIR [4]. Simon's minimax two-stage design with 10% one-sided type I error and 10% type II error rate is used for study design as follows. We will consider 43% incidence of grade II-IV

acute GVHD (aGVHD) not warranting further study. We will use 23% incidence rate as a promising result to pursue further study. With these study parameters, 15 evaluable patients will be investigated in the first stage. If 7 or more of 15 evaluable patients experience the grade II-IV acute GVHD, the treatment will be stopped. Otherwise, once 9 or more of 15 patients completed the +90 (+/-14) days follow up without experiencing the grade II-IV acute GVHD 23 additional evaluable patients (a total of 38 evaluable patients) will be enrolled. If the number of patients experiencing the grade II-IV aGVHD is 12 or less, (≤31.6% incidence rate at day 100), the null hypothesis will be rejected and the regimen will be considered promising. An evaluable patient for primary endpoint is defined as patients who receive at least one dose of PANO per protocol and who neither relapse nor die of any reasons without experiencing grade II-IV aGVHD until day 100. A non-evaluable patient will be replaced and we anticipate a total of 44 patients will be accrued in order to account for 10% of competing risks (relapse or death without GVHD) at day 100.

12.2. Stopping criteria for toxicity

A patient who receives at least one dose of the study drug (PANO) per protocol is evaluable for toxicity. As shown in Table 9, the accrual will be suspended if *c* or more of *n* patients experience CTCAE (v.4) grade 3 or higher toxicity unexpected with transplant, where *n* is the number of evaluable patients and *c* is the number of patients who experience CTCAE (v.4) grade 3 unexpected toxicity or higher. Assuming true toxicity rate is 20% and the type I error rate is 5%, the stopping boundary was computed by Ivanova et al[49]. If true toxicity rate is 20%, 40% and 50%, then the probability of early stopping is 4.9%, 79% and 98%, respectively.

Table 9. Boundary for early stopping rule for toxicity

n	3	4	5	6	7	8	9	10	11	1
c	3	4	4	5	5	5	6	6	6	
n	21	22	23	24	25	26	27	28	29	3
c	9	10	10	10	11	11	11	11	12	1

Note: 1. *n* = # of patients who receive at least one dose of study drug

2. *c* = # of patients who experience CTCAE (v4) Grade 3 or higher toxicity unexpected to HCT

12.3 Population for Primary and Secondary Endpoints

Patients who receive at least one dose of the study drug (PANO) per protocol will be eligible for primary and secondary endpoints.

12.4 Analysis Plan for Primary Endpoint

The cumulative incidence rate of grade II-IV acute GVHD at day 100 is the primary endpoint of the study and will be estimated along with one-sided 90%

confidence interval using the approach proposed by Gray[50]. The log-log transformation will be used to compute the point-wise confidence intervals. The death and relapse will be considered as competing risks of acute GVHD.

12.5 Analysis Plan for Secondary Endpoints

The demographic and clinical characteristics of subjects will be summarized using descriptive statistics; mean, median, standard deviation and range for continuous measures and counts and frequencies for categorical measures. Adverse events will be recorded, including the details of the duration and the severity. Time-to-event data such as overall survival (OS) and relapse-free survival (RFS) is measured from the date of transplantation. OS and RFS will be analyzed using the Kaplan-Meier method[51]. The cumulative incidence of relapse and non-relapse mortality will be computed by Gray method. Death and relapse will be considered as the competing risks of chronic GVHD and the cumulative incidence of chronic GVHD will be measured by Gray method. The association of time-to-event data with and without competing risks will be explored by the method by Fine and Gray[52] and the Cox proportional hazards regression model[53], respectively.

For correlative studies (appendix F) results will be compared to an external control patient subset that will be treated with TAC/SIR with same inclusion and exclusion criteria as this protocol except that they will not be treated with PANO. For the regulatory T-cell repopulation, the profiles of regulatory T cell counts will be analyzed using generalized linear models for repeated measures based on generalized estimating equations. The comparisons of profiles of T-cells and DC cells with external control will be made using the Satterthwaite t-test.

The analysis of the secondary endpoints is considered exploratory and no multiplicity adjustment is planned for it. A two-sided p-value of <0.05 is considered statistically significant.

If we compare a total of 30 patients (**15 in treatment group and 15 in external control**) that will achieve 83% power to detect an effect size (mean difference divided by standard deviation) of 1.1 between two groups.

If we compare a total of 40 patients (20 in PANO group and 20 in external control) will achieve 87% power to detect an effect size (mean difference divided by standard deviation) of 1 between two groups.

13 Procedures and instructions

13.1 Publication of results: Any formal presentation or publication of data from this trial may be published after review and comment by Novartis and prior to any outside submission. Novartis must receive copies of any intended

communication in advance of publication (at least fifteen working days for presentational materials and abstracts and thirty working days for manuscripts). These requirements acknowledge Novartis' responsibility to provide peer input regarding the scientific content and conclusions of such publications or presentations. Principal Investigation/Institution shall have the final authority to determine the scope and content of its publications, provided such authority shall be exercised with reasonable regard for the interests of Novartis and, in accord with the trial contract and shall not permit disclosure of Novartis confidential or proprietary information.

13.2 **Disclosure and confidentiality**: The investigator agrees to keep all information provided by Novartis in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided by Novartis (investigators' brochures and other material) will be stored appropriately to ensure their confidentiality. The information provided by Novartis to the investigator may not be disclosed to others without direct written authorization from Novartis, except to the extent necessary to obtain informed consent from patients who wish to participate in the trial.

13.3 **Discontinuation of study**: Novartis reserves the right to discontinue any study support under the conditions specified in the clinical trial agreement.

13.4 **Ethics and Good Clinical Practice**: This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice, as described in Novartis standard operating procedures and: (1) ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community. (2) US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations); and (3)Declaration of Helsinki and amendments, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects).The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

13.5 **Institutional Review Board/Independent Ethics Committee**. Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation. Any amendments to the protocol, other than administrative ones, must be reviewed by Novartis approved by this committee.

13.6 **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.

14 REFERENCES

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2. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood*. 1998;92(7):2303-2314.
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APPENDIX A:

1. Acute GVHD organ staging

Stage	Skin	GI	Liver
1	< 25% rash	Diarrhea > 500ml/d or persistent nausea	Bilirubin 2-3mg/dl
2	25-50%	> 1000 ml/d	Bilirubin 3-6 mg/dl
3	> 50%	> 1500 ml/d	Bilirubin 6-15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

Grade	Skin	GI	Liver
I	Stage 1-2	0	0
II	Stage 3 or	Stage 1 or	Stage 1
III	---	Stage 2-4	Stage 2-3
IV	Stage 4	---	Stage 4

2. Grading of Chronic GVHD (NIH Criteria)

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† ☐ **SCORE % BSA** *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS** **Symptom score**:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **%** FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):* _____				

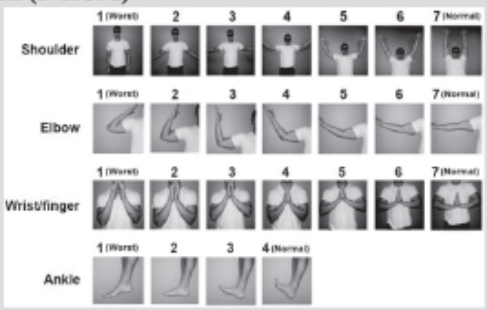
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



3. Categories of Acute and Chronic GVHD

Categories of Acute and Chronic GVHD			
Category	**Time of Symptoms after HCT**	**Presence of Acute GVHD Features**	**Presence of Chronic GVHD Features***
Acute GVHD			
Classic acute GVHD	≤100 d	Yes	No
Late-onset acute GVHD	>100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

*as described herein:

Signs and Symptoms of Chronic GVHD

Organ or Site	**Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)**	**Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)**	**Other Features***	**Common (Seen with Both Acute and Chronic GVHD)**
Skin	Poikiloderma	Depigmentation	Sweat impairment	Erythema
	Lichen planus-like features		Ichthyosis	Maculopapular rash

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
	Sclerotic features		Keratosis pilaris	Pruritus
	Morphea-like features		Hypopigmentation	
	Lichen sclerosis-like features		Hyperpigmentation	
Nails		Dystrophy		
		Longitudinal ridging, splitting, or brittle features		
		Onycholysis		
		Pterygium unguis		
		Nail loss (usually symmetric; affects most nails)†		

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy)	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes)	
		Scaling, papulosquamous lesions		
			Premature gray hair	
Mouth	Lichen-type features	Xerostomia		Gingivitis
	Hyperkeratotic plaques	Mucocele		Mucositis
	Restriction of mouth opening from sclerosis	Mucosal atrophy		Erythema
		Pseudomembranes†		Pain
		Ulcers†		
Eyes		New onset dry, gritty, or painful eyes‡	Photophobia	
		Cicatricial conjunctivitis	Periorbital hyperpigmentation	
		Keratoconjunctivitis sicca‡		
		Confluent areas of punctate keratopathy		

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
			Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features	Erosions†		
	Vaginal scarring or stenosis	Fissures†		
		Ulcers†		
GI tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus†		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children)

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Liver				Total bilirubin, alkaline phosphatase >2 × upper limit of normal† ALT or AST >2 × upper limit of normal†
Lung	Bronchiolitis obliterans diagnosed with lung biopsy	Bronchiolitis obliterans diagnosed with PFTs and radiology‡		BOOP
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis‡	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia	

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
			Lymphopenia	
			Hypo- or hypergammaglobulinemia	
			Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions	
			Ascites	
			Peripheral neuropathy	
			Nephrotic syndrome	
			Myasthenia gravis	

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
			Cardiac conduction abnormality or cardiomyopathy	

GVHD indicates graft-versus-host disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BOOP, bronchiolitis obliterans-organizing pneumonia; PFTs, pulmonary function tests; AIHA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura.

* Can be acknowledged as part of the chronic GVHD symptomatology if the diagnosis is confirmed.

† In all cases, infection, drug effects, malignancy, or other causes must be excluded.

‡ Diagnosis of chronic GVHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

Appendix B

General Guidance:

- In generally, medications listed in Table 1 should be avoided while medications listed in Tables 2 and 3 are to be used with caution when co-administered with PANO.

- It is of great importance to avoid combining drugs listed below in Table 1 and Table 2 (CYP3A inhibitors) especially in the presence of electrolyte abnormalities, notably decreased potassium or magnesium levels commonly associated with diuretic usage.

1 Medications which have a risk of causing Torsades de pointes ventricular arrhythmia should be avoided

Patients who are currently receiving treatment of the medications in Table 1 and cannot either discontinue from this treatment or switch to an alternative medication prior to enrollment, will be excluded from the study. Patients may not begin PANO treatment with any of the medications listed in Table 1 unless this is discussed with and approval is granted by Novartis. It may be agreed to temporarily discontinue PANO treatment (e.g., for 72 hours) during administration with these drugs or withheld medications in Table 1 for at least 72 hours when PANO is to be administered and missed dose(s) should be administered until completion of the trial specified PANO schedule.

This is not a comprehensive list of medications which may prolong the QT interval or have a risk of causing Torsades de pointes. This list of medications was developed in collaboration with an external cardiology consultant, and represents those medications which are deemed to have an unacceptable risk of co-administration with PANO.

The following website may be referenced as a supplemental guide for drugs which have been associated with Torsades de pointes or prolonging the QT interval but at this point lack substantial evidence for causing Torsades de pointes:

http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm#.

Medications listed on the website which do not appear in Table 1 may be used with caution at the discretion of the investigators.

The serotonin ($5HT_3$) antagonists, often used as antiemetics, such as ondansetron dolasetron, (also are known CYP2D6 substrates, see Table 3), or granisetron have been associated with Torsades de points and QT prolongation but have not been shown to cause Torsades de pointes. Therefore, $5HT_3$ antagonists are not per se prohibited but close monitoring for signs and symptoms of QT prolongation is recommended. Caution is to be exercised when using these or other agents that may prolong QT intervals in combination with PANO.

Table 1. Medications which have a risk of causing Torsades de pointes to be avoided

All Class IA antiarrhythmics • quinidine • procainamide • disopyramide • any other class IA antiarrhythmic drug
All Class III antiarrhythmics amiodarone* sotalol bretylium disopryramide dofetilide ibutilide any other class III antiarrhythmic drug
Antibiotics Macrolide antibiotics* • erythromycin • clarithromycin • telithromycin Quinolone antibiotics* sparfloxacin
Antipsychotics thioridazine mesoridazine chlorpromazine pimozide
Antimalarials • halofantrine • chloroquine
Miscellaneous drugs • arsenic trioxide • astemizole • bepridil • domperidone

• levomethadyl • methadone • pentamidine IV* • droperidol
*Note: azithromycin, ciprofloxacin, levofloxacin, pefloxacin, ofloxacin, tosufloxacin, difloxacin, temafloxacin, fleroxacin, acrosoxacin, nalidixic acidand enoxacin are allowed. Inhaled pentamidine can be used for PCP prophylaxis (less likely to induce QTc prolongation). If amiodarone is used to the discretion of the treating physician, close monitoring of QTcF is recommended as clinically indicated.

2 Medications which are known strong CYP3A4/5 inhibitors to be used with caution Table 2.

Table 2 Medications which are known strong CYP3A4/5 inhibitors to be used with caution

Macrolide antibiotics* • clarithromycin • telithromycin • troleandomycin • erythromycin
Antifungals (azoles)* • ketoconazole • itraconazole • fluconazole
Antidepressants • nefazodone
Calcium channel blockers* • diltiazem • verapamil
HIV protease inhibitors: • indinavir • nelfinavir • ritonavir • saquinavir
Miscellaneous drugs or products • aprepitant

• grapefruit product or juice or grapefruit
* azithromycin, voriconazole, ketoconazole, regular orange juice and dihydropyridine calcium channel blockers (e.g. amlodipine, felodipine, nicardipine, nifedipine) are allowed.

This is not a comprehensive list of medications which may inhibit CYP3A4/5. Additional updated versions with moderate and weak CYP3A inhibitors, which are meant to be used as a guide, may be found at the following website: http://medicine.iupui.edu/clinpharm/DDIs

3 Medications which are known CYP2D6 substrates to be used with caution

Panobinostat was also shown to be a CYP2D6 inhibitor (K_i 0.17 µM) *in vitro*. Thus, clinical drug-drug interaction study with panobinostat as CYP2D6 inhibitor and dextromethorpan as CYP2D6 substrate was recently conducted in study CLBH589B2109.

Multiple panobinostat doses increased C_{max} and AUC of dextromethorphan by a mean of 1.8- and 1.6-fold respectively, but with no change in T_{max} in 17 cancer patients. An approximately 2-fold increase in dextromethorphan AUC upon co-administration with panobinostat indicated that *in vivo* CYP2D6 inhibition of panobinostat is weak.

As the study was conducted using a sensitive CYP2D6 substrate which resulted in a weak inhibition, drugs with a large therapeutic index such as anti-emetics, anti-hypertensives, and anti-depressants are generally safe to be co-administered with panobinostat.

Patients should be carefully monitored for potential signs and symptoms of toxicity and may require dose titration or dose reduction of a sensitive CYP2D6 substrate which also have a narrow therapeutic window (e.g., the ratio of toxicity exposure is ≤ 2-fold higher than the efficacious or therapeutic exposure).

Table 3-1 Medications which are known CYP2D6 substrates to be used with caution

Beta blockers (listed below):	Antipsychotics (listed below):
carvedilol	aripiprazole
metoprolol	haloperidol
propafenone	perphenazine
timolol	risperidone
Antidepressants (listed below):	thioridazine
amitriptyline	zuclopenthixol
chlormipramine	amphetamine
desipramine	alprenolol
imipramine	bufuralol

fluoxetine	chloropheniramine
paroxetine	Antiarrhythmics (listed below):
venlafaxine	quinidine
bupropion	lidocaine
duloxetine	mexiletine
Antiemetics (listed below):	propafenone
	Others:
	oxycodone
metoclopramide	codeine
	hydrocodone
	terbinafine
	promethazine
	tamoxifen
	Tramadol
Use of antiemetics ondansetron and dolasetron is contraindicated (granisetron use as an antiemetic is permitted).	

This is not a comprehensive list of CYP2D6 substrates. Additional updated versions of this list, which are meant to be used as a guide, may be found at the following website: http://medicine.iupui.edu/clinpharm/DDIs 1 Synold TW, Takimoto CH, Doroshow JH, Gandara D, Mani S, Remick SC, Mulkerin DL, Hamilton A, Sharma S, Ramanathan RK, Lenz HJ, Graham M, Longmate J, Kaufman BM, Ivy SP. Dose-Escalating and Pharmacological Study of Oxaliplatin in Adult Cancer Patients with Impaired Hepatic Function: A National Cancer Institute Organ Dysfunction Working Group Study, Clin Cancer Res. 2007 *13;* 3660

APPENDIX C. Study calendar for patients treated with TAC+SIR+PANOor with TAC+SIR (PANO has been discontinued due to GVHD)

Test and Procedures	Screening	-6/-5[7]	-1	+28	+60	+90	+120	+150	+180	+270	+365
				+/- 3days		+/- 14 days				+/- 30 days	
Medical history, complete physical exam, weight, vital signs, performance status by Karnofsky.	X			X	X	X	X	X	X	X	X
Transplant Vital organ testing.[1]	X										
BSA- Height.	X										
Consent signing	X										
TSH[4], free T4[4]	X										X
Coagulation Profile (PT, PTT, Fibrinogen[4])	X										
PANO (PO) administration[5]											
Electrocardiograms (EKGs)[2]	X	X	X								
Biochemistries[3]	X										
Cholesterol, LDL, HDL, triglycerides, and uric acid[4]	X										
GVHD organ assessments				X	X	X	X	X	X	X	X
Record immune suppression				X	X	X	X	X	X	X	X
AE and SAE				X	X	X	X	X	X	X	X
Research Studies[6] (see Appendix F)											

[1] LVEF by MUGA and/or echocardiogram, PFT, CT chest, CT sinus, bone marrow, liver function tests, creatinine clearance, serum pregnancy test, infectious disease panel as per SOP. All must meet **inclusion criteria** detailed in body of protocol. **Exclusionary factors** must be confirmed as negative upon screening evaluation, as detailed in the protocol.

[2] Electrocardiograms (EKGs): see section 5.6.

[3] Biochemistry:: BUN, creatinine, sodium, potassium, chloride, CO_2 (HCO_3), glucose, calcium, albumin, total protein, total bilirubin, alkaline phosphatase, LDH, AST/SGOT, ALT/SGPT, phosphorous and magnesium, and uric acid. If total bilirubin is greater than the upper limit of normal, direct and indirect bilirubin should be performed. Biochemistry tests should be obtained after patient has fasted if possible.

[4] test specific to study- cost covered by study budget

[5]PANO administration: Patients will be treated with PANO 5 MG PO TIW (3 doses about 48 hours apart in a 7 day period) QW for 26 weeks starting prior to stem cell infusion. See section 5.7.1.3 and Appendix G for further details.

[6]TAC will be administered starting on day -3 and SIR on day -1 following our institutional guidelines for dosing. See section 5.7.1.1 and 5.7.1.2.

[6]Research samples will be obtained on days-30 to -5,15±3, 28±3, 90±14 and 365±45

[7]See Appendix G with details of PANO administration depending on conditioning regimen

APPENDIX D
Karnofsky Performance Status Criteria
Able to carry on normal activity; no special care is needed

100	Normal; no complaints; no evidence of disease	(ECOG 0)
90	Able to carry on normal activity	(ECOG 0)
80	Normal activity with effort	(ECOG 1)

Unable to work; able to live at home, cares for most personal needs; a varying amount of assistance is needed

70	Cares for self; unable to carry on normal activity or to do active work	(ECOG 1)
60	Requires occasional assistance but is able to care for most needs	(ECOG 2)
50	Requires considerable assistance and frequent medical care	(ECOG 2)

Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly

40	Disabled; requires special care and assistance	(ECOG 3)
30	Severely disabled; hospitalization indicated, although death not imminent	(ECOG 3)
20	Very sick; hospitalization necessary	(ECOG 4)
10	Moribund; fatal process progressing rapidly	(ECOG 4)

APPENDIX E. Comorbid Conditions
Comorbidity score per Sorror, et al.

Comorbidity	Definitions of comorbidities included in the new HCT-CI	HCT-CI weighted scores
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1
Cardiac‡	Coronary artery disease,§ congestive heart failure, myocardial infarction, or EF ≤ 50%	1
Inflammatory bowel disease	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 × ULN, or AST/ALT > ULN to 2.5 × ULN	1
Obesity†	Patients with a body mass index > 35 kg/m^2	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 transplantation	2
Moderate pulmonary‡	DLco and/or FEV_1 66%-80% or dyspnea on slight activity	2
Prior solid tumor‡	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer	3
Heart valve disease	Except mitral valve prolapse	3
Severe pulmonary‡	DLco and/or FEV_1 ≤ 65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic‡	Liver cirrhosis, bilirubin > 1.5 × ULN, or AST/ALT > 2.5 × ULN	3

*composite score obtained by summing total points
*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.
*psychiatric disturbance: only those with psychiatric disturbance (such as depression and/or anxiety) requiring therapy or treatment will be assigned a point on this measure.
Non-relapse mortality per Sorror, et al

	Training set			Validation set		
		NRM			NRM	
Score	Patients, %	HR* (95% CI)	2-year, %	Patients, %	HR* (95% CI)	2-year, %
0	38	1	9	38	1	14
1	17	1.66 (0.9-3.1)	14	18	1.57 (0.7-3.3)	22
2	17	3.48 (2.0-6.0)	27	17	1.26 (0.6-2.8)	19
3	17	6.09 (3.7-10.1)	41	15	3.95 (2.1-7.5)	41
4 or more	11	6.93 (4.0-12.0)	43	13	3.05 (1.5-6..2)	40

APPENDIX F

Translational research Introduction:
The goals of correlative studies are to test the pharmacodynamics of PANO administered in for GVHD prevention. We will determine whether PANO abrogates HCT induced cytokine storm, if there is a correlation between HDAC enzymatic activity inhibition and clinical GVHD prevention; we will establish whether there is enhanced proteins/histones acetylation in T-cell subsets that may be responsible for GVHD prevention and effects on immune reconstitution. Samples will be collected with the AM blood draw whenever possible. Samples will be labeled with the patient's name and Moffitt unique patient number (UPN), date and time of draw, and transported immediately by courier to the Moffitt Cancer Center Research laboratory no. 22324, in the Stabile Building at the Moffitt Cancer Center and/or to Novartis pharmaceutical (If PK performed. Samples acquisition, results and statistics will be recorded into a Microsoft Excel database.

Data for each correlative study performed from the whole study population (patients receiving TAC+SIR +PANO) will be presented over time and compared to data from similar "external control" patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO. We are going to accrue **n=20** subjects as "external control". These patients will be consented with an specific "external control" consent.

Immune Reconstitution after TAC/SIR/PANO:

To describe the kinetics of total T cells, CD4+ (T helper) and CD8+ (T cytotoxic), Th1, Th2, Th17, regulatory T-cells (Tregs), CD19+ (B cells), CD56 (NK cells) and DCs cell subsets recovery following TAC/MTX/PANO treatment FACS analysis will be performed on lysed whole blood samples with previously established multicolor antibodies panels. FACS Calibur flow cytometer will be used for acquisition and analysis will be performed with FlowJo software. All appropriate compensation tubes, including fluorescence minus 1, will be prepared from normal donor blood cells. The absolute lymphocyte subset counts will be determined using the formula: (absolute subset count) x (percentage of events positive for the TEST). To test circulating monocytes and/or DCs we will establish a lineage negative (Lin-) panel consisting of CD3, CD19, CD16, 14 and CD56 antibodies. Monocytes will be characterized by CD14+, 16+ (or CD16-), CD11c-, CD86- and CD123 low/neg cells. Dentritic cells subsets will be gated on the Lin- and HLA-DR+ gate. Monocytes derived DCs (CD11c+, CD86+, CD123-), plasmocytoid DC (CD11c-, CD86+, CD123++) and 6-sulfoLacNac DC (slanDC) (M-DC8+/1c-/11c+14- 16+ 45RA+ 5aR+).

In addition we will test for type-2 myeloid dendritic cells by BDCA-3 (CD141) expression. Absolute cell counts will be enumerated using the Becton Dickinson TruCOUNT tubes with MultiSET software. Flow cytometry and absolute cell counts will be enumerated before PANO administration (days -30 to -5, 15(+/-3), 28(+/-3), 90(+/-14) and 365(+/-45) in order to determine changes in the absolute cell numbers over time. In a group of patients we will correlate the flow cytometry findings with Western blot acetylation analysis of PBMC at the same time points. Data for each cell subset from the whole study population will be presented over time and compared to data from similar external control patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

-

Histone acetylation of T cells and DC subsets with TAC/SIR/PANO

To determine the level of protein acetylation and more specifically of hyper-acetylated histones (H3 and/or H4) in total T cells, T-regs, CD4/CD8 and DC subsets in peripheral blood we will use multi parameter flow cytometry assay. In a group of patients we will correlate the flow cytometry results by histone acetylation measured by Western blot analysis of PBMC. We speculate that TAC/SIR/PANO will lead to an overall increase in the number of acetylated proteins. It remains to be determined which T-cell subsets will be more susceptible to HDACi with PANO at this point. In summary, whole blood will be collected and separated in 100µL aliquot and/or PBMC (5-10x10^6) will be obtained after Ficoll-Paque or Buffy coat/RBC lysis separation. For multiparameter flow staining, an aliquot containing at least 1 x 10^6 cells will be used per tube if possible. Cells will be washed in PBS and fixed with 0.4% paraformaldehyde in PBS for 5-10 minutes at 37ºC and washed with buffer. The fixed cells will be re-suspended in permeabilization buffer for 5 minutes (0.4% Triton X-100 in wash buffer) and then washed. After fixation and permeabilization, cells will be stained with anti-acetylated-lysine or anti-acetylated histone antibodies as well as with a variety of lymphocytes markers (CD4, CD8, CD3, CD25 and/or CD117) and apoptosis detection markers including annexin, 7-AAD and/or caspase 3. Some antibodies may be directly labeled with fluorochrome prior to their use to minimize secondary antibody staining background. All compensation tubes, including fluorescence minus 1, will be prepared from normal donor blood cells. Test will be done before PANO administration on day -5, and on days 30 to -5, 15(+/-3), 28(+/-3), 90(+/-14) and 365(+/-45). Data for histone acetylation and DC subset from the whole study population will be presented over time and compared to data from similar external control patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

T reg immune-reconstitution, Foxp3+ expression and acetylation with TAC/SIR/PANO

T-regs will be measured with the 259D Biosciences Biolegend Foxp3 kit antibody that identifies $CD4^+CD25^+$ Tregs and/or by FACS as determined by the lack of CD127 expression. Since Foxp3 requires cell membrane permeabilization, standard viable dyes are not applicable. The Live/Dead Fixable Dead Cell Violet Stain (L/D) from Molecular Probes will be used for exclusion of dead cells. We propose that one of the benefit conferred by TAC/SIR/PANO-based immune suppression results from apoptosis of alloreactive donor non-Treg CD4+ cells while sparing T regulatory cells that mitigate GVHD risk and promote immune tolerance. Accordingly, we will study the proportion of Treg/total CD4+ cells. We will also measure the expression of *FOXP3* and *IDO* mRNA in PBMC by RT-PCR. Test will be done before PANO administration on day -30 to -5, and on days 30 to -5, 15(+/-3), 28(+/-3), 90(+/-14) and 365(+/-45). Data for T-reg immune reconstitution and acetlylation from the whole study population will be presented over time and compared to data from similar external control patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

Inflammatory cytokine expression profiling with TAC/SIR/PANO

In order to rapidly and accurately measure the expression levels of up to 40 cytokines/chemokines and other relevant proteins in GVHD inflammation (cytokine storm) we will use a human inflammation antibody array assay. The technology is designed based on the sandwich immunoassay principle. A panel of antibodies (capture) is immobilized in the specific spot locations on the surface of membrane. Incubation of array membranes with biological samples results in capturing cytokines by corresponding antibodies. The bound cytokines are detected with a cocktail of biotinylated antibodies. Signals are then visualized using chemiluminescence. We hypothesize that HDACi will reduce the level of expression of pro-inflammatory cytokines like TNF/IFN and increase level of tolerogenic cytokines (TGFb-b /IL-10). We will use a Raybiotech's Human inflammation antibody array (catalog #, AAH-INF -3-8) to detect the following cytokines IL-1, 2, 3, 4, , 6, 7, 8, 10, 11, 12, 13, 15, 16, 17 , IFN, TNF-α, TNF-β, TNF RI, s TNF RII, MCP1-2, M-CSF, MIP, GM-CSF, G-CSF among others proteins.
We will also measure serum levels of soluble IL-2 receptor α using a commercially available ELISA kit. Test will be done before PANO administration on day 30 to -5, 15(+/-3) and 28(+/-3). Data for inflammatory cytokines from the whole study population will be presented over time and compared to data from similar external control patients

treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

GVHD Biomarkers

ELISA-based analysis will be used to determine whether a marker panel comprised of IL-2Rα , TNFR1, HGF, IL-8, Elafin, REG-3α and ST2 levels tested prospectively at 30 to -5, and 15(+/-3) after allogeneic HCT is able to predict for the development of acute GVHD. Test will be done before PANO administration on day –30 to 5, and on days 15+/-1. Data for GVHD biomarkers from the whole study population will be presented over time and compared to data from similar external control patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

Stat-3 activation and acetylation, STAT-5 and S6 phosphorylation

To determine the functional impact of HDACi on PBMCs we will measure total STAT-3, phosphorylated STAT3 and acetylated STAT-3 by WB and flow cytometry in PBMC and gated T/DCs cells populations in patients and external controls on day 30 to -5, 15(+/-3), 28(+/-3), 90(+/-14) and 365(+/-45). T-cell STAT5 and S6 phosphorylation will also be measured among the external controls on day +30+/-3 and day +90+/314. Data for stat -3/stat-5and S6 phosphorylation and/or acetylation subset from the whole study population will be presented over time and compared to data from similar external control patients treated TAC/SIR with identical inclusion and exclusion criteria except that will not be treated with PANO.

Note: The data gathered on these results will be used for future analysis not considered in the context of this proposal. Thus, after identifying changes in phenotype and the expression pattern of immune-related genes upon PANO/TAC/SIR treatment, we will expand our research to investigate the role of PANO in their regulation.

List of Test to be performed in patients treated with TAC+SIR+PANO and "external control patients" treated with TAC+SIR only:

TEST	-30 to -5	15(+/-3)	28(+/-3)	90(+/-14)	365(+/-45)
ImmuneReconstitution (T, DCs and Tregs)	x	x	x	x	x
Histone Acetylation (T and DCs)	x	x	x	x	x
STAT-3 activation and acetylation	x	x	x	x	x
Cytokine profiling	x	x	x		
Biomarker profiling	x	x			

*Note: T-cell STAT5 and S6 phosphorylation will also be measured among the external controls on day +28 (+/- 3) and day +90(+/-10).

Appendix G.

Panobinostat administration as per conditioning regimen. Patients will be treated with PANO 5 MG PO TIW (3 doses about 48 hours apart in a 7 day period) QW for 26 weeks starting prior to stem cell infusion. During the study, PANO will be administered orally as once daily dose. Patients should receive their once-a-day oral dose of PANO approximately at the same time each day in AM. Each dose of PANO should be taken with about 8 oz / 240 ml glass of water. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit or grapefruit juice and seville (sour) oranges during the entire study. If the patient forgets to take his/her dose on scheduled treatment day or cannot take it due to mucositis and/or HCT related GI toxicities (nausea/vomit), then he/she should take PANO on that same day within 16 hours after the missed dose if possible. After more than 16 hours, that day's dose should be withheld, and the patient should wait to take PANO until the next scheduled treatment day (i.e., patients should be instructed not to try to make-up the missed dose after 16 hours). If mucositis and/or GI toxicity preclude PANO oral intake, patient are allowed to hold up to 3 doses and administration should be resumed once mucositis and/or toxicity resolves. Patient should complete missed doses and then continue treatment as planned per protocol extending PANO administration period.

Busulfan and Fludarabine Chemotherapy

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
-5 BU FLU PANO(1)	-4 BU FLU	-3 BU FLU PANO(2)	-2 BU FLU	-1 PANO(3)	0	+1
+2	+3 PANO	+4	+6 PANO	+7	+8 PANO	

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
			-6 BU FLU PANO(1)	-5 BU FLU	-4 BU FLU PANO(2)	-3 BU FLU
-2 PANO(3)	-1	0	+1 *PANO*	+2	+3 *PANO*	+4
+5	+6 PANO	+7	+8 PANO		+10 PANO	

Fludarabine and Melphalan Chemotherapy

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
			-5 FLU/MEL PAN0	-4 FLU/MEL	-3 FLU/MEL PANO	-2 FLU/MEL
-1 Rest PANO	0	+1	+2 PANO	+3	+4 PANO	+5
+6	+7 PANO	+8	+9 PANO	+10	+11 PANO	+12

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
				-5 FLU/MEL PAN0	-4 FLU/MEL	-3 FLU/MEL PANO
-2 FLU/MEL	-1 Rest PANO	0	+1	+2	+3 PANO	+4
+5	+6 PANO	+7	+8 PANO	+9	+10 PANO	+11

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
					-5 FLU/MEL PAN0	-4 FLU/MEL
-3 FLU/MEL PANO	-2 FLU/MEL	-1 Rest PANO	0	+1	+2 PANO	+3
+4	+5 PANO	+6	+7 PANO	+8	+9 PANO	+10

Fludarabine and Melphalan Chemotherapy

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
						-5 FLU/MEL PAN0
-4 FLU/MEL	-3 FLU/MEL PANO	-2 FLU/MEL	-1 Rest PANO	0	+1	+2
+3 PANO	+4	+5	+6 PANO	+7	+8 PANO	+9
+10	+11 PANO	+12	+13 PANO		+15 PANO	

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
5 FLU/MEL PAN0	-4 FLU/MEL	-3 FLU/MEL PANO	-2 FLU/MEL	-1 Rest PANO	0	+1
+2	+3 PANO	+4	+5 PANO	+6	+7 PANO	+8
+9	+10 PANO	+11	+12 PANO	+13	+14 PANO	+15

Busulfan and Fludarabine Chemotherapy

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
-5 BU FLU PANO(1)	-4 BU FLU	-3 BU FLU PANO(2)	-2 BU FLU	-1 PANO(3)	0	+1
+2	+3 PANO	+4	+6 PANO	+7	+8 PANO	

Sunday	Monday	Tuesday	Wed	Thursday	Friday	Saturday
			-6 BU FLU PANO(1)	-5 BU FLU	-4 BU FLU PANO(2)	-3 BU FLU
-2 PANO(3)	-1	0	+1 *PANO*	+2	+3 *PANO*	+4
+5	+6 PANO	+7	+8 PANO		+10 PANO	