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Phase II Trial of Inhibition of Dipeptidyl Peptidase (DPP)-4 with Sitagliptin for the Prevention of Acute Graft-versus-Host Disease Following Allogeneic Hematopoietic Stem Cell Transplantation

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1.0 BACKGROUND

Acute graft-versus-host disease (GvHD) remains one of the most significant causes of morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT) for hematological malignancies. Using standard GvHD prophylaxis regimens, the incidence of grade II-IV acute GvHD ranges from 35-50% with human leukocyte antigen (HLA)-matched related donors, and 40-70% with unrelated donors.[1-3] While acute GvHD can be eliminated through the use of *in vivo* or ex-vivo depletion of T cells, relapse increased. However, using T cell-replete grafts, the effect of acute GvHD on the competing risks of transplant-related mortality (TRM) and relapse incidence has been clarified to a significant extent by a large analysis of 4,174 patients undergoing HLA-matched sibling transplants.[4] In this large study, although GvHD was protective against relapse, patients developing severe (grades II-IV) GvHD had a net inferior survival to patients without acute GvHD or developing only grade I (mild) acute GvHD,[4] indicating that the effect of severe GvHD more than mitigated the protective effects on relapse. Therefore, novel strategies preventing grades II-IV acute GvHD are likely to improve overall outcome of patients undergoing allogeneic HSCT.

1.1 Dipeptidyl peptidase (DPP)-4

DPP-4 is a homodimeric type II transmembrane glycoprotein identical to leucocyte surface antigen CD26, and is also present in a soluble enzymatically active for form in plasma. CD26/DPP-4 has dipeptidylpeptidase activity that selectively removes the N-terminal dipeptide from peptides with proline or alanine at the penultimate position. DPP-IV is involved in a broad range of biological processes, including modulation of insulin release and metabolism,[5] modulating stromal-derived factor (SDF)-1 important in homing and engraftment of stem cells,[6] hematopoietic cytokines,[6] and T immune functions.[7,8] Specific DPP-4 inhibitors (e.g., sitagliptin) are now clinically available and approved for type 2 diabetes mellitus. We are investigating the clinical efficacy of sitagliptin to enhance engraftment of umbilical cord blood (UCB) transplantation.[9]

In the immune system, CD26/DPP-4 is expressed on a specific population of $CD4^+CD45RO^+$ memory T cells, and is upregulated after T cell activation.[10-12] CD26 is also associated with T cell signal transduction processes as a co-stimulatory molecule (Figure 1).[7] Crosslinking of CD26 and CD3 with solid phase immobilized monoclonal antibodies (but not CD3 alone) enhances T cell co-stimulation, proliferation, and IL-2 production.[12,13] Further studies demonstrated that recombinant soluble CD26 (rsCD26) enhances proliferative responses of peripheral blood lymphocytes to stimulation with soluble antigen, and that this effect requires DPP-4 enzymatic activity.[8] Subsequently it was shown that the target cells of rsCD26 were CD14+ monocytes, and rsCD26 up-regulates CD86 on monocytes.

The function of CD26/DPP-4 in T cell activation is shown in Figure 1. The ligand for CD26/DPP-4 is caveolin-1, an integral membrane protein, expressed on a wide variety of cells, including antigen-presenting cells (APC).[8,14] In APC, loaded antigens are trafficked in the cell through caveolae (vesicular invaginations of the membrane), and caveolin-1 is transported along with the peptide-MHC complex to the cell surface where it is expressed for interaction with cognate T cells.[8,15] Upon binding CD26/DPP-4 on T cells, caveolin-1 is phosphorylated, which leads to the up-regulation of CD86 and enhanced co-stimulation of T cells, as described in Figure 1.[16,17] CD26-medated T-cell signaling, therefore, provides a positive co-stimulatory loop for action of CD28.[17] Within T cells, CD26/DPP-4-caveolin-1 leads to T cell proliferation and IL-2 production (Figure 1).

CD26+ lymphocytes are reported to be increased in blood of acute GvHD patients, and infiltrate tissues involved by acute GvHD.[18] In mouse models, depleting monoclonal antibodies against CD26 prevent GvHD.[18] However, as DPP-4 enzymatic activity of CD26 is required for interaction with caveolin-1, and in turn APC/T-cell signaling, we hypothesize that inhibition of DPP-4 enzymatic activity may be a novel therapeutic approach for preventing acute GvHD.

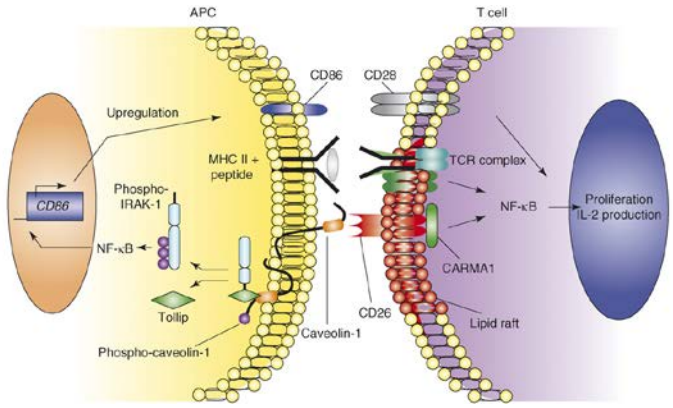


Figure 1. Co-stimulatory function of CD26/DPP-IV in T cell activation. After antigen uptake, a portion of caveolin-1 is exposed on the outer cell surface and aggregates in the APC-T cell contact area in lipid rafts. Aggregated caveolin-1 then binds its specific ligand, CD26, resulting in caveolin-1 phosphorylation. Phospho-caveolin-1 transduces a signal into the APCs, leading to dissociation of IRAK-1 and Tollip, followed by activation of NF-kB, and finally resulting in CD86 upregulation and T cell costimulation. In T cells, binding by specific MHC-peptide complexes leads to TCR signal transduction. Additionally, caveolin-1 on the APC ligates CD26 dimers on the T cell surface, resulting in the recruitment of lipid rafts in the plasma membrane and the recruitment of CARMA1 to the cytosolic portion of CD26. Ultimately, these steps lead to the activation of NF-kB, to T cell proliferation, and to IL-2 production. Enzymatic DPP-IV activity is required for these functions. (Adapted from *Trends in Immunology* 2008, 29: 295-301).

1.2 Sitagliptin: a specific DPP-4 inhibitor approved for clinical use

Sitagliptin is an approved DPP-4 inhibitor for clinical use. It is available in an oral formulation only and has been approved by the Food and Drug Administration (FDA) for treatment of type 2 diabetes mellitus.[19] As antihyperglycemic agents, DPP-4 inhibitors increase active levels of incretin peptides, including glucagon-like peptide (GLIP)-1 and glucose-dependent insulinotropic peptide (GIP). The beneficial effects of GLIP-1 and GIP on glucose homeostasis are limited by the short half-life of these peptides due to rapid inactivation by DPP-4. Sitagliptin has been found to be highly selective for DPP-4, and demonstrates at least a 2600-fold margin over its activity against the closely related enzymes DPP-8 and DPP-9.[20] This is important as in animal studies inhibition of DPP-8 or 9 by selective inhibitors or by non-selective DPP-4 inhibitors was associated with multiorgan toxicity.[21]

Pharmacology of sitagliptin: Sitagliptin phosphate is chemically described as 7-[(3*R*)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3 (trifluoromethyl)-1,2,4-triazolo[4,3-a] pyrazine phosphate (1:1) monohydrate. Sitagliptin enhances the effects of the incretin hormones GIP and GLIP-1, which are secreted in in response to food and have a role in regulating glucose homeostasis.[22] Activation of GIP and GLIP-1 receptors on pancreatic β-cells leads to increased levels of cyclic AMP and intracellular calcium with subsequent glucose dependent insulin secretion. Also, sustained receptor activation leads stimulation of β-cell proliferation and resistance to apoptosis. GIP and GLIP-1 are rapidly inactivated by DPP-4, and following administration of sitagliptin, post-prandial levels of active GLIP-1 are increased and activity is prolonged. While the effect on glucose homeostasis has been the clinical driving force for developing sitagliptin in Type 2 diabetes mellitus, DPP-4 also modulates other biological activities. In addition to and independent of its enzymatic activity in plasma, DPP-IV is a membrane-spanning peptidase that is widely distributed in numerous tissues and T-cells, B-cells, and natural killer cells.

Sitagliptin is a potent, reversible inhibitor of DPP-4/CD26. In vitro studies show that sitagliptin has high selectivity for DPP-4 (IC_{50}, 18 nM). Affinity for other proline-specific peptidases, DPP-8 (IC_{50}, 48,000 nM) and DPP-9 (IC_{50}, >100,000 nM), is low.[20] Low affinity for these peptidases is of particular importance since in preclinical studies, inhibition of DPP-8 and DPP-9 has been associated with severe toxicities, including alopecia, blood dyscrasias, multi-organ

histopathologic changes, and mortality in rats; and gastrointestinal toxicity in dogs.[21] Likewise, whereas other nonselective DPP-4 inhibitors have been associated with the development of necrotic skin lesions in preclinical studies involving monkeys, no treatment-related skin toxicity was observed in a 3-month study in monkeys treated with sitagliptin.

Pharmacokinetics and pharmacodynamic studies in normal volunteers and patients with diabetes mellitus: Sitagliptin has been tested extensively in healthy volunteers and in patients with type 2 diabetes mellitus and found to be safe and well tolerated.[19,23-25] Importantly, as incretin stimulation of insulin release is glucose dependent, the risk of hypoglycemia is minimal and has not been observed in healthy volunteers given high doses (600 mg/day) of sitagliptin.[24] Hypoglycemia, however, may occur when sitagliptin is administered in combination with an insulin secretagogue (e.g., sulfonylureas) or insulin therapy, indicating the need for close monitoring [26]

The *pharmacokinetics* of sitagliptin in healthy subjects has been reported to be comparable with those observed in patients with type 2 diabetes.[24,25] Key pharmacokinetic parameters in healthy subjects provided by the manufacturer are summarized in Table 1. The sitagliptin plasma area under the curve (AUC) is increased in a dose-dependent manner following both single dose (1.5-600 mg)[25] and multidose (25-600 mg QD and 300 mg BID)[23] in healthy volunteers. Absorption is not appreciably affected by food, and sitagliptin may be dosed without regard to meals.

Sitagliptin does not appear to undergo extensive metabolism. Studies of metabolism and excretion of [^{14}C]-sitagliptin in healthy subjects indicated that the parent drug comprised the majority of plasma (78-90%) and urinary (84-88%) radioactivity.[27] Six metabolites were detected in small amounts, but have been found to have several hundred-fold less activity against DPP-4 and are not, therefore, believed to contribute to the pharmacologic activity of sitagliptin.[27] Approximately 80% of the dose is cleared by the kidneys, and renal clearance is independent of dose.[23,25] Moderate hepatic impairment has minimal effect on sitagliptin pharmacokinetics.[28]

Table 1. Pharmacokinetic parameters in healthy subjects

Parameter	Value
Bioavailability	87%
Volume of distribution	~198 L
Protein binding	38%
T_{max}	1-4 hours
Metabolism	Minimal hepatic metabolism
Elimination	87% urine (~79% unchanged);
	13% feces
Apparent terminal $t_{1/2}$	~12.4 hours
Renal clearance	~350 ml/min

In clinical studies involving healthy volunteers, treatment with sitagliptin was associated with dose-dependent inhibition of DPP-IV activity.[23,25] Furthermore, in rodent models of diabetes, near maximal glucose lowering effects have been observed with 80% or greater inhibition of plasma DPP-4 activity.[29] In a study of 70 healthy normal volunteers receiving multiple oral doses (up to 600 mg per day) of sitagliptin for up to 10 days have shown that although the terminal half-life of the drug ranged from 11.8 to 14.4 hours, sustained inhibition of plasma DPP-4 enzyme activity for at least 24 hours after each dose was observed only at higher doses.[23] Sitagliptin produced a dose-dependent inhibition of plasma DPP-4 enzyme activity, with greater than 90% inhibition seen at the highest dose of 600 mg/day. [23]

Reported adverse events of sitagliptin in patients with diabetes mellitus: Reported clinical evaluation of sitagliptin has been essentially restricted to patients with type 2 diabetes mellitus. In all large clinical trials reported, sitagliptin has been very well tolerated. In a phase II, randomized, double blind, placebo and active-controlled parallel group study, where treatment was for 12 weeks, there was no difference in the incidence of adverse events between the sitagliptin (up 100 mg per day) and placebo groups.[30] In a phase III trial comparing sitagliptin 100 mg/day, sitagliptin 200 mg/day, or placebo for 24 weeks, the incidence of drug-related adverse events were slightly higher in the sitagliptin treated patients and were reported in 9.7%, 10.8%, and 7.5%, respectively.[31] The adverse events occurring at a frequency of 2% or more and with a higher incidence in one or both of the sitagliptin groups compared to placebo, included constipation, nasopharyngitis, pharyngitis, pharyngolaryngeal pain, urinary tract infection, myalgia, arthralgia, hypertension, and dizziness.[31] A slight increase (4.2% and 4.7%) in white blood cell count was reported in the sitagliptin arms, but was not considered clinically significant. Similar mild biochemical changes, including a 3-4% increase in creatinine kinase (CK) and a 3-4 IU/ml decrease in alkaline phosphatase were also observed in patients treated with sitagliptin, but were not of any clinical significance.[31] In a second Phase III double-blind, placebo controlled trial comparing sitagliptin at doses of 100 mg/day, 200 mg/day, and placebo for 18 weeks, drug-related clinical adverse events were slightly higher in the *placebo* treated group (10.2%, 8.3% and 17.3%, respectively).[32] No serious adverse events were reported during the study. There was no significant difference in the incidence of hypoglycemia between groups. Adverse events occurring at a higher frequency in the sitagliptin groups compared with placebo were nasopharyngitis, back pain, osteoarthritis, and pain in extremities.[32] Clinically insignificant changes in white blood cell count (increase of 5-10% from baseline), alkaline phosphatase (decrease of 5-10% from baseline), and in uric acid (increase of ~12 µmol/l) were observed in patients treated with sitagliptin.

In the **post-marketing** experience, reports of acute pancreatitis, acute renal failure, hypoglycemia (when sitagliptin has been used concurrently with insulin secretagogues or insulin), and allergic and hypersensitivity reactions have been reported (Januvia Prescribing Information, Merck Sharp & Dohme Corp.). While case reports have documented acute pancreatitis in patients receiving sitagliptin, a large analysis of pooled clinical trials failed to confirm that the incidence of this adverse event occurs more commonly among diabetic patients treated with sitagliptin.[33]

1.3 Clinical results of DPP-4 inhibition using high-dose sitagliptin

We have studied the clinical effect of DPP-4 inhibition using high doses of the specific inhibitor sitagliptin for enhancing the engraftment of umbilical cord blood (UCB) transplants. In a pilot trial, we recently reported the safety of high-dose sitagliptin (600 mg per day) in the setting of myeloablative chemoradiotherapy and allogeneic transplantation using UCB.[9] Pharmacokinetics (PK) and pharmacodynamic studies associated with this trial showed that while the PK parameters of sitagliptin appeared to be similar to those previously reported in patients with diabetes mellitus and normal volunteers,[23] the dose of 600 mg per day used did not result in sustained inhibition of plasma DPP-4 as was expected.[23] Further, the kinetics of engraftment was significantly associated with the extent of plasma DPP-4 inhibition,[9] suggesting that plasma DPP-4 inhibition may be a good surrogate for the efficacy of sitagliptin in this context. Pharmacokinetic-pharmacodynamic modeling indicated that improved inhibition of DPP-IV could be better achieved using multiple daily dosing.[34]

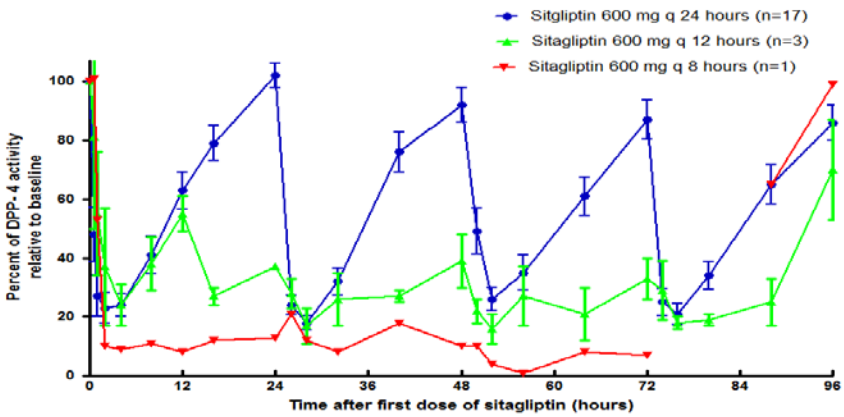


Figure 2. Plasma DPP-4 activity (mean values ± standard error) as a percentage of baseline following different sitagliptin dosing schedules. Inhibition of DPP-4 activity was not sustained using 600 q 24 hours. In a dose-escalation study, sitagliptin given q 12 or q 8 hours resulted in greater inhibition of DPP-4 activity.

Based on the pharmacodynamic data from the pilot trial, a dose-escalation study was subsequently performed, testing sitagliptin doses of 600 mg every 12 hours, and 600 mg every 8 hours. While sitagliptin dosing of 600 mg every 12 hours was well tolerated, grade 5 dose-limiting toxicity (capillary leak syndrome and multiorgan failure) was observed at 600 mg every 8 hours.[35] More sustained inhibition of plasma DPP-IV activity was observed with twice daily dosing of sitagliptin (Figure 2).[35] Based on these studies, we have initiated a phase II clinical trial of *in vivo* DPP-4 inhibition using sitagliptin at 600 mg every 12 hours starting Day -1 through Day +3 (total 10 doses) in adult patients with hematological malignancies undergoing UCB transplantation, with the engraftment as the primary endpoint (ClinicalTrials.gov identifier: NCT01720264). The results from the first 8 patients treated at the higher dose have confirmed the safety of 600 mg every 12 hours dosing of sitagliptin, with no toxicity related to sitagliptin observed.

While not the primary endpoint of the pilot study, an important observation was the very low incidence of acute GvHD.[9] Of 17 patients receiving one and two antigen mismatched UCB transplants, and treated with the specific DPP-IV inhibitor sitagliptin, only 1 (6%) patient developed acute grade II GvHD on day +259 while tapering immunosuppression after a median follow-up of 259 (range, 84-736) days at time of reporting.[9] This low incidence of severe acute GvHD is significantly lower than previously reported for patients receiving UCB transplants, even when anti-thymocyte globulin (ATG) has been used as part of the preparative regimen.[36-38] The remarkably low incidence of severe GvHD observed in this trial has prompted us to take our clinical observations back to the bench to further study DPP-4 as a potential target for prevention of acute GvHD.

1.4 Preclinical results for DPP-4 as a target for GvHD

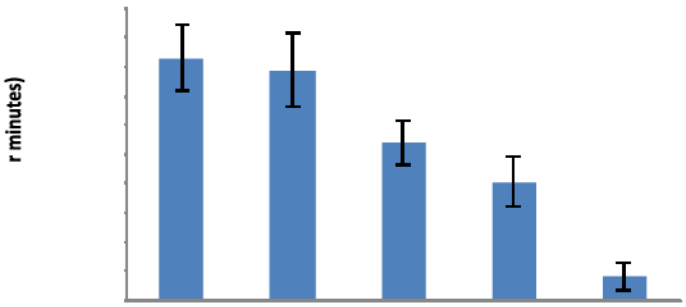


Figure 3. Sitagliptin inhibits alloreactive T cell proliferation in mixed lymphocyte reactions in a dose-dependent manner (see text).

Sitagliptin inhibits T cell activation in response to allogeneic stimulation. We assessed the proliferation of T cells in mixed lymphocyte reactions using monocytes as stimulator cells, both obtained from random donor buffy coats obtained from the Indiana Blood Center. Freshly (immuno-magnetically) purified $CD3^+$ T cells were treated different concentration (150-1500 ng/ml; concentrations we have easily and safely achieved *in vivo* in allogeneic transplant patients[9]) with sitagliptin for 30 minutes, washed, and then co-cultured with allogeneic irradiated peripheral blood mononuclear cells (PBMC) for 4 days. Proliferation in T cells was assessed by H3-thymidine uptake (added to last 24 hours of culture). As shown in Figure 3, there was a dose-dependent inhibition of T cell proliferation with sitagliptin. Treatment of PBMC stimulator cells, as opposed to T cells, with sitagliptin had no effect on proliferation (not shown).

Donor T cells from $CD26^{-/-}$ knockout mice induce less GvHD in transplanted mice. In a well-established MHC-mismatched (BL6□BALB/c) mouse transplant model, BALB/c recipient mice were lethally irradiated (800 cGy) and transplanted with $2x10^6$ bone marrow (BM) cells from donor wild type (WT) or $CD26^{-/-}$ BL6 mice, with or without $5x10^6$ purified $CD3^+$ spleen cells (n=10 per group). As shown in Figure 4, BLAB/c mice receiving BL6 $CD26^{-/-}$ BM alone remained healthy and free of GvHD. While all BALB/c mice who received BM + $CD3^+$ spleen from WT donors rapidly died from GvHD within 20 days, there was significantly protection from GvHD in BALB/c mice receiving BM + $CD3^+$ spleen cells from $CD26^{-/-}$ BL6 mice, suggesting that CD26 on donor T cells plays a significant role in mediating acute GvHD. Unlike in human recipients,[9] sitagliptin (and other DPP-4 inhibitors such as Diprotin A) block DPP-4 enzymatic activity for only 2-3 hours (i.e., significantly less sustained enzymatic blockade) in this mouse model requiring frequent daily dosing by gavage making such in vivo preclinical testing not feasible. Nonetheless, the aggregate of our clinical, ex-vivo MLR studies, and in vivo results in CD26-/- mouse transplant studies, shown above, strongly suggest that blocking CD26/DPP-4 activity will likely also protect from GvHD.

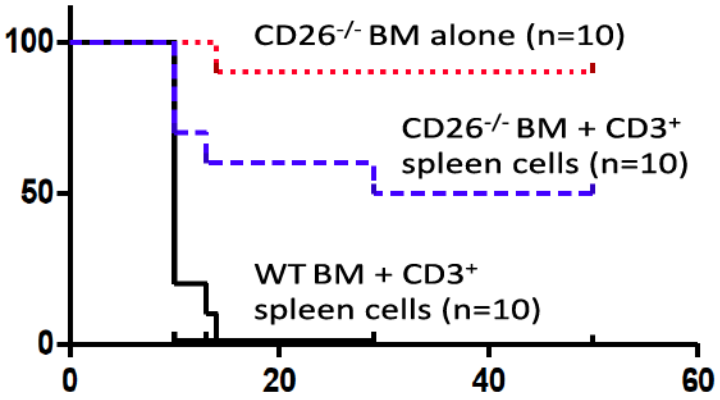


Figure 4. CD26 knockout (CD26-/-) BL6 donor CD3 cells cause significantly less GvHD and improved survival compared with WT BL6 CD3 cells in BL6➔BALB/c mouse transplant model ($P<0.01$) (see text)

1.5 Rationale for a current clinical trial of sitagliptin for prevention of acute GvHD

The combination of sirolimus and tacrolimus is an accepted standard regimen for prevention of acute GvHD in patients undergoing allogeneic hematopoietic cell transplantation,[39,40] and has been the institutional standard regimen at Indiana University since 2007 for patients undergoing myeloablative transplants for hematological malignancies. A randomized phase III trial comparing sirolimus and tacrolimus with tacrolimus and methotrexate showed the regimens to be equivalent for prevention of acute grades II-IV GvHD, although the sirolimus containing regimen was associated with less of the most severe (grades III-IV) acute GvHD,[39] with no increase in relapse. A recent meta-analysis has also confirmed the potential of the sirolimus and tacrolimus regimen to reduce the incidence of severe acute GvHD.[40] The reported frequency of grades II-IV acute GvHD by day 100 after transplantation following sirolimus and tacrolimus prophylaxis has varied in different series and trials (up to 43%), with the efficacy of the regimen being similar in both matched related and well-matched unrelated donor transplants.[40] Therefore, the development of a novel regimen with a potential to reduce the incidence of grades II-IV acute GvHD to less than 20% to be a significant advance and be worthy of further study in future comparative studies.

Most of what is known about the pathophysiology of GvHD has been gained largely from mouse models. Acute GvHD is initiated by infused donor T cells that recognize alloantigen expressed by recipient antigen presenting cells (APCs).[41-43] This recognition results in the activation, differentiation, and expansion of donor T cells in the secondary lymphoid tissues and subsequent migration to target organs to cause profound tissue damage.[42,44-47] Therefore, it is expected that this event occurs quite early after transplant when host APCs are still present, before they are replaced by donor-derived cells. Indeed, several studies have shown that activation of alloreactive T cells that induce acute GvHD begins quite early post-transplantation following radiation or high-dose chemotherapy,[48] with highest levels of expansion of donor dendritic cells (DCs), known to play a key role in inducing acute GvHD, occurring by day +3, and highest levels of inflammatory cytokines (interleukin-2, interferon-γ, and tumor necrosis factor-α) occurring at day +5.[48] Further, the number of DCs recovered to normal by day +21. While the expansion and activation kinetics of immune cells in human acute GvHD development are not known, an early occurrence of activation of alloreactive T cells is consistent with our clinical observation in UCB transplants where short term sitagliptin administration appeared to reduce the incidence of acute GvHD.[9] Therefore, in the absence of definitive kinetics data in the human, and based on data derived from mouse models, we propose to continue sitagliptin dosing in this trial until day +14 after transplantation.

As noted above, there is strong rationale from our pre-clinical data and clinical observation in UCB transplantation to study sitagliptin for prevention of GvHD. We have shown that high-dose sitagliptin at a dose of 600 mg every 12 hours is safe and well-tolerated in the setting of myeloablative conditioning, and produces acceptable inhibition of DPP-4 activity *in vivo* (as performed under IND 114587). Therefore, in this trial we propose to test short-term sitagliptin in combination with standard sirolimus and tacrolimus for prevention of severe acute GvHD.

2.0 OBJECTIVES

2.1 Primary Objective

Evaluate the efficacy of sitagliptin in reducing the incidence of grade II-IV acute GvHD by day +100 post-transplant in patients undergoing allogeneic hematopoietic stem cell transplantation and receiving standard sirolimus and tacrolimus GvHD prophylaxis. We will test the null hypothesis H0: $p_0 \geq 0.30$ versus the alternative H1: $p_1 < 0.15$, where p is the probability of grades II-IV acute GvHD by day +100.

2.2 Secondary Objectives

The following descriptive secondary objectives will be studied:

- Describe the tolerability and potential toxicity of sitagliptin.
- Describe the cumulative incidence of grades II-IV acute GvHD by day +100.
- Describe the cumulative incidence of grades III-IV acute GvHD.
- Describe the engraftment kinetics of absolute neutrophil count and platelets.
- Describe the incidence of infections occurring during the 100 days post-transplant.
- Describe non-relapse mortality (NRM) at day +30, +100, and 1 year post-transplant.
- Describe overall survival.
- Describe the incidence of chronic GvHD.
- Describe the cumulative incidence of relapse of the primary hematological malignancy.

2.3 Exploratory Objectives

The following exploratory studies will be conducted (see Section 13.0):

- Describe immune cell reconstitution
- Describe changes in plasma soluble CD26 antigen (sCD26) and plasma DPP-4 activity from baseline in patients undergoing allogeneic transplantation, as a possible biomarker for development of GvHD
- Explore for any association between inhibition of plasma DPP-4 activity following sitagliptin administration and development of acute GvHD by comparing activities in patients who develop GvHD with those who do not.

3.0 ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

3.1.1 Patients with any of the following hematologic malignancies:

- Acute myeloid leukemia (AML) with any of the following:
  - In first remission (CR1) with intermediate risk or high-risk cytogenetic and/or molecular features.
  - Patients in second or subsequent complete remission (CR2, CR3, etc.).
  - Primary refractory or relapsed AML with no more than any one of the following adverse additional features according to modified CIBMTR criteria:[49]
    - Duration of first CR < 6 months
    - Poor risk cytogenetics or molecular features (FLT-3 internal tandem duplication (ITD); complex karyotype with ≥3 clonal abnormalities, 5q-/-5, 7q-/-7, 11q23 abnormalities, inv(3), monosomal karyotype)
    - Circulating peripheral blood blasts at time of enrollment
    - Karnofsky performance status <90%
- Acute lymphoblastic leukemia (ALL) with any of the following:
  - In CR1 or subsequent complete remission (CR2, CR3, etc.)
  - Primary refractory or relapsed ALL with no more than one of the following adverse features according to modified CIBMTR criteria:[49]
    - Second or subsequent relapse

- Bone marrow blasts >25% at time of enrollment
- Age >40 years

- Myelodysplasia with a Revised International Prognostic System Score (IPSS-R) of greater than 3 at the time of evaluation for transplantation (see Table below).

Variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good		Good		Intermediate	Poor	Very poor
BM blasts(%)	≤ 2		>2 to <5		5 to 10	>10	
Hb (g/dl)	≥ 10		8 to <10	<8			
PLTS (x10^9/l)	≥100	50 to <100	<50				
Neutrophils (x10^9/l)	≥0.8		<0.8				

IPSS-R cytogenetics prognostic grouping to assign sub-score:

- Very Good: -Y, del(11q)
- Good: normal karyotype, del(5q), del(12p), del(20q)
- Intermediate: del(7q) as single abnormality, +8, +19, any other abnormality not in other categories
- Poor: -7, inv(3), double abnormality including -7/del(7q), complex with 3 abnormalities per clone
- Very Poor: complex karyotype with more than 3 abnormalities per clone

- Chronic myelogenous leukemia (CML) with one of the following criteria:
  - Accelerated phase, defined by any of the following:
    - Blasts 10-19% in peripheral blood white cells or bone marrow
    - Peripheral blood basophils at least 20%
    - Persistent thrombocytopenia (<100 x 10^9/l) unrelated to therapy, or persistent thrombocytosis (>1000 x 10^9/l) unresponsive to therapy
    - Increasing spleen size and increasing white blood cell (WBC) count unresponsive to therapy
    - Cytogenetic evidence of clonal evolution (i.e., the appearance of an additional genetic abnormality that was not present in the initial specimen at the time of diagnosis of chronic phase)
  - Chronic phase provided a complete hematologic remission was not achieved by 3 months or a complete cytogenetic remission by 18 months and the patient had received at least 2 tyrosine kinase inhibitors.
- Patients with aggressive non-Hodgkin's lymphoma (NHL), including diffuse large cell lymphoma, mediastinal B-cell lymphoma, transformed lymphoma, mantle cell lymphoma, and peripheral T cell lymphoma, who also have one of the following criteria:
  - Failure to achieve complete remission to primary induction therapy
  - Relapsed and refractory to at least one line of salvage systemic therapy
  - Failed stem cell collection
- Patients with Hodgkin's lymphoma meeting one of the following criteria:
  - Primary refractory (failure to achieve complete remission to primary induction therapy)
  - Relapsed and refractory to at least one line of salvage systemic therapy
  - Failed stem cell collection

3.1.2 Patient age ≥ 18 to ≤ 60 years

3.1.3 Karnofsky Performance status ≥ 70%
3.1.4 Patients must also receive a full myeloablative preparative regimen (Patients treated with either total body irradiation (TBI)-based or high-dose chemotherapy only regimens are eligible other than high-dose busulfan containing regimens or regimens that include anti-thymocyte globulin or other T cell depleting antibodies).
3.1.5 Patients receiving allogeneic peripheral blood stem cell (PBSC) grafts from HLA-matched (5/6 and 6/6 matches) siblings or from well matched unrelated donors (9/10 or 10/10 matches at HLA-A, B, C, DRB1 and DQB1 by high resolution typing) are included. All grafts will be unmanipulated (i.e., no T cell depleted or CD34 selected grafts).
3.1.6 No uncontrolled bacterial, viral or fungal infection at time of enrollment defined as currently taking medication and progression of clinical symptoms
3.1.7 No HIV disease (Patients with immune dysfunction are at a significantly higher risk of infection from intensive immunosuppressive therapies.)
3.1.8 Non-pregnant and non-nursing
3.1.9 Required baseline values within 60 days prior to admission:
- LVEF ≥ 45%
- DLCO ≥ 50% of predicted (corrected for hemoglobin)

3.1.10 Required baseline laboratory values within 16 days prior to admission:
- Estimated creatinine clearance ≥60 ml/min
- Serum total bilirubin ≤ 2 x upper limit of normal value (ULN)
- AST and ALT ≤ 2 x ULN (unless determined by treating physician to be related to underlying malignancy)

3.1.11 Signed written informed consent (Patient must be capable of understanding the investigational nature, potential risks and benefits of the study, and able to provide valid informed consent.)
3.1.12 Patients must otherwise fulfill institutional criteria for eligibility to undergo myeloablative allogeneic stem cell transplantation

3.2 Exclusion Criteria

3.2.1 Symptomatic uncontrolled coronary artery disease or congestive heart failure
3.2.2 Severe hypoxemia with room air PaO2 < 70, supplemental oxygen dependence, or DLCO < 50% predicted
3.2.3 Patients with active central nervous system involvement
3.2.4 Prior allogeneic or autologous hematopoietic stem cell transplant in past 12 months
3.2.5 Patients with diabetes mellitus requiring insulin secretagogues and/or insulin
3.2.6 Patients with hypertriglyceridemia with serum triglyceride level ≥500 mg/d (lipid lowering drugs may be used to control level).
3.2.7 Patients who have hypersensitivity to sitagliptin
3.2.8 Patients with a history of pancreatitis
3.2.9 Patients with symptomatic cholelithiasis
3.2.10 Patients with a current dependence on alcohol (characterized by a physical addiction to alcohol that interferes with physical or mental health, and social, family or job responsibilities)

4.0 REGISTRATION PROCEDURES AND INFORMED CONSENT

4.1 Registration Procedures

Patients who appear to be eligible for this trial will undergo the Informed Consent Process and be screened for eligibility utilizing the Eligibility Criteria. The original signed IRB approved Informed Consent Document and completed eligibility checklist will be forwarded to the Clinical Trials Office designee for eligibility verification and registration in the OnCore® database. Notification will be sent to the principal investigator, treating physician and research nurse when registration is complete to confirm registration and inform them of patient ID number.

4.2 Informed Consent

The patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts as objectively as possible. Consent will be obtained using the IRB approved consent.

Written informed consent will be obtained from all subjects before initiation of any study-specific procedures. Procedures performed as part of the subject's routine clinical management and obtained prior to signing informed consent may be utilized for screening or baseline purposes provided the procedure was performed within the timeframe specified in the protocol.

5.0 TREATMENT PLAN

This is an open label phase II study. Although the myeloablative preparative regimen is not prescribed, it is anticipated that most patients will receive total body irradiation (TBI) plus etoposide (TBI/VP16), or high-dose thiotepa plus cyclophosphamide according to institutional standards. Regardless of the preparative regimen, all patients will receive tacrolimus and sirolimus for GvHD prophylaxis, which includes the study drug sitagliptin:

Day -3: **Tacrolimus** is initiated on day -3. *Tacrolimus may be given according to institutional practice at the investigator's discretion.* However, it is recommended that tacrolimus be given at a starting dose of 0.02 mg/kg/day IV as a continuous infusion, and then modified to target serum levels of 5-10 ng/ml. Serum levels should be monitored according to institutional practice (typically at least three times weekly until discharge, then at times of outpatient clinic visits). Tacrolimus may be switched to PO dosing when the patient is able to tolerate oral intake satisfactorily. Note that concurrent use of agents such as itraconazole, voriconazole or fluconazole (at doses > 200 mg) may inhibit the metabolism of tacrolimus, and thus increase tacrolimus levels. Initial dosing may be decreased in order to account for increased levels related to use of 'azole' agents. In addition, it is recommended to check tacrolimus levels twice weekly when these agents are initiated concurrently.

Sirolimus is started on day -3. *Sirolimus may be given according to institutional practice at the investigator's discretion.* However, it is recommended that sirolimus be given at a starting dose of 4 mg PO, and then modified to target serum levels of 5-10 ng/ml. Serum levels should be monitored according to institutional practice (typically at least three times weekly until discharge, then at times of outpatient clinic visits). Initial dosing may be decreased in order to account for increased levels related to use of 'azole' agents.

Day -1: **Sitagliptin** 600 mg q 12 hours PO starting on Day -1 to be administered between 8:00 am and 10:00 am then given every 12 hours (total 32 doses) through day +14.

In the absence of acute GvHD, begin tapering of both tacrolimus and sirolimus on Day +100 as tolerated with a goal of stopping by Day +180. The rate of taper may be adjusted for presence of signs and symptoms of GvHD. Mycophenolate mofetil may be substituted for tacrolimus or sirolimus if any toxicity related to these drugs arises (e.g., renal failure, hemolytic microangiopathy, allergic rash, etc.).

6.0 DOSE ADJUSTMENT

The dose of **sitagliptin** should be adjusted for **altered renal dysfunction** according to the creatinine clearance as calculated by the Cockcroft-Gault formula:

$$\text{Creatinine Clearance (CrCl)} = \frac{140 - Age}{SeCr} \times \frac{Weight}{72}$$

where Age is in years, Serum Creatinine (Se Cr) in mg/dl, and Weight in in kilograms (kg). For females multiply result by 0.85.

Adjustment:

CrCl ≥ 50 ml/min, no dose adjustment

CrCl ≥ 30 to < 50 ml/min, reduce by 50% (300 mg every 12 hours)

CrCl < 30 ml/min (including dialysis), reduce by 75% (150 mg every 12 hours)

The dose of sitagliptin will not be adjusted for liver dysfunction.

Discontinuation of sitagliptin

Sitagliptin will be **discontinued** if the patient develops any of the following:

- Pancreatitis
- Hypersensitivity reaction to sitagliptin
- Any grade 3-4 organ toxicity thought to be related or possibly related to sitaglipin

7.0 ANCILLARY THERAPY

7.1 Full supportive care.

Patients should receive *full supportive care*, including transfusions of blood and blood products, antibiotics, antiemetics, etc., when appropriate **according to institutional standards**.

7.2 Use of Palifermin.

Palifermin may be used for prevention of severe mucositis **according to institutional preferences (usually for TBI containing regimens)**. If used, palifermin will be administered according to the following schedule:

Palifermin 60 mcg/kg/day IV bolus on the three consecutive days prior* to the preparative regimen (i.e., days -8, -9, -10 if TBI-based regimen is used; OR days -9, -10, -11 if chemotherapy only regimen used) AND again for three days after infusion of peripheral blood stem cell (PBSC) (i.e., on days 0 to +2).

*MUST have a minimum of 24 hours between LAST dose of Palifermin and FIRST dose of either preparative regimen.

7.3 Pneumocystis carinii prophylaxis.

Prophylaxis against pneumocystis infection will be **according to institutional standards**. (An acceptable regimen includes, but is not restricted to cotrimoxazole one DS tablet once daily on Mondays, Wednesdays and Fridays. Patients allergic/intolerant to cotrimoxazole may receive dapsone or inhaled pentamidine instead). Prophylaxis will commence on engraftment (defined as achievement of ANC ≥ $0.5x10^9$/l for three consecutive days) and continue until at least day +180 post-transplant. This may be continued beyond Day +180 at the discretion of the treating physician. For patients who develop chronic GvHD (after Day +180), it is recommended that pneumocystis prophylaxis be continued for an extended period at the discretion of the treating physician.

7.4 Herpes Zoster prophylaxis.

All patients will receive acyclovir (or equivalent) for day +1 until day +180 following transplantation **according to institutional standards**.

7.5 Cytomegalovirus (CMV) prophylaxis.

Prophylaxis against CMV infection will be **according to institutional standards**. Either prophylaxis using drug therapy or monitoring for CMV reactivation and pre-emptive therapy upon activation may be used. It is recommended that patients will be monitored weekly for reactivation of CMV using a sensitive assay (e.g., quantitative PCR, ppp65 antigenemia, or hybrid capture assay) until day +100 (see Section 10.5 for recommended frequency of monitoring after day 100). Acceptable pre-emptive therapy includes Foscarnet (90 mg/kg BID), ganciclovir (10 mg/kg/day), or valganciclovir until resolution. Doses of drugs are adjusted for renal function and myelosuppression.

7.6 Fungal prophylaxis.

Patients will receive prophylaxis to cover yeasts and mold infections **according to institutional standards and at the investigator's discretion**. The recommended regimen is fluconazole 400 mg PO QD from day +1 to day +100. Acceptable alternatives are voriconazole, micafungin, caspofungin, lipid preparations of amphotericin B.

8.0 STUDY SCHEDULE

8.1 Guidelines for Pre-Study Testing

To be completed within 60 DAYS before day of admission:

- MUGA scan or ECHO
- Pulmonary function tests
- HIV, HTLV, hepatitis B and C serology
- Bone marrow aspirate (only for patients with leukemia or MDS to document remission- if patient is known to be in relapse, bone marrow aspirate will not be done)

To be completed within 16 DAYS before day of admission:

- Liver profile to document total bilirubin, AST and ALT levels ≤ 2 x upper limits of normal values (unless determined by the treating physician to be related to the underlying cancer).
- Estimated creatinine clearance (CrCl) to document ≥ 60 ml/min
- Pregnancy test (serum or urine β-hCG) for all women of childbearing potential

8.2 Screening / Pre-Transplant and Treatment Schedule

	Prior to start of Preparative Regimen	Time points peritransplant						Follow-up
		Days -1 to +14	+30 days (±3)	+100 days (±7)	+180 days (±7)	+270 days (±7)	+365 days (±7)	+5 years[7]
Sitagliptin treatment		X[1]						
Patient Evaluation								
H&P**	X	X	X	X	X	X	X	
CBC**	X	X	X	X	X	X	X	
CMP**	X	X	X	X	X	X	X	
GvHD evaluation		X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X
Soluble (s) CD26 antigen	X	X[3]	X	X	X	X	X	
Plasma DPP-4 activity (PD)	X	X[4]	X	X	X	X	X	
Plasma sitagliptin (PK)		X[5]						
Immune cell analysis		X (day +14)	X	X	X	X	X	
BAFF		X (day +14)	X	X	X	X	X	
AE assessment[6]	X	X	X	X				

[1]Sitagliptin is administered 600 mg every 12 hours from day -1 to day +14.

[2]Highest grade of acute or severity of acute GvHD and/or chronic GvHD is recorded (see Section 9.0).

[3]sCD26 is measured at baseline (prior to start of preparative regimen), day -1, day 0, day 7, day 14 during this period, and thereafter as indicated.

[4]DPP-4 activity is measured at baseline (prior to start of preparative regimen); day -1 pre-dose of sitagliptin, then 2, 4, and 6 hours after first dose; thereafter activity is measured 30 minutes before each morning dose and 2 hours post-morning dose through last dose on day +14.

[5]Plasma sitagliptin levels are measured at baseline (day -1 pre-dose of sitagliptin), then 2, 4, and 6 hours after first dose; thereafter activity is measured 30 minutes before each morning doseand 2 hours post-morning dose through last dose on day +14.

[6]Refer to Section 10.4 for a complete list of AEs that will be captured until day +100.

[7]Patients will be followed annually per institutional standards beginning year 2 through year 5 for development and severity of chronic GvHD, late-onset acute GvHD, relapse or progression of primary malignancy, and survival.

** Patients will be evaluated clinically by history and physical examination (H&P) and laboratory testing for safety and toxicity during routine standard of care assessments. These will occur at

minimum frequencies of daily during preparative regimen until discharge from hospital, then weekly until day +30, every two weeks until day +100, and then monthly thereafter until day +365. CBC, complete blood count; CMP, complete metabolic panel.

9.0 DEFINITIONS AND ASSESSMENT OF PRIMARY ENDPOINT: ACUTE GVHD

The primary endpoint of the study will be the development of acute GvHD in the first 100 days post- transplant. Patients will be monitored for the development of acute GvHD at least every second day until day +28 or discharge, then at each subsequent clinic visit as outpatient. Beyond day +100, patients will be followed at their routine clinic visits for the development of chronic GvHD (a secondary endpoint).

9.1 Evaluation of acute GvHD

GvHD must be documented by biopsy of at least one of the organs involved (skin, gut, or liver). Each organ will be staged using standard criteria, as outlined below (see Section 8.2). The character and extent of skin involvement will be determined by examination. The rules of nine will be used to estimate skin surface area involved. Staging is also based on the extent and character of the skin; for example, the presence or absence of bullae. Gastrointestinal GvHD requires 24-hour stool volume for staging. In addition, a history will be performed to document the absence or presence of abdominal pain, nausea, and vomiting. Patients will also be evaluated for the presence of ileus. Hepatic staging will be determined by elevation of serum total bilirubin. The grade of acute GvHD used for evaluation of the therapy will be the maximum grade developed during the *entire* period of evaluation.

9.2 Grading of acute GvHD

Acute GvHD should be graded according to standard clinical criteria as outlined in Appendix II.

10.0 DEFINITIONS OF SECONDARY ENDPOINTS

10.1 Time to engraftment of neutrophils

The time to engraftment of neutrophils is defined as the time from day 0 to the date of the first of three consecutive days after transplantation during which the absolute neutrophils count (NEUTROPHILS + BANDS) is at least 0.5 $x10^9$/l. Patients surviving at least 14 days after transplant will be evaluable for this endpoint.

10.2 Time to engraftment of platelets

The time to engraftment of platelets is defined as the time day 0 to the date of the first of 7 consecutive days of an unsupported (i.e., sustained for at least 7 days without transfusion) platelet count of at least 20 $x10^9$/l post-transplant (i.e., Day 0). The date of the first of 7 consecutive days of an unsupported platelet count of at least 50 $x10^9$/l will also be noted.

10.3 Chronic Graft versus Host Disease

Assessment of chronic GvHD will be according to the National Institutes of Health (NIH) scoring system.[50,51] Briefly, a clinical categorical system (0-3) is used for scoring of individual organs that describes the severity for each affected organ taking functional impact into account. Eight organs (skin, mouth, eyes, gastrointestinal [GI] tract, liver, lungs, joints, and female genital tract) are assessed. In general, a score of 0 means no manifestations/symptoms, a score of 1 indicates no significant impairment of function or activities of daily living (ADL), a score of 2 reflects significant impairment of ADL but no major disability, and a score of 3 indicates significant impairment of ADL with major disability. The scoring is clinical and the only mandated

laboratory tests for its completion are liver function tests, although pulmonary function tests will be performed only when indicated by symptoms. Global severity (mild, moderate, severe) is calculated from these scores according to the number and severity of organs reported:

Mild: 1 or 2 organs (except lung) with score 1.

Moderate: ≥3 organs with score 1, or lung score 1, or 1 or more organs with score 2.

Severe: any organ with a score 3, or lung score 2.

10.4 Hematological and Non-Hematological toxicity

Assessment of toxicity and adverse events will be based upon the descriptions and grading scales of the revised NCI Common Terminology Criteria for Adverse events (CTCAE) version 4.0. A copy of the CTCAE version 4.0 is available from (http://ctep.info.nih.gov).

For non-hematological toxicity, biochemical changes that are reversible with simple supplementation or treatment (e.g., electrolyte abnormalities, hyperglycemia, asymptomatic liver enzyme elevations, serum creatinine fluctuations related to hydration status, etc.) will not be captured. However, all cases of **hypoglycemia** will be recorded and their potential attribution to sitagliptin will be captured. The following major toxicities will be also recorded in the first 100 days:

- **Sinusoidal obstruction syndrome (SOS).** The diagnosis of SOS is will be based on the McDonald criteria.[52] Hepatic SOS is defined by the occurrence of 2 or more of the following criteria before day 30: (1) total bilirubin >2 mg/dl; (2) painful hepatomegaly; and (3) *unexplained* weight gain of >2% from baseline. No other reasonable explanation for these signs could be present at the time of diagnosis. Clinical grading of VOD will be according to Bearman criteria:[53]

  Mild: Self-limiting (resolving within day 100), no therapy required

  Moderate: Self-limiting (resolving within day 100) but requiring therapy, including diuretics for fluid retention, narcotic analgesia for painful hepatomegaly.

  Severe: VOD persisting to day 100 or causing hepatic failure or death.

- **Interstitial pneumonitis.** The incidence of grade 3-4 pulmonary toxicity, within the first 100 days post-transplantation, will be described.
- **Pancreatitis**
- **Incidence of infections during first 100 days post-transplant.** Infectious episodes will be documented as “proven” if an organism is isolated or confirmed by serological, molecular, or histological evidence, or “suspected” if there is documented fever and organ-related changes without isolated organism. The sites of infection will also be described. Infections to be reported include, but are not limited to, blood stream bacterial infections, pneumonia, all fungal infections, viral reactivation or infection (including CMV, EBV, HHV-6, BK, adenovirus, RSV, etc.), and any sepsis resulting in organ failure regardless of origin or organism isolation. Simple neutropenic fever will not be captured.
- **Thrombotic microangiopathy (TMA).** TMA will be defined according to the Blood and Marrow Transplantation Clinical Trials Network (BMT-CTN) consensus criteria, and requires all of the following criteria for diagnosis[57]
  - Schistocytosis (≥2 schistocytes in high power field) on peripheral blood

- Increased LDH
- Doubling of serum creatinine or ≥50% decrease in creatinine clearance from baseline
- Negative direct and indirect Coomb's test.

All cases of grade 3-4 TMA will reported annually to the Food and Drug Administration (FDA) in the annual report.

- **Transfusion requirements during first 100 days.** It is expected that almost all patients undergoing myeloablative conditioning will develop grade 4 hematological toxicities. However, the number of units of red blood cells and platelets transfused in the first 100 days post-transplant (or longer if no engraftment has occurred) will be recorded and monitored.

10.5 Long-term follow-up

Beyond day +100, patients will be followed up for development and severity of chronic GvHD, late-onset acute GvHD, relapse or progression of primary malignancy, and survival annually for up to 5 years (beginning year 2). Causes of death should be recorded as either related to relapse or progression of malignancy, acute or chronic GvHD, infection in the presence or absence of GvHD, other transplant-related complication, or unrelated to transplantation (i.e., incidental).

11.0 DRUG FORMULATION, AVAILABILITY, AND PREPARATION

11.1 Sitagliptin (Januvia™)

11.1.1 Availability

Sitagliptin is a specific DPP-4 inhibitor that is approved by the FDA for the treatment of type 2 diabetes mellitus. In this protocol, sitagliptin will be purchased commercially and provided by the study.

11.1.2 Preparation

Sitagliptin is commercially available as 25 mg, 50 mg, and 100 mg tablets. Sitagliptin will be supplied by the study and provided to patients free of charge.

11.1.3 Storage and stability

Sitagliptin tablets should be stored at 20-25°C (68-77°F), with excursions permitted to 15-30°C (59-86°F).

11.1.4 Administration

Sitagliptin may be administered with or without food.

11.1.5 Toxicity

The most common toxicity that occurred in clinical trials of patients with type 2 diabetes mellitus and in healthy volunteers are as follow:

Side effects occurring with a frequency of 1% to 10%: headache (5%), diarrhea (3%), upper respiratory tract infection (6%), and nasopharyngitis (5%).

Side effects occurring with an incidence less than or equal to placebo: abdominal pain (2%), hypoglycemia (1%), nausea (1%), neutrophils increased, and elevation of serum creatinine.

During controlled clinical trials in healthy subjects, single doses of up to 800 mg

sitagliptin were administered. Maximal mean increases in QTc of 8.0 msec were observed in one study at a dose of 800 mg sitagliptin, a mean effect that is not considered clinically important. There is no experience with doses above 800 mg in humans.

There have been post-marketing reports of acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis. If pancreatitis is suspected, promptly discontinue sitagliptin.

There have been post-marketing reports of acute renal failure, sometimes requiring dialysis. Dosage adjustment is recommended in patients with moderate or severe renal insufficiency and in patients with ESRD. Assessment of renal function is recommended prior to initiating sitagliptin and periodically thereafter.

There is an increased risk of hypoglycemia when sitagliptin is added to an insulin secretagogue (e.g., sulfonylurea) or insulin therapy. Consider lowering the dose of the sulfonylurea or insulin to reduce the risk of hypoglycemia.

There have been post-marketing reports of serious allergic and hypersensitivity reactions in patients treated with sitagliptin such as anaphylaxis, angioedema, and exfoliative skin conditions including Stevens-Johnson syndrome. Onset of these reactions occurred within the first 3 months after initiation of treatment with sitagliptin, with some reports occurring after the first dose. In such cases, promptly discontinue sitagliptin, assess for other potential causes and institute appropriate monitoring and treatment.

11.1.6 Drug interactions

In clinical studies in diabetic patients, sitagliptin did not meaningfully alter the pharmacokinetics of metformin, glyburide, simvastatin, rosiglitazone, warfarin, or oral contraceptives, providing *in vivo* evidence of a low propensity for causing drug interactions with substrates of CYP3A4, CYP2C8, CYP2C9, and organic cationic transporter (OCT).

Sitagliptin had a minimal effect on the pharmacokinetics of digoxin. Following administration of 0.25 mg digoxin concomitantly with 100 mg of sitagliptin daily for 10 days, the plasma AUC of digoxin was increased by 11%, and the plasma C_{max} by 18%.

Effects of Other Drugs on Sitagliptin: Clinical data described below suggest that sitagliptin is not susceptible to clinically meaningful interactions by co-administered medications:

Metformin: Co-administration of multiple twice-daily doses of metformin with sitagliptin did not meaningfully alter the pharmacokinetics of sitagliptin in patients with type 2 diabetes.

Cyclosporine: A study was conducted to assess the effect of cyclosporine, a potent inhibitor of p-glycoprotein, on the pharmacokinetics of sitagliptin. Co-administration of a single 100 mg oral dose of sitagliptin and a single 600 mg oral dose of cyclosporine increased the AUC and Cmax of sitagliptin by approximately 29% and 68%, respectively. These modest changes in sitagliptin pharmacokinetics were not considered to be clinically meaningful. The renal clearance of sitagliptin was also not meaningfully altered. Therefore, meaningful interactions would not be expected with other p-glycoprotein inhibitors.

11.2 Tacrolimus (Prograf®)

11.2.1 Availability

Tacrolimus is commercially available as an injection (5 mg/ml; 1 ml ampoules) and as oral capsules (1 mg and 5 mg).

11.2.2 Preparation

Tacrolimus injection must be diluted prior to IV infusion with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 µg/ml. Solutions should be prepared in non-PVC plastic or glass. Tacrolimus injection and diluted solutions of the drug should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

11.2.3 Administration

Oral therapy should be started as soon as possible as per protocol and 8 to 12 hours after stopping intravenous therapy. Oral doses will be administered twice a day.

11.2.4 Storage and stability

Tacrolimus capsules and injection should be stored at controlled room temperature, 15-30°C (59-86°F).

11.2.5 Toxicity

In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Chest pain was reported in 19%. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%); and dizziness (19%). Tremor and headache may respond to a dosage reduction. Visual changes, agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15% of tacrolimus-treated patients. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%), hypophosphatemia (49%), and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. Hyperuricemia has been reported in greater than 3% of tacrolimus-treated patients. Gastrointestinal adverse effects of tacrolimus have included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%) and diarrhea (37% to 72%). Nephrotoxicity was reported in 38% and 52% of liver and kidney transplant patients, respectively. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients. Abnormal liver function tests have been reported in 6% to 36% of patients; ascites was reported in 7% to 27% of these patients.

Other miscellaneous effects that have occurred in clinical trials include pain (24% to 63%), fever (19% to 48%), asthenia (11% to 52%), back pain (17% to 30%), and peripheral edema (12% to 36%). The incidence of hyperglycemia is 17% and may require therapy with insulin. Other less frequently occurring effects (greater than 3%) includes abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus injection contains cremophor which in other drugs has been associated with anaphylaxis. Because

tacrolimus is an immunosuppressive, the risk of opportunistic infections is increased.

11.2.6 Drug Interactions

Tacrolimus is metabolized by cytochrome P450 3A4. Drugs that are inhibitors (e.g., itraconazole) or inducers (e.g., phenytoin) of 3A4 might be expected to increase or decrease tacrolimus concentrations, respectively. This could result in increased or decreased effect of tacrolimus.

11.3 Sirolimus (Rapamycin; Rapamune®)

11.3.1 Availability and preparation

Sirolimus is commercially available as solution for oral use as 1 mg/ml (60 ml) (containing ethanol 1.5% to 2.5%; packaged with oral syringes and a carrying case), as 1 mg and 2 mg tablets.

11.3.2 Administration

The oral solution should be mixed with at least 2 ounces of water or orange juice. No other liquids should be used for dilution. Patient should drink diluted solution immediately. The cup should then be refilled with an additional 4 ounces of water or orange juice, stirred vigorously, and the patient should drink the contents at once. Sirolimus should be taken 4 hours after cyclosporine oral solution, cyclosporine capsules, or tacrolimus capsules.

11.3.3 Storage and stability

Store Sirolimus capsules at controlled room temperature, 15-30°C (59-86°F).

11.3.4 Toxicity

The Incidence of many adverse effects is dose related. The following are reported adverse events associated with Sirolimus.

Common

Cardiovascular: Peripheral edema (54% to 64%), hypertension (39% to 49%), peripheral edema (54% to 64%), edema (16% to 24%), chest pain (16% to 24%)

Central nervous system: Fever (23% to 34%), headache (23% to 34%), pain (24% to 33%), insomnia (13% to 22%)

Dermatologic: Acne (20% to 31%), rash (10% to 20%)

Endocrine & metabolic: Hyperlipidemia (38% to 57%), hypercholesterolemia (38% to 46%), hypophosphatemia (15% to 23%), hypokalemia (11% to 21%)

Gastrointestinal: Diarrhea (25% to 42%), constipation (28% to 38%), abdominal pain (28% to 36%), nausea (25% to 36%), vomiting (19% to 25%), dyspepsia (17% to 25%), weight gain (8% to 21%)

Genitourinary: Urinary tract infection (20% to 33%)

Hematologic: Anemia (23% to 37%), thrombocytopenia (13% to 40%)

Neuromuscular & skeletal: Weakness (22% to 40%), arthralgia (25% to 31%), tremor (21% to 31%), back pain (16% to 26%)

Renal: Serum creatinine increased (35% to 40%)

Respiratory: Dyspnea (22% to 30%), upper respiratory infection (20% to 26%), pharyngitis (16% to 21%)

Uncommon:

Cardiovascular: Atrial fibrillation, CHF, facial edema, hypervolemia, hypotension, palpitation, peripheral vascular disorder, postural hypotension, syncope, tachycardia, thrombosis, vasodilation, venous thromboembolism

Central nervous system: Chills, malaise, anxiety, confusion, depression, dizziness, emotional lability, hypoesthesia, hypotonia, neuropathy, somnolence

Dermatologic: Dermatitis (fungal), hirsutism, pruritus, skin hypertrophy, dermal ulcer, ecchymosis, cellulitis, skin carcinoma

Endocrine & metabolic: Cushing's syndrome, diabetes mellitus, glycosuria, acidosis, dehydration, hypercalcemia, hyperglycemia, hyperphosphatemia, hypocalcemia, hypoglycemia, hypomagnesemia, hyponatremia, hyperkalemia (12% to 17%)

Gastrointestinal: Enlarged abdomen, anorexia, dysphagia, eructation, esophagitis, flatulence, gastritis, gastroenteritis, gingivitis, gingival hyperplasia, ileus, mouth ulceration, oral moniliasis, stomatitis, weight loss

Genitourinary: Pelvic pain, scrotal edema, testis disorder, impotence

Hematologic: Leukocytosis, polycythemia, TTP, hemolytic-uremic syndrome, hemorrhage, leukopenia (9% to 15%)

Hepatic: Abnormal liver function tests, alkaline phosphatase increased, ascites, LDH increased, transaminases increased

Local: Thrombophlebitis

Neuromuscular & skeletal: Arthrosis, bone necrosis, CPK increased, leg cramps, myalgia, osteoporosis, tetany, hypertonia, paresthesia

Ocular: Abnormal vision, cataract, conjunctivitis

Otic: Ear pain, deafness, otitis media, tinnitus

Renal: Albuminuria, bladder pain, BUN increased, dysuria, hematuria, hydronephrosis, kidney pain, tubular necrosis, nocturia, oliguria, pyelonephritis, pyuria, nephropathy (toxic), urinary frequency, urinary incontinence, urinary retention

Respiratory: Asthma, atelectasis, bronchitis, cough, epistaxis, hypoxia, lung edema, pleural effusion, pneumonia, rhinitis, sinusitis

Miscellaneous: Abscess, diaphoresis, facial edema, flu-like syndrome, herpes simplex, hernia, infection, lymphadenopathy, lymphocele, lymphoproliferative disease, peritonitis, sepsis, increase in serum lipids (cholesterol and triglycerides).

11.3.5 Drug interactions

Sirolimus is a substrate of CYP3A4, and weakly inhibits CYP3A4. The following drugs have been found to interact with sirolimus:

Antifungal agents, imidazoles (itraconazole, ketoconazole, voriconazole): May increase the levels/effects of sirolimus. Concurrent use is not recommended, or levels must be closely monitored.

Calcineurin inhibitors (cyclosporine, tacrolimus): Concurrent therapy may increase the risk of HUS/TTP/TMA. Cyclosporine capsules (modified) or cyclosporine oral solution (modified) increase Cmax and AUC of sirolimus during concurrent therapy, and cyclosporine clearance may be reduced during concurrent therapy. Sirolimus should be

taken 4 hours after cyclosporine oral solution (modified) and/or cyclosporine capsules (modified).

Clarithromycin: May increase serum concentrations of sirolimus. Concurrent use not recommended.

CYP3A4 inducers: CYP3A4 inducers may decrease the levels/effects of sirolimus. Example inducers include aminoglutethimide, carbamazepine, nafcillin, nevirapine, phenobarbital, phenytoin, and rifamycins. Concurrent use is not recommended.

CYP3A4 inhibitors: May increase the levels/effects of sirolimus. Example inhibitors include azole antifungals, clarithromycin, diclofenac, doxycycline, erythromycin, imatinib, isoniazid, nefazodone, nicardipine, propofol, protease inhibitors, quinidine, telithromycin, and verapamil. Concurrent use is not recommended.

Calcium channel antagonists: Diltiazem may increase serum concentrations of sirolimus; monitor. Verapamil and nicardipine may share this effect.

Erythromycin: May increase serum concentrations of sirolimus. Concurrent use is not recommended.

Rifampin: May decrease serum concentrations of sirolimus. Concurrent use is not recommended.

12.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE

Although not a primary endpoint of the study, formal evaluation of response and disease status will be performed on at Days +30 (if not in remission at time of transplant), +100, +180, +270 and +365. After 1 year post transplant, disease evaluation will be according to institutional standards of care until progression and/or death until year 5. Patient may be assessed more frequently at the discretion of the treating physician.

12.1 Response criteria for patients with AML and ALL

12.1.1 Complete Remission (CR): defined by the presence of all of the following:

- Bone marrow cellularity > 20% with maturation of all cell lines, blasts ≤ 5% of total nucleated cells, and absence of Auer rods (for patients with AML).
- No extramedullary leukemia (e.g., CNS or soft-tissue involvement)

12.1.2 Treatment Failure: defined as failure to achieve CR.

12.1.3 Relapse: defined by any of the following factors after being in CR:

- Reappearance of circulating blast cell in the peripheral blood.
- More than 5% blasts in the bone marrow, not attributable to another cause.
- Development of extramedullary leukemia

12.2 Response criteria for patients with myelodysplasia

12.2.1 Complete Remission (CR): defined by the presence of all of the following:

- Bone marrow cellularity > 20% with maturation of all cell lines, blasts ≤ 5% of total nucleated cells and no morphological or cytogenetic evidence of dysplasia.

12.2.2 Treatment Failure: defined as failure to achieve CR.

12.2.3 Relapse: defined by any of the following factors after being in CR:

- Reappearance of circulating blast cell in the peripheral blood.

- >5% blasts in the bone marrow not attributable to another cause and/or reappearance of any cytogenetic abnormality previously present.

12.3 Response criteria for patients with CML

12.3.1 Complete hematological response (CHR): The complete disappearance of all signs and symptoms of the disease including palpable splenomegaly. The peripheral blood white cell (WBC) and platelet counts must be within institutional normal ranges. Bone marrow aspirate differential must have ≤ 5% blast cells.

12.3.2 Partial Hematological Response (PHR):

- A decrease in the WBC count by at least 50% and to < 20,000/μl.

OR

- The achievement of CHR except for

a) Persistence of palpable splenomegaly; or

b) Persistence of immature cells (>5% myelocytes, promyelocytes, or blasts) in peripheral blood; or

c) Thrombocytosis (exceeding the upper limit of normal), which must have decreased by more than 50% of pretreatment levels.

12.3.3 Cytogenetic response: Patients in CHR may be further classified in terms of cytogenetic response, as assessed from a bone marrow aspirate, as follows:

- **Complete cytogenetic response (CCyR):** 100% normal metaphases.
- **Partial cytogenetic response (PCyr):** > 65% normal metaphases (i.e., 1-34% Ph+ metaphases).
- **Major cytogenetic response (MCyR):** Includes both complete and partial cytogenetic response (i.e., < 35% Ph+ metaphases).
- **Minor cytogenetic response (MiCyR):** 1-65% normal metaphases (i.e., 35-99% Ph+ metaphases).

In the rare typical CML patients who do not have a Ph+ chromosome demonstrated by classical cytogenetics, but have a demonstrable BCR/ABL translocation at diagnosis, the “cytogenetic” response will be assessed by **fluorescent in-situ hybridization (FISH) analysis**. In this case, responses are defined as:

- **Complete response by FISH:** A 100% reduction in the cells positive for the translocation of BCR and ABL (i.e., 0% cells positive).
- **Partial response by FISH:** A reduction greater than 50% but less than 100% of cells positive for the BCR/ABL translocation.

12.3.4 Molecular response: Patients who have achieved a cytogenetic response will be evaluated for a molecular response by RT-PCR analysis for minimal residual disease. A complete molecular response is defined as no detectable BCR/ABL transcript.

12.4 Response criteria for patients with Hodgkin’s or non-Hodgkin’s Lymphoma

12.4.1 Complete Response (CR): defined by the presence of all the following criteria

- Complete disappearance of all detectable clinical and radiographic evidence of target lesions and disappearance of all disease-related symptoms if present prior

to therapy, as well as normalization of those biochemical abnormalities (e.g., LDH, etc.) definitely assignable to NHL.

- All lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm prior to therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter prior to treatment must have decreased to ≤ 1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of their greatest transverse diameters (SPD).
- The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. (No normal size can be specified, however, because of the difficulties in evaluating splenic and hepatic size.) Any macroscopic nodules in any organs detectable on imaging studies should no longer be present. Similarly, other organs considered to be enlarged prior to therapy due to involvement of lymphoma (i.e., kidneys, liver, etc.) must have decreased in size.
- If the bone marrow was involved by lymphoma prior to treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of same site.

12.4.2 Complete Response Uncertain (CRu): Complete response/uncertain will include those patients who have met the criteria in Section 12.4.1 bullet points 1 and 3, but with one or more of the following:

- A residual node > 1.5 cm in greatest transverse diameter that has regressed more than 75% in the SPD. Individual nodes that were previously confluent must have regressed more than 75% in their SPD compared with the size of the original mass.
- Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

12.4.3 Partial Response (PR):

- A decrease of ≥ 50% in the SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase in the size of other nodes, liver, or spleen.
- Splenic and hepatic nodules must regress by at least 50% in SPD.
- With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.
- Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive, the cell type should be specified in the report, e.g., large-cell lymphoma.
- No new sites of disease.

12.4.4 Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

12.4.5 Progressive Disease:

- 50% or more increase from nadir in the SPD of any previously identified abnormal node for PRs or non-responders.
- Appearance of any new lesion during or at the end of therapy.

13.0 CORRELATIVE STUDIES AND SAMPLE SUBMISSION

13.1 Laboratory Correlative Studies

13.1.1 Pharmacokinetic studies of sitagliptin. Plasma levels of sitagliptin will be performed by Dr. David Jones' Laboratory at the Clinical Pharmacology Analytical Core of the Indiana University Simon Cancer Center. Plasma sitagliptin levels will be assayed by high-turbulence liquid chromatography online extraction method,[54] and detected by mass spectroscopy (API 4000, Applied Biosystems, Toronto, Canada) using selected reaction monitoring with turbo-ionspray interface in the positive ion mode, as adapted by Dr. Jones' Laboratory (limit of assay 1.23 nmol/l). PK analysis will be performed both non-compartmentally, as well as by population pharmacokinetics-based approaches. Additional analyses will include the development of a pharmacokinetic-pharmacodynamic model linking sitagliptin concentrations to DPP4 activity to subsequent development of grades 2-4 acute GvHD by day +100, and engraftment as measured by neutrophils post-transplant. To facilitate these analyses, additional variables from previously collected laboratory values will be extracted from the medical record from the period immediately preceding transplant to time of engraftment. Values to be extracted will include but are not limited to liver function tests, kidney function tests and CBC with differential.

Timepoints of collection will be Day -1 before first dose of sitagliptin and then 2, 4 and 6 hours after the FIRST dose. Thereafter, samples are to be collected 30 minutes BEFORE and 2 hours AFTER each morning dose (+/- 10 minutes for all timepoints Day -1 thru Day +14). Samples will be processed and stored at the Research Laboratory at the Clinical Trials Office at IUSCC until transfer to Dr. Jones' laboratory for analysis. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.1.2 Pharmacodynamic studies of plasma CD26/DPP-IV activity. We will use plasma CD26/DPP-IV activity as a surrogate to provide some level of confidence that biologically active levels of the drug are achieved using the dose and schedule administered by demonstrating target inhibition. However, to explore whether plasma CD26/DPP-IV inhibition will provide a good surrogate marker, we will correlate DPP-IV activity with plasma levels of sitagliptin and subsequent development of grades 2-4 acute GvHD by day +100, and engraftment as measured by neutrophils post-transplant.

Plasma DPP-IV activity will be assayed in Dr. Farag's laboratory using a modification of the chromogenic assay we have previously used to assess DPP-IV activity on CD34+ UCB cells,[55] and previously reported by others.[23] Plasma DPP-IV activity will be assayed by incubating 4 µl of plasma with the chromogenic substrate Gly-Pro-*p*-nitroanilide (400 µM) (Gly-Pro-pNA; Sigma, St. Louis, MO) at 37°C in 96-well microplates and determining the amount of nitroanilide (pNA) released in the supernatant by measuring absorbance at 390 nm over time. Absorbance will be measured at 390 nm on a microplate spectrofluorometer (SpectraMax 190; Molecular Devices, Menlo Park, CA). The change in absorbance between each 30 second interval will be averaged over 10 minutes to calculate the slope for each sample. Enzyme activity is defined as the slope

(in mOD/min) from 4 to 14 minutes. The mean percentage inhibition of DPP-IV activity relative to baseline following sitagliptin administration will be plotted against time.

Timepoints of collection will be at baseline (i.e., prior to the start of the preparative regimen), Day -1 before first dose of sitagliptin and then 2, 4 and 6 hours after the FIRST dose. Thereafter, samples are to be collected 30 minutes BEFORE and 2 hours AFTER each morning dose through Day +14 (+/- 10 minutes for all timepoints Day -1 thru Day +14). Then samples will be collected Day +30 (± 3 days), +100, +180, +270 and +365 (± 7 days at all other timepoints).

Samples will be processed and stored at the Research Laboratory at the Clinical Trials Office at IUSCC until transfer to Dr. Farag's laboratory for analysis. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.1.3 Soluble CD26 (sCD26) antigen levels. There is currently no conclusive information about the behavior of antigen expression, sCD26 concentration, and enzymatic function under disease conditions, particularly in transplantation. Some studies have suggested that sCD26/DPP-IV activity may be a promising biomarker for monitoring the immune status in solid organ transplantation.[56] There are no studies investigating the changes in sCD26 or DPP-IV activity in the setting of hematopoietic stem cell transplantation. Therefore, in addition to plasma DPP-IV activity, we will also explore the change in sCD26 antigen in the peri-transplant process. Plasma sCD26 will be assayed at defined points using the Human sCD26 Platinum ELISA kit from eBiosciences (San Diego, CA).

Timepoints of collection will be prior to the start of the preparative regimen, Days -1, 0, +7, +14, and then Day +30 (± 3 days), +100, +180, +270 and +365 (± 7 days at all other timepoints).

Samples will be processed and stored at the Research Laboratory at the Clinical Trials Office at IUSCC until transfer to Dr. Farag's laboratory for analysis. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.1.4 Immune reconstitution studies. We will also investigate the effect of DPP-4 inhibition by sitagliptin on immune cell reconstitution at defined time points after transplantation. The following lymphocyte subsets will be assessed by multiparameter flow-cytometry in Dr. Farag's laboratory:

- CD3+T cells (CD3+CD4+, and CD3+CD8+)
- CD3+ T cells expressing CD26 (CD4+CD26+. And CD8+CD26+)
- T_{reg} cells (CD3+CD4+CD25+FOXP3+)
- CD4+ T cell subsets (CD45RA+CD27+, CD45RA+CD27-CD62L+, CD45RA-CD27+CD62L+, CD45RA-CD27+CD62L-, CD45RA-CD27-)
- CD8+ T cell subsets (CD45RA+CD27+, CD45RA+CD27-CD62L+, CD45RA-CD27+CD62L+, CD45RA-CD27+CD62L-, CD45RA-CD27-)
- B cells (CD19)
- NK cells (CD3-CD56+)

In addition, we will assess plasma BAFF (B cell activating factor from the tumor necrosis factor) as BAFF levels and BAFF/B cell ratio have been correlated with development of chronic GvHD. Plasma BAFF levels will be assayed at defined time points using the Human BAFF Instant ELISA kit from eBiosciences (San Diego, CA) in Dr. Farag's laboratory.

Samples will be processed and stored at the Research Laboratory at the Clinical Trials Office at IUSCC until transfer to Dr. Farag's laboratory for analysis. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.1.5 Microchimerism analysis. Chimerism will be assayed on unfractionated whole blood and in CD3 selected (T-cell) cells using polymerase chain reaction for amplification of short tandem repeat markers, as currently performed routinely for patients undergoing standard allogeneic stem cell transplantation.

Timepoints of collection will be prior to starting study treatment, samples must be collected from the donor cells AND the patient for baseline analysis of short tandem repeats (STR). In addition, samples will also be collected from the patient on Day +30 (± 3 days), +100, +180, +270 and +365 (± 7 days at all other timepoints).

Microchimerism samples will be processed at the institution's local laboratory according to standard practice (STR analysis is preferred).

14.0 ADVERSE EVENTS AND REPORTING GUIDELINES

14.1 Definitions of Adverse Events

14.1.1 Adverse Event (AE). An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a drug, without any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose. Adverse events will be graded according to the NCI Common Toxicity Criteria, Version 4.0.

14.1.2 Suspected Adverse Reaction (SAR). Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. Suspected adverse reactions are the subset of all adverse events for which there is a reasonable possibility that the drug caused the event.

Examples of types of evidence that would suggest a causal relationship between the drug and the adverse event:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson's Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

14.1.3 Adverse Reaction (AR). An adverse reaction is any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

14.1.4 Serious Adverse Event (SAE). An adverse event or suspected adverse reaction is considered "serious" if it results in any of the following outcomes:

- Results in death
- Is life-threatening. Life-threatening is defined as an adverse event or suspected adverse reaction that places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization

  NOTE: Hospitalizations that are not considered SAEs are:

  - Hospitalization planned prior to first administration of study drug
  - Hospitalization for elective treatment of a pre-existing condition unrelated to the study medication
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.

14.1.5 Unexpected Adverse Event. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

This definition relies entirely on the adverse events or suspected adverse reactions listed in the investigator brochure for the particular drug under investigation (or elsewhere in the general investigational plan if an investigator brochure is not required or available) as the basis for determining whether newly acquired information generated from clinical trials or reported from other sources is *unexpected*. This means that events not listed for

the particular drug under investigation in the investigator brochure are considered "unexpected" and those listed are considered "expected." When new adverse event information is received, it is the sponsor's responsibility to determine whether the event is "unexpected" for IND safety reporting purposes. In the clinical trial setting, there has been some confusion with the term "expected" as it has been used to mean "anticipated" for the disease being treated or population being studied rather than "listed in the investigator brochure." For example, some adverse events can be anticipated to occur as a result of a disease or in an older population (e.g., cancer-related deaths in a cancer trial, strokes or acute myocardial infarctions in an older population). However, for reporting purposes, these anticipated events are not "expected" because they are not listed in the investigator brochure (i.e., the test drug is not suspected or known to cause them).

Adverse events listed in the investigator brochure as occurring with members of the same class of drugs, or as anticipated from the pharmacological properties of the drug, would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the angiotensin-converting enzyme (ACE) inhibitor class and angioedema would be described in the investigator brochure as a class effect, a case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes until it is included in the investigator brochure as occurring with the drug under investigation.

14.1.6 Determining Attribution to the Investigational Agent(s)

Attribution: An assessment of the relationship between the AE and the medical intervention. CTCAE does not define an AE as necessarily *"caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly **NO**T related
	Unlikely	The AE is **doubtfully related**
Related to investigational agent/intervention	Possible	The AE **may be related**
	Probable	The AE **is likely related**
	Definite	The AE **is clearly related**

14.2 Adverse Event (AE) Reporting Requirements:

Grade III/IV and all related adverse events (AEs) will be recorded from the time of first study drug administration and for at least 30 days after treatment discontinuation. All AEs considered related to trial medication will be followed until resolution, return to baseline, or deemed clinically insignificant, even if this occurs post-trial.

The following AEs are expected as part of the normal process of a high dose chemotherapy and radiotherapy containing preparative regimens for bone marrow transplant. They will not be collected or reported on source documents, regardless of grade: alopecia, nausea and vomiting, fatigue, thrombocytopenia, anemia and neutropenia. Further, non-hematological toxicity, biochemical changes that are reversible with simple supplementation or treatment (e.g., electrolyte abnormalities, hyperglycemia, asymptomatic liver enzyme elevations, serum creatinine fluctuations

related to hydration status, etc.) will not be collected or reported on source documents. However, time to engraftment of neutrophils and platelets and all cases of hypoglycemia, GVHD, SOS, interstitial pneumonitis, pancreatitis, incidence of infections, TMA, and transfusion requirements regardless of attribution and as described in Section 10.0 will be collected as endpoints for the secondary objectives of this study. If any of these events are deemed related to the study drug, they will be reported on source documents.

14.2.1 Reporting to the FDA. Per CFR 312.32 (c), the investigator-sponsor of the IND must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any adverse experience. There are two types of reports to the FDA: 7-day and 15-day reports.

15-Day IND Reports:

The investigator-sponsor of the IND must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any:

- **suspected adverse reaction** that is ***both***
- **serious *and***
- **unexpected**

Each written notification shall be made as soon as possible, and no later than **15 calendar** days after the investigator-sponsor's initial receipt of the information.

7-Day Reports:

The investigator-sponsor must notify FDA and all participating investigators in a written IND safety report of any adverse experience:

- **fatal or life-threatening experience** that is ***both***
- **associated with use of the drug *and***
- **unexpected**

The FDA will be notified as soon as possible but no later than **7 calendar** days after initial receipt of the information.

Report Content:

Each written notification may be submitted on FDA Form 3500A or in a narrative format and must bear prominent identification of its contents, i.e., "IND Safety Report". For purposes of this protocol, the **MedWatch Report Form (FDA 3500A mandatory reporting), along with FDA Form 1571, and a cover letter** submitted to the appropriate FDA division, will serve as the written IND safety report. Follow-up information to a safety report should be submitted as soon as the relevant information is available.

Submit:

- MedWatch Report Form (FDA 3500A)
- FDA Form 1571
- Cover Letter

The IUSCC Protocol Development Coordinator should be contacted to assist with all FDA submissions and will be provided with a copy of all events that are reported to the FDA. All IND submissions will be maintained in a master file in the Clinical Trials Office of the IU Simon Cancer Center.

14.2.2 Reporting to the IRB

Unanticipated problems involving risks to subjects or others will be reported **promptly** to

the IRB if they:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the serious adverse event does not meet all three (3) criteria listed above, the event does not have to be promptly reported to the Indiana University IRB. However, it should be reported at the time of continuing review.

Prompt reporting of unanticipated problems to the IRB is defined as within 5 days from becoming aware of the event.

14.2.3 Reporting to the IUSCC Data Safety Monitoring Committee:

Regardless of study sponsorship, the study team must enter all initial and follow-up SAE, expedited, and noncompliance reports into OnCore® for review by the DSMC chair and/or coordinator. Expedited reports may include IRB Prompt Report Forms, AdEERS reports, MedWatch, and additional SAE forms as required by the sponsor. When follow-up information is received, a follow-up report should also be created in OnCore®. This DSMC reporting requirement is **in addition to any other** regulatory bodies to be notified (i.e. IRB, FDA, pharmaceutical company, etc.). The DSMC chair and/or coordinator will review all SAE, expedited, and noncompliance reports monthly.

15.0 DATA AND SAFETY MONITORING PLAN

Investigators will conduct continuous review of data and patient safety. **Weekly** review meetings for high-risk trials are required and will include the principle investigator, clinical research specialist and/or research nurse (other members per principle investigator's discretion). **Weekly** meeting summaries should include review of data and patient safety by including the number of patients, significant toxicities as described in the protocol, dose adjustments and responses observed. Summaries are to be submitted to DSMC@iupui.edu weekly and reviewed monthly. A summary template can be found in Appendix I.

15.1 Study Auditing and Monitoring

All trials conducted at the IUSCC are subject to auditing and/or monitoring. Reports will be reviewed by the full DSMC at the time of study review (Reference Risk Table in full DSMC Charter).

15.2 Early Study Closure

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the patient warrant early closure of the study, this recommendation should be made in writing to the Data Safety Monitoring Committee. Alternatively, the DSMC may initiate suspension or early closure of the study based on its review.

The DSMC has streamlined the reporting process by utilizing reports from OnCore. This has allowed direct view of reports within the Clinical Trials Management System (CTMS); thus discontinuing paper reports. SAE reports are entered into OnCore and reviewed by the DSMC chair and/or coordinator monthly. Findings will be reported to the full DSMC at the time of study review.

15.3 Study Accrual Oversight

Accrual data will be entered into the IU Simon Cancer Center OnCore system. The Protocol Progress Committee (PPC) reviews study accrual twice per year while the PPC coordinator

reviews accrual quarterly.

15.4 Protocol Deviations

Protocol deviations are entered into OnCore and reviewed by the DSMC chair and/or coordinator monthly. Findings will be reported to the full DSMC at the time of study review.

16.0 STATISTICAL CONSIDERATIONS

16.1 General Considerations

Statistical analysis of this study will be the responsibility of Biostatistics and Data Management Core at IUSCC. Parameter estimates and relevant summary statistics will be reported where appropriate. For continuous variables, summary statistics will include number of patients, mean, median, standard deviation, minimum and maximum. Categorical endpoints will be summarized using number of patients, frequency, and percentages. Missing data will not be imputed.

Additional exploratory analyses of the data will be conducted as deemed appropriate. Changes from this analysis plan will not require an amendment to the protocol unless it changes a significant feature of the protocol.

16.2 Study Design

The trial is an open label Simon minimax two-stage Phase II trial of a fixed dose of oral sitagliptin to assess the safety and efficacy in preventing grades II-IV acute GvHD in patients undergoing matched related and unrelated allogeneic peripheral blood stem cell transplantation for hematological malignancies.

CTCAE Version 4.0 will be used to summarize adverse events in the assessment of safety and tolerability of the combination therapy.

16.3 Sample Size

The **primary endpoint** of this phase II study is the incidence of grade II-IV acute GvHD by day +100. Patients who expire before day +100 from other causes and have not developed any GvHD will not be evaluable for the primary endpoint and will be replaced. However, such patients will be evaluable for other secondary endpoints (see below), and will be included in evaluation for the stopping rule of non-relapse mortality (see below).

This study uses a Simon minimax two-stage design. The primary objective is to assess if DPP-4 inhibition using sitagliptin will reduce the incidence of grade II-IV acute GvHD by day +100 from 30% to 15% or less. In effect, this is the same as inversing the proportion of patients *without* acute grade II-IV GvHD by day +100 from 70% to 85% or more. In statistical terms, we are testing the null hypothesis H_0: $p_0 \leq 0.70$ versus the alternative hypothesis H_1: $p_1 \geq 0.85$, where p is the probability of being without acute grade II-IV GvHD by day +100. We will use a minimax optimal stage design with a one-sided type I error set to 0.1, and a type II error rate set to 0.2. In the *first* stage, 23 evaluable patients will be entered. If 6 or more develop acute grade II-IV GvHD by day +100 (i.e., 17 or fewer of these patients have no or only grade I acute GvHD by day +100), the study will be stopped in favor of the null hypothesis. On the other hand, if only less than 6 develop acute grade II-IV GvHD by day +100, an additional 13 evaluable patients will be accrued for a total of 36 evaluable patients. In the final analysis**,** *if fewer than 8 of the 36 evaluable patients develop acute grade II-IV GvHD by day +100, the null hypothesis will be rejected*, and we will conclude that DPP-IV inhibition with sitagliptin for prevention of severe GvHD is worthy of further study.

With an expected non-relapse mortality rate of 10-15% in the first 100 days post transplantation, we expect to enroll approximately 40-43 patients to have 36 patients evaluable for the primary endpoint.

16.4 Patient Characteristics and Significant Protocol Violations

16.4.1 Patient Characteristics

Patient demographics and patient baseline characteristics will be listed and summarized for all patients enrolled, including age, gender, and race. For these baseline characteristics, counts, means, medians, standard deviation, minimum and maximum values will be presented as appropriate.

16.4.2 Significant Protocol Violations

The IUSCC will closely monitor the study to identify and evaluate any violation of good clinical practices (GCP) or clinically important protocol violations. Clinically important protocol violation will be defined as those deviations from the protocol that could affect patient safety, data integrity, or the conclusions drawn from the study. Important protocol violations and any actions to be taken regarding the exclusion of patients or affected data from specific analyses will be summarized and listed.

16.4.3 Patient Disposition

Patient disposition will be tabulated and will show the number of patients enrolled, and the number of patients completing each stage of the study. All reasons for discontinuation will be listed and summarized.

16.4.4 Treatment Exposure/Compliance will be summarized using descriptive statistics for all enrolled patients.

16.5 Analysis Plan

All patients who receive at least one dose of sitagliptin will be analyzed for the primary and secondary efficacy endpoints and for safety endpoints of toxicity, including mortality and stopping criteria (see Section 16.6). However, as noted below, patients who expire before day +100 of causes other than acute GvHD will not be considered evaluable for the primary endpoint, and will be replaced. These patients, however, will still be analyzed for secondary endpoints, safety, and taken into account in the determining the stopping rule (see Section 16.6).

16.5.1 Safety and Tolerability

To evaluate the safety and tolerability of sitagliptin, summaries of treatment related adverse events in the population will be tabulated. All adverse events (AEs) will be presented in incidence tables coded by CTCAE term. An adverse event will be considered treatment related if it occurred on or after date of first dose of sitagliptin, and was possibly, probably, or definitely related to treatment. All adverse events will be recorded until off study date. All deaths recorded in this study will be listed and summarized, and the cause documented.

16.5.2 Analysis of Primary Objective

The primary objective is to investigate if DPP-IV inhibition using sitagliptin can result in a 20% decrease in the incidence of acute grade II-IV GvHD by day +100 following matched-related or well-matched unrelated allogeneic PBSC transplantation. Patients who expire before day +100 of causes other than acute GvHD will not be considered evaluable for the primary endpoint, and will be replaced. Patients who develop acute

grades II-IV GvHD after day +100 (usually in the setting of tapering of immune suppressive drugs) will not be included in the estimate of the primary endpoint, but will de described separately.

Two-sided 95% confidence intervals (calculated using the Jennison-Turnbull method) will be calculated to estimate the proportion of patients who develop acute grade II-IV acute GvHD.

16.5.3 Analysis of Secondary and Exploratory Objectives

For the *secondary objectives*, non-hematological toxicity (other than nausea, vomiting, non-GvHD related diarrhea, and electrolyte abnormalities) will be graded and described according to The National Cancer Institute Common Terminology Criteria for Adverse events (CTCAE) version 4.0 (http://ctep.cancer.gov), and will be described in terms of frequency. Attribution to sitagliptin will also be described. In addition, the incidence and types of infections (viral, bacterial, fungal, etc.) will be tabulated and described.

In addition to the description of acute GvHD rate for the primary endpoint, the cumulative incidences of all grades of acute GvHD, grade II-IV acute GvHD, and grade III-IV acute GvHD by day +100 will be described using a competing risk analysis with deaths from causes other than GvHD considered as a competing risk.

Patients surviving at least 100 days will be evaluable for chronic GvHD. The cumulative incidence of chronic GvHD (total, and mild, moderate, severe) will be described using deaths from causes other than chronic GvHD considered a competing risk

The time to engraftment of neutrophils will be defined as the time from day 0 of transplant to the first of three consecutive days after transplantation during which the ANC is $\geq 0.5 \times 10^9$/l. The time to engraftment of platelets will be defined as the time from day 0 to the first of seven consecutive days after transplantation during which the platelet count is $\geq 20 \times 10^9$/l without transfusion. The cumulative incidence of engraftment of neutrophils and platelets will be described using competing risk analysis with death before engraftment as a competing risk.

The cumulative incidence of non-relapse mortality will be described with death from relapse considered a competing risk (the same model will be used to describe the cumulative incidence of relapse). Overall survival will be described using the Kaplan-Meier method. Actuarial survival at day +30, +100, +180, and 1 year with 95% confidence intervals will be estimated.

For the *exploratory objectives*, the trajectories of immune cell reconstitution, plasma sCD26, and plasma DPP-4 activity for each patient will be plotted graphically over time, and inhibition of plasma DPP-4 activity will be explored as a predictor of a GHVD using the same approach, denoting in the plots for whom and when did a GHVD occur.

16.6 Stopping Criteria

We wish to insure that patients being treated with this approach do not experience unacceptable levels of transplant-related mortality (assessed by non-relapse mortality) within 180 days of transplantation. The expected 180-day non-relapse mortality (NRM) for patients undergoing matched sibling and well-matched volunteer unrelated donor PBSC transplants is approximately 30-35%. Therefore, in this protocol, we wish to insure that the 180 day NRM is 35% or less. To do this, we will compute binomial probabilities after each five patients either, reaching 180 days post-transplant, or dying prior to 180 days post-transplant. If the lower 90% confidence interval of the proportion becomes greater than 35%, the trial will be suspended. The following table lists the number of patients dying of NRM within 180 days of transplant to require suspension for

unacceptable NRM.

Number of patients treated evaluable for NRM at 180 days	Suspend if the number of patients experience non-relapse mortality by day +180
5	4
10	6
15	8
20	11
25	13
30	15

17.0 REFERENCES

1. Nash RA, Antin JH, Karanes C, et al: Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood 96:2062-8, 2000

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 92:2303-14, 1998

3. Weisdorf DJ, Anasetti C, Antin JH, et al: Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. Blood 99:1971-7, 2002

4. Gratwohl A, Brand R, Apperley J, et al: Graft-versus-host disease and outcome in HLA-identical sibling transplantations for chronic myeloid leukemia. Blood 100:3877-86, 2002

5. Gerich J: Pathogenesis and management of postprandial hyperglycemia: role of incretin-based therapies. Int J Gen Med 6:877-895, 2013

6. Broxmeyer HE, Hoggatt J, O'Leary HA, et al: Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. Nat Med 18:1786-96, 2012

7. Ohnuma K, Dang NH, Morimoto C: Revisiting an old acquaintance: CD26 and its molecular mechanisms in T cell function. Trends Immunol 29:295-301, 2008

8. Ohnuma K, Takahashi N, Yamochi T, et al: Role of CD26/dipeptidyl peptidase IV in human T cell activation and function. Front Biosci 13:2299-310, 2008

9. Farag SS, Srivastava S, Messina-Graham S, et al: In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. Stem Cells Dev 22:1007-15, 2013

10. Fleischer B: A novel pathway of human T cell activation via a 103 kD T cell activation antigen. J Immunol 138:1346-50, 1987

11. Fox DA, Hussey RE, Fitzgerald KA, et al: Ta1, a novel 105 KD human T cell activation antigen defined by a monoclonal antibody. J Immunol 133:1250-6, 1984

12. Morimoto C, Torimoto Y, Levinson G, et al: 1F7, a novel cell surface molecule, involved in helper function of CD4 cells. J Immunol 143:3430-9, 1989

13. Tanaka T, Camerini D, Seed B, et al: Cloning and functional expression of the T

cell activation antigen CD26. J Immunol 149:481-6, 1992

14. Fleischer B: CD26: a surface protease involved in T-cell activation. Immunol Today 15:180-4, 1994

15. Ohnuma K, Uchiyama M, Hatano R, et al: Blockade of CD26-mediated T cell costimulation with soluble caveolin-1-Ig fusion protein induces anergy in CD4+T cells. Biochem Biophys Res Commun 386:327-32, 2009

16. Ohnuma K, Yamochi T, Uchiyama M, et al: CD26 up-regulates expression of CD86 on antigen-presenting cells by means of caveolin-1. Proc Natl Acad Sci U S A 101:14186-91, 2004

17. Ohnuma K, Uchiyama M, Yamochi T, et al: Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem 282:10117-31, 2007

18. Hatano R, Ohnuma K, Yamamoto J, et al: Prevention of acute graft-versus-host disease by humanized anti-CD26 monoclonal antibody. Br J Haematol 162:263-77, 2013

19. Rosenstock J, Zinman B: Dipeptidyl peptidase-4 inhibitors and the management of type 2 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes 14:98-107, 2007

20. Kim KR, Rhee SD, Kim HY, et al: KR-62436, 6-{2-[2-(5-cyano-4,5-dihydropyrazol-1-yl)-2-oxoethylamino]ethylamino}nicotinonitr ile, is a novel dipeptidyl peptidase-IV (DPP-IV) inhibitor with anti-hyperglycemic activity. Eur J Pharmacol 518:63-70, 2005

21. Lankas GR, Leiting B, Roy RS, et al: Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. Diabetes 54:2988-94, 2005

22. Zerilli T, Pyon EY: Sitagliptin phosphate: a DPP-4 inhibitor for the treatment of type 2 diabetes mellitus. Clin Ther 29:2614-34, 2007

23. Bergman AJ, Stevens C, Zhou Y, et al: Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. Clin Ther 28:55-72, 2006

24. Herman GA, Bergman A, Stevens C, et al: Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 91:4612-9, 2006

25. Herman GA, Stevens C, Van Dyck K, et al: Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther 78:675-88, 2005

26. Triplitt C, Wright A, Chiquette E: Incretin mimetics and dipeptidyl peptidase-IV inhibitors: potential new therapies for type 2 diabetes mellitus. Pharmacotherapy 26:360-74, 2006

27. Vincent SH, Reed JR, Bergman AJ, et al: Metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [14C]sitagliptin in humans. Drug Metab Dispos 35:533-8, 2007

28. Stevens C, Bergman A, Liu Q: Lack of clinically significant effect of moderate hepatic insufficiency on the pharmacokinetics of MK-0431 (sitagliptin), a dipeptidylpeptidase-IV inhibitor. Clin Pharmacol Ther 79:P49, 2006

29. Kim D, Wang L, Beconi M, et al: (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)- yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent,

orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J Med Chem 48:141-51, 2005

30. Scott R, Wu M, Sanchez M, et al: Efficacy and tolerability of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy over 12 weeks in patients with type 2 diabetes. Int J Clin Pract 61:171-80, 2007

31. Aschner P, Kipnes MS, Lunceford JK, et al: Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care 29:2632-7, 2006

32. Raz I, Hanefeld M, Xu L, et al: Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. Diabetologia 49:2564-71, 2006

33. Engel SS, Williams-Herman DE, Golm GT, et al: Sitagliptin: review of preclinical and clinical data regarding incidence of pancreatitis. Int J Clin Pract 64:984-90, 2010

34. Velez de Mendizabal N, Strother RM, Farag SS, et al: Modelling the Sitagliptin Effect on Dipeptidyl Peptidase-4 Activity in Adults with Haematological Malignancies After Umbilical Cord Blood Haematopoietic Cell Transplantation. Clin Pharmacokinet, 2013

35. Davis T, Farag SS: Targeting Homing to Enhance Engraftment following Umbilical Cord Blood Stem Cell Transplantation, in Ballen K (ed): Umbilical Cord Blood Transplantation and Banking, Springer, 2014

36. Eapen M, Rocha V, Sanz G, et al: Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol 11:653-60, 2010

37. Laughlin MJ, Eapen M, Rubinstein P, et al: Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 351:2265-75, 2004

38. Rocha V, Crotta A, Ruggeri A, et al: Double cord blood transplantation: extending the use of unrelated umbilical cord blood cells for patients with hematological diseases. Best Pract Res Clin Haematol 23:223-9, 2010

39. Cutler C, Logan B, Nakamura R, et al: Tacrolimus/sirolimus vs tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. Blood 124:1372-7, 2014

40. Ziakas PD, Zervou FN, Zacharioudakis IM, et al: Graft-versus-host disease prophylaxis after transplantation: a network meta-analysis. PLoS One 9:e114735, 2014

41. Matte CC, Liu J, Cormier J, et al: Donor APCs are required for maximal GVHD but not for GVL. Nat Med 10:987-92, 2004

42. Sung AD, Chao NJ: Concise review: acute graft-versus-host disease: immunobiology, prevention, and treatment. Stem Cells Transl Med 2:25-32, 2013

43. Shlomchik WD, Couzens MS, Tang CB, et al: Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science 285:412-5, 1999

44. Anderson BE, McNiff J, Yan J, et al: Memory CD4+ T cells do not induce graft-versus-host disease. J Clin Invest 112:101-8, 2003

45. Chen BJ, Cui X, Sempowski GD, et al: Transfer of allogeneic CD62L- memory T cells without graft-versus-host disease. Blood 103:1534-41, 2004

46. Foster AE, Marangolo M, Sartor MM, et al: Human CD62L- memory T cells are less responsive to alloantigen stimulation than CD62L+ naive T cells: potential for adoptive immunotherapy and allodepletion. Blood 104:2403-9, 2004

47. Wen HS, Wang JM, Zhou H, et al: Migration and activation of T cells during development of graft-versus-host disease in a mouse model. Transplant Proc 45:713-8, 2013

48. Sadeghi B, Al-Hashmi S, Hassan Z, et al: Expansion and activation kinetics of immune cells during early phase of GVHD in mouse model based on chemotherapy conditioning. Clin Dev Immunol 2010:142943, 2010

49. Duval M, Klein JP, He W, et al: Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. J Clin Oncol 28:3730-8, 2010

50. Jagasia MH, Greinix HT, Arora M, et al: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant 21:389-401 e1, 2015

51. Moon JH, Sohn SK, Lambie A, et al: Validation of National Institutes of Health global scoring system for chronic graft-versus-host disease (GVHD) according to overall and GVHD-specific survival. Biol Blood Marrow Transplant 20:556-63, 2014

52. McDonald GB, Sharma P, Matthews DE, et al: Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. Hepatology 4:116-22, 1984

53. Bearman SI, Anderson GL, Mori M, et al: Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation. J Clin Oncol 11:1729-36, 1993

54. Zeng W, Fisher AL, Musson DG, et al: High-throughput liquid chromatography for drug analysis in biological fluids: investigation of extraction column life. J Chromatogr B Analyt Technol Biomed Life Sci 806:177-83, 2004

55. Christopherson KW, 2nd, Hangoc G, Broxmeyer HE: Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. J Immunol 169:7000-8, 2002

56. Leicht S, Shipkova M, Klett C, et al: CD26/dipeptidyl peptidase IV: a comparative study of healthy persons and kidney transplant recipients before and early after transplantation. Clin Biochem 46:1383-8, 2013

57. Ho VT, Cutler C, Carter S, et al. Blood and marrow transplant clinical trials network committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 11: 571-75, 2005.

APPENDIX I

DSMC Meeting Summary Template

Meeting Minutes Form for DSMC

send to dsmc@iupui.edu or file in binder

Meeting Date:			
Team/Program: (include meeting sign in sheet)			
Protocol & Status (open/closed to accrual) (one protocol per sheet)			
PI:			
	Y	N	
Weekly and Monthly meetings should include discussion on data, dose levels, accrual numbers, deviation summaries and SAE reports (per IUSCC DSMP).			
Has accrual been reviewed and entered into Oncore?			
Have all SAE's been entered into Oncore?			
Is there documentation for study discontinuation?			
Have all deviations been entered into OnCore?			
Have study deviation summaries been reviewed by the team ***(CTO continue to keep deviation logs signed by PI) ?***			
Record any dose limiting toxicities (DLT's) on this form for any phase I investigator initiated trial, HOG or on a multi-site trial in which IUSCC is the lead site.			
If any of your answers are "NO" please explain in the space below.			

***Notes**	

This form is to be used for Investigator Initiated and HOG trials (High risk weekly, Moderate risk Monthly, Low risk quarterly)

APPENDIX II

IU Health Acute GvHD Assessment Tool

34075
CH-6083 (APR 13)
Page 1 of 1

Indiana University Health

ACUTE GRAFT VERSUS HOST DISEASE ASSESSMENT TOOL (Page 1 of 1)

Please check the maximum grade of GVHD over the stated period of time.

Date: _____ Date of SCT: _____

Person Completing the Form: _____

Performance Status (ECOG/Lansky/Karnofsky): _____

Assessment: From ___/___/___ to ___/___/___ ☐ **Inpatient**

☐ **Oupatient. Date of assessment as listed above.**

☐ **No GVHD** **Date Initial Onset:** _____ ☐ **Onset date previously reported**

ACUTE GVHD GRADING SCALE: Based on modified Keystone Grading Scale. *– Mark the Appropriate Boxes –*

Stage	Skin	[a]Liver	[b]GI (Adult)	[b]GI (Peds)
0	☐ None attributed to GVHD	☐ Not GVHD	☐ None attributed to GVHD	☐ None attributed to GVHD
1	☐ Maculopapular rash, less than 25% of body surface	☐ Bili 2-3mg/dL	☐ 500-999mL diarrhea/day, or persistent nausea with histo logic evidence of GVHD in stomach or duodenum	☐ Diarrhea 280-555mL/m2/day, ***OR;*** persistent nausea with histologic evidence of GVHD in stomach or duodenum
2	☐ Maculopapular rash, 25-50% of body surface	☐ Bili 3.1-6mg/dL	☐ 1000-1499mL diarrhea/day	☐ Diarrhea 556-833mL/m2/day
3	☐ Maculopapular rash, more than 50% of body surface	☐ Bili 6.1-15mg/dL	☐ 1500 or more mL diarrhea/day	☐ Diarrhea more than 833mL/m2/day
4	☐ Generalized erythroderma with bullous formation	☐ Bili greater than 15mg/dL	☐ Severe abdominal pain with or without ileus	☐ Severe abdominal pain, with or without ileus

a. Total bilirubin downgrade one stage if additional cause of bilirubin elevation has been documented.
b. Downgrade one stage if an additional cause of diarrhea has been documented

Overall Clinical Grading of Severity of Acute GVHD**

Grade	Degree of Organ Involvement
0	☐ None attributed to GVHD
I	☐ Stage 1-2 rash and no liver or gut involvement
II	☐ Stage 3 rash, or stage 1 liver involvement, or stage 1 gut involvement
III	☐ None to stage 3 skin rash with stage 2-3 liver, or stage 2-4 gut involvement
IV	☐ Stage 4 skin rash, or stage 4 liver involvement

Histopathology Results *(Confirmed or Pending)*

Immunosuppressive Medications for This Period:

☐ CSA ☐ FK-506 ☐ MMF ☐ Sirolimus ☐ Systemic Corticosteroids

☐ Topical Corticosteroids ☐ Infliximab ☐ Daclizumab ☐ Other: _____

☐ Comments: _____

I have reviewed the above documentation and concur with the findings.

_____ _____ M.D. Date: ___/___/___

Assessed By *Bone Marrow Transplant Attending*

ACUTE GRAFT VERSUS HOST DISEASE ASSESSMENT TOOL (Page 1 of 1) | Medical Record Copy | B-34

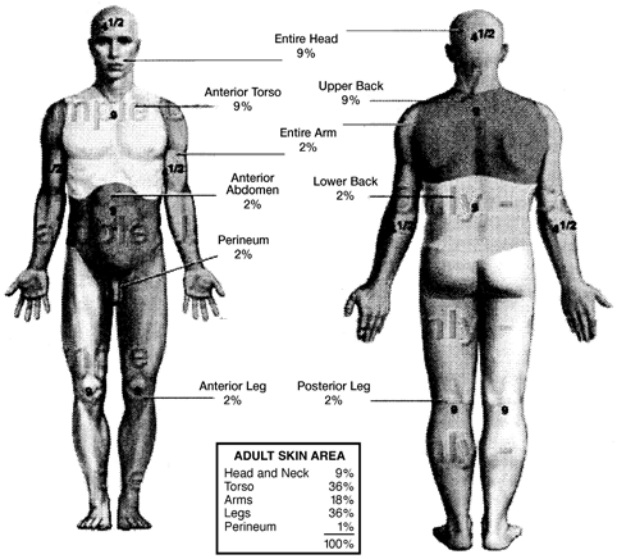
*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

**Adapted from Thomas *et al*, New England J. Medicine, 292:895-902, 1975.

+ Shulman *et al*, The American J of Medicine, 69:204-216, 1980.

Lansky Score	Performance Status
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restrictions of and less time spent in play activities
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities.
10	No play; does not get out of bed
0	Unresponsive

RULE OF NINES FOR BODY AREAS



ADULT SKIN AREA

Head and Neck	9%
Torso	36%
Arms	18%
Legs	36%
Perineum	1%
	100%

Karnofsky Score	General Category	Performance Status
100	Able to carry on normal activity, no special care needed	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, no special care needed	Able to carry on normal activity, minor signs or symptoms of disease
80	Able to carry on normal activity, no special care needed	Normal activity with effort, some signs or symptoms of disease
70	Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	Cares for self, unable to carry on normal activity or to do work
60	Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	Requires occasional assistance from others but able to care for most needs
50	Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	Requires considerable assistance from others; frequent medical care
40	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing	Disabled, requires special care and assistance
30	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing	Severely disabled, hospitalization indicated; death not imminent
20	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing	Very sick, hospitalization necessary, active supporting treatment necessary
10	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing	Moribund
0		Dead

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, *e.g.,* light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead