Targeting Cross-presentation with Peginterferon alfa-2a to Enhance Anti-leukemic Responses after Allogeneic Transplantation in High Risk Acute Myeloid Leukemia

Version 5.0

Blood and Marrow Transplantation Program

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1.0 OVERVIEW

This protocol is an open label, single arm, non-randomized, phase I / II clinical trial investigating the use of commercially available pegylated interferon alpha-2a (peg-IFN-α, Pegasys®, Genentech) for prevention of relapse in acute myeloid leukemia (AML) at very high risk for relapse or not in remission at the time of allogeneic hematopoietic stem cell transplantation (HCT). The inability to attain remission status following induction therapy for AML remains a significant problem and is associated with poor outcomes. While HCT remains a curative option, its effectiveness in the setting of relapsed or primary refractory AML is significantly diminished due to high rates of relapse.

The anti-leukemic properties of HCT are primarily attributed to the combined effects of 1) pre – transplant chemotherapy (termed conditioning) and 2) the immunologic effects of donor cells (termed graft-versus-leukemia or GVL). While increasing the intensity of conditioning reduces relapse, this strategy has historically been associated with greater toxicity. Alternatively, improving GVL without added toxicity (particularly graft-versus-host disease) represents an alternative strategy for limiting relapse and improving outcomes, which is the primary aim of this protocol.

IFN-α, FDA approved for viral hepatitis, has demonstrated anti-tumor activity in several hematologic malignancies including myeloproliferative diseases and CML.. IFN-α has also shown feasibility in the treatment of post HCT relapse by eliciting durable clinical responses, without significant toxicity. Recent insights from pre-clinical studies in HCT have now identified a central role of IFN-α in enhancing tumor cell antigen presentation, thereby promoting leukemia specific T cell responses (GVL) without GVHD. In this protocol, we propose to administer peg-IFN-α to prevent relapse by increasing GVL responses in patients with relapsed and refractory AML not in remission at the time of HCT.

2.0 STUDY OBJECTIVES

2.1 Primary Clinical Objectives:

Phase I: To determine a phase II dose of peg-IFN-α in patients with AML not in remission at HCT.

Phase II: To determine the incidence of relapse at six months post HCT for patients receiving the phase II dose of peg-IFN-α.

2.2 Secondary Clinical Objectives:

To estimate the rate of primary engraftment by day +28 post HCT.

To estimate the cumulative incidence of non-relapse mortality.

To estimate the cumulative incidence of relapse.

To estimate overall and disease free survival.

To estimate the incidence of viral infection.

To estimate the cumulative incidence of acute GVHD at six months

To estimate the cumulative incidence of chronic GVHD.

2.3 Biologic/Exploratory Objectives:

To analyze numbers of leukemia antigen specific T cells in donors and patients during and after peg-IFN-α.

To analyze numbers and activation status of BDC3A+ dendritic cells (DCs) in donors and patients during and after peg-IFN-α.

To analyze the pharmacodynamic effects of peg-IFN-α on signaling within the JAK / STAT pathway.

To analyze serum inflammatory markers and cellular immune subsets during and after peg-IFN-α.

To measure *in vitro* T cells responses during and after peg-IFN-α.

To characterize genomic and transcriptomic features associated with non-remission AML and any subsequent relapse after HCT with peg-IFN-α.

To measure minimal residual disease by molecular methods during and after peg-IFN-α in bone marrow and peripheral blood genomic DNA, plasma cell-free DNA and urinary DNA.

To measure spatial and temporal dynamics of bone marrow ablation and engraftment after HCT using quantitative MRI.

3.0 BACKGROUND

3.1 Allogeneic HCT for AML not in remission

Allogeneic hematopoietic stem cell transplantation (HCT) is a curative therapy for many high risk hematologic malignancies including acute myeloid leukemia (AML)(1). In recent years, the numbers of HCT have increased to greater than 20,000 annually with AML representing the most common indication (2, 3). Although outcomes have improved by reducing conditioning toxicity, infection, and acute graft-versus-host disease (aGVHD); relapse remains the principle cause of treatment failure (2, 4)**(FIGURE 1).** Relapse rates after HCT range from 25 - 65% in patients with AML and is determined by a number of factors including conditioning intensity, cytogenetic risk, molecular profile and status of disease (5-10). Patients with AML not in remission (e.g. relapsed or refractory to induction therapy) consistently have the highest incidence of relapse, exceeding 50% in the first year after HCT (8, 11, 12). Likewise, detection of minimal residual disease by flow cytometry or persistence of unfavorable clonal cytogenetic abnormalities at morphologic remission (< 5% myeloblasts) have similarly correlated with high post HCT relapse rates and poor survival(13, 14). More recently, adverse cytogenetic and / or molecular risk by or monosomal karyotype have been described as independent predictors of poor disease free survival even for HCT in remission(15, 16). In these settings, high relapse together with commonly encountered HCT related mortality have resulted in disease free survivals of approximately 10 – 20% (12, 17). Furthermore, the median survival is 3 months when relapse occurs after HCT due to ineffective therapies (18). Considering 30 - 40% of AML patients do not obtain remission after induction therapy, the scope of this problem is significant. Thus, while HCT remains the lone curative therapy for relapsed and refractory AML (11), treatment strategies that limit relapse after HCT are urgently needed to improve outcomes.

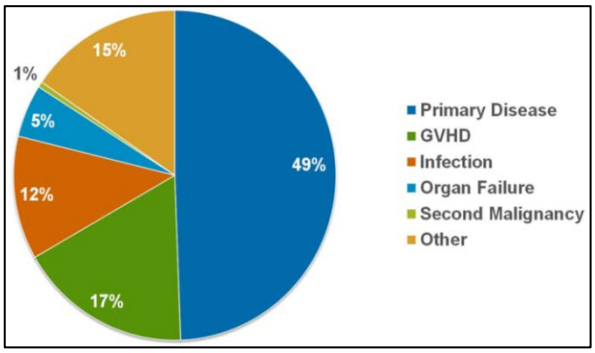

FIGURE 1: Causes of Mortality after sibling donor allogeneic HCT. CIBMTR Summary Slides, 2013.

3.2 Approach to relapse for AML not in remission: conditioning therapy

We have previously approached the problem of relapse after HCT by attempting to increase the anti-leukemic properties without adding significant toxicity to myeloablative transplant conditioning. Clofarabine, a second generation purine nucleoside analog, had

previously demonstrated potent anti-leukemic properties in treatment of relapsed AML (19, 20). Patients with relapsed and refractory hematologic malignancies, predominantly AML not in remission at HCT (N = 31), were treated on a phase I/II clinical trial with myeloablative clofarabine and busulfan (CloBu4)(21). This regimen demonstrated significant early activity in AML with 94% of patients achieving remission post HCT. Despite early responses, 44% of patients eventually relapsed after HCT. The two-year overall survival (OS) was 35%, which compared favorably to historical outcomes of approximately 10 - 15% for AML patients with similar characteristics treated with conventional myeloablative conditioning (17). In order to confirm these observations, CloBu4 was tested in a multicenter trial of relapsed / refractory AML (22). This trial has now completed accrual (N = 74) and again demonstrated a very high day 30 post HCT CR rate of 90%, with relatively low toxicity (non-relapse mortality of 20% at one year). However, at early follow up the incidence of relapse was higher than in our initial phase I / II experience, estimated to be 50% at six months and 60% at one year **(FIGURE 2)**. Therefore, although CloBu4 may be associated with higher CR rates, less toxicity and possibly improved overall survival when compared to traditional myeloablative regimens, ultimately a pattern of high early relapse is observed (9). This suggests that myeloablative conditioning may be effective at inducing initial leukemia free states in patients with refractory AML, unfortunately, in many instances these remissions cannot be sustained.

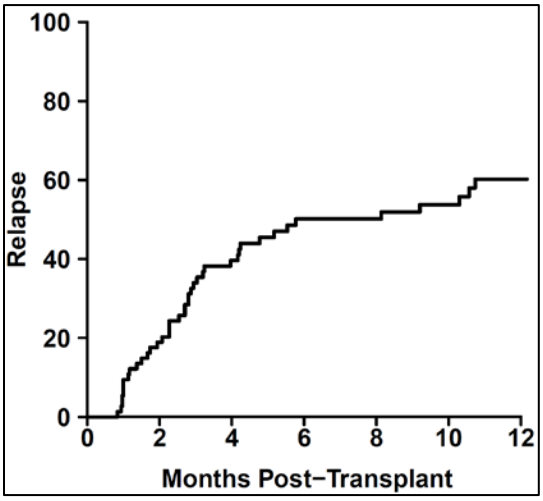

FIGURE 2: Cumulative incidence of relapse of AML after myeloablative conditioning with CloBu4.

3.3 Timing of relapse and post HCT interventions

In AML not in remission, relapse is an early event, predominately occurring within the first six months post HCT. In our combined experience using CloBu4 conditioning (N = 105), 60% of all relapses occurred within 100 days post HCT, and 82% by six months, and 91% by one year post HCT. Therefore, although late events occur, estimating early relapse (e.g. six months) appears to be a good predictor of overall rates. Furthermore, considering these kinetics, therapeutic strategies aimed at preventing relapse might ideally have the greatest impact if administered very early after HCT.

3.4 Increasing the graft-versus-leukemia (GVL) effects of HCT to limit relapse

3.4.1 Biology of GVL

The clinical observation of high remission rates after myeloablative conditioning coupled with subsequent relapse suggests the occurrence of a transient minimal residual disease

state that may be amenable to further immune based strategies. Since the anti-leukemic properties of HCT result from conditioning *and* GVL, enhancing GVL effects might also prevent relapse. GVL reflects a complex immunologic process that is primarily mediated by immune effectors such as T and NK cells from the donor graft. In order to effectively mount anti-tumor responses, however, donor T cells must recognize both tumor specific antigens (TSAs) *and* alloantigens (23). However, naïve donor T cells initially administered in the HCT inoculum are not activated (or primed) against TSAs and are easily rendered tolerant (24, 25). Although tumor cells themselves may illicit immune responses, we have shown that antigen presenting cells (APCs) play a pivotal role in orchestrating effective T cell specific responses against tumor cells, **Reddy, Nat Med. 2005 Nov;11(11):1244-9 and Toubai, Blood. 2013 May 16;121(20):4231-41** (23, 26).

3.4.2 Cross-presentation in GVL

Both host and donor APCs play important roles in mediating GVHD (27, 28), but APCs of host origin are particularly implicated in GVL (23, 26, 28). These interactions facilitate potent CD8+ T cell responses that can be directed at both allo-antigens and TSAs (23, 29). Specifically, a subset of host APCs, CD8α+ DCs, are amongst the most specialized at presenting exogenous antigens in the context of class I major histocompatibility complex (MHC class I), a process termed cross-priming or cross-presentation that is crucial for inducing cytotoxic T cell responses (30). Due to their similar specialization in cross-presentation of soluble and cell associated antigens to CD8+ T cells, human blood DC antigen 3 (BDCA3+) DCs or CD141+ DCs are putative human homologs to murine CD8α+ DCs (31, 32).

Host DCs persist early after HCT, but are eventually replaced by donor cells in the first several weeks after HCT (33-35). Experimental models of donor lymphocyte infusion indicate that GVL responses are optimized in the mixed host / donor chimeric state; presumably due to DC presentation of recipient allo-antigens and TSAs (36). This suggests that in addition to the rapid pace of relapse, there is a biologic basis for early post HCT interventions that promote GVL through DC cross-presentation. Although *ex vivo* activation of T cells to TSAs may circumvent the requirement for host DCs and generate effective GVL, significant cost and infrastructure are needed for clinical translation (37). There also remains limited knowledge as to whether *ex vivo* approaches will mimic the robust memory responses observed with *in vivo* activation.

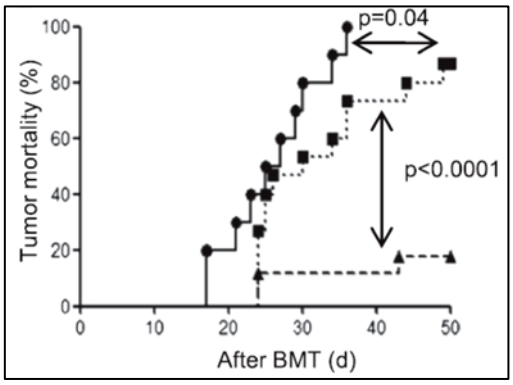

Figure 3: Absence of GVL CD8+ DCs mitigates GVL responses. Syngeneic animals (circles), allogeneic animals (triangles), and squares allogeneic Batf3-/- mice (squares).

3.4.3 Methods to enhance cross presentation: Polyl:C and Type I Interferon.

We have shown that host CD8α+ DCs play a critical role in mediating murine GVL (26). In multiple models of HCT, allogeneic mice deficient in CD8α+ DCs (Batf3$^{-/-}$) consistently

demonstrated significantly impaired tumor clearance compared to allogeneic and syngeneic controls, despite developing GVHD at severities equivalent to wild-type animals (**FIGURE 3**). Thus, CD8α+ DCs appear necessary for generating effective GVL responses, but distinct from the generalized alloreactivity of HCT. Likewise, we have shown in subsequent experiments that loss of CD8α+ DCs coincided with a concomitant decline in tumor specific CD8+ T cells. Additional signals acting upon DCs can significantly influence whether cross presentation results in T cell responses or inactivation (tolerance). Toll-like receptor 3 (TLR3), expressed predominantly on CD8α+ DCs, represents one signal that has been shown to greatly amplify the cross-presentation of exogenous viral antigens and promote cytotoxic CD8+ T cell responses (38). We treated HCT recipient animals with the immune adjuvant polyinosinic:polycytidylic acid (polyI:C), a Toll-like receptor 3 (TLR3) agonist, to increase DC cross presentation. In these models of GVL we observed a significant increase in GVL effects *without* aggravating GVHD (**FIGURE 4**). ***Importantly, anti-tumor effects were optimized in the context of simultaneous alloantigen responses and were not observed in the syngeneic controls.*** Collectively, these results confirm the relevance of cross-presentation by host CD8α+ DCs for mediating experimental GVL, in the context of allogeneic HCT.

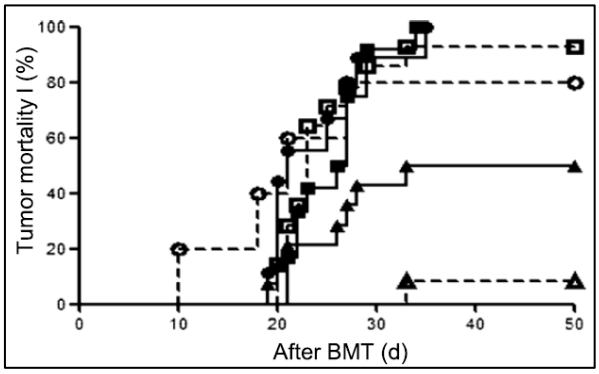

Figure 4: Use of TLR3 agonist PolyI:C increases GVL responses. Allogeneic animals with PolyI:C (Triangle / hatched line) and without PolyI:C (Triangle / solid line). Treatment had no effect on GVL in syngeneic animals (circles) or TLR3 $^{(-/-)}$ animals (squares)

Multiple lines of evidence suggest type I interferons (e.g. IFN-α/β), long available clinically, mediate their anti-tumor effects in part through cross-presentation by CD8 α+ DCs and subsequent antigen specific CD8+ T cell responses (39). Type I IFNs released by virally infected cells directly act upon and are secreted by DCs promoting activation, differentiation, co-stimulatory molecule expression and cross-presentation (40, 41). Notably, PolyI:C itself is a potent inducer of type I IFN secretion by DCs (42). Likewise, type I IFN signaling is necessary for DC activation following PolyI:C (43). In the absence of type I IFN signaling, CD8α+ DC priming of T cells and anti-tumor responses are markedly impaired. This was demonstrated in experiments using conditional knockouts of the IFN receptor (IFNAR1), where the absence of IFN- α/β signaling in CD8 α + DCs resulted in loss of anti-tumor activity and cross-presentation to CD8+ T cells (44). Furthermore, in murine HCT models similar *interruption* of IFN signaling unexpectedly increased GVHD, but as expected, abrogated anti-tumor immunity. In contrast, administration of exogenous type I IFN lessened GVHD and enhanced cytotoxic CD8+ T cell responses (45). **Taken together, these results suggest that signaling by type I IFN is critical for CD8α+ DC cross-presentation. Therefore, careful administration of exogenous type I IFN early after allogeneic HCT in patients with AML not in remission might enhance GVL effects by increasing cross-presentation by DCs.**

3.5 Clinical use of Type I Interferons in AML and HCT.

IFN-α is a type I interferon that is FDA approved for chronic hepatitis B and C and has been used in the treatment of several solid and hematologic malignancies including chronic myeloid leukemia, myeloproliferative neoplasms and AML (46-49). In AML, IFN-α has been used primary alone or in combination for remission induction or maintenance after remission. IFN-α has also been studied in a limited fashion as treatment for post HCT relapse in AML, with several reports of patients achieving durable remissions (50). To date, experiences in AML after HCT have been exclusively with unmodified IFN preparations which possess a very short biologic half-life. Because patients with post HCT relapse often harbor a large burden of disease, it is postulated that immune interventions that promote GVT may be best optimized in minimal residual disease states; such as the early post HCT period. To our knowledge, only two examples of IFN-α prophylaxis exist after HCT, but none specifically for enhancement of GVL. In one study, leukocyte IFN was administered after engraftment to patients with acute lymphoblastic leukemia (ALL) to prevent CMV reactivation (51). While CMV rates were similar between groups, relapse in IFN treated patients was lower. In another study, eleven patients (two with AML) received IFN-α at various doses in the context of T cell depleted HCT (52). Importantly, and consistent with pre-clinical evidence, neither study reported an increased risk for GVHD. Thus, while clinical experience with IFN-α for relapse *prevention* after HCT is limited, these studies suggest feasibility.

3.6 Pegylated IFN-α

Despite responses in advanced AML, the efficacy of IFN-α has yet to be clearly demonstrated. To date, the large heterogeneity of treatments, small patient numbers and diverse preparations have contributed to varied results (50). IFN-α dose and duration of activity may be relevant for optimizing *in vivo* activity (either direct or immunologic). For example, higher serum levels of IFN-α (>3000 IU/ml) may correlate with clinical response in AML (53). More recently, *in vivo* models of AML show prolonged IFN-α exposure improved activity but the underlying mechanisms of these effects are yet to be described (54). At approved doses, modern polyethylene glycol-conjugated (pegylated) formulations now used clinically provide reliable and evenly sustained IFN-α exposure at serum levels known to exert anti-leukemic activity (55). In the clinic, there is now considerable experience with peg-IFN-α formulations for treatment of advanced myeloproliferative neoplasms. In one report of AML arising from antecedent myelofibrosis, peg-IFN-α (without chemotherapy) induced a durable remission lasting > 18 months (56). These observations suggest a potential benefit in anti-tumor activity with use of peg-IFN-α and suggest modern long-acting formulations may be optimal for enhancing GVL after HCT.

In general, due to their improved efficacy, ease of administration and tolerability at approved dosages commercially available peg-IFN-α preparations (peginterferon alfa-2a; PEGASYS®, Genentech, Inc. and peginterferon alfa-2a; PEGINTRON®, Merck & Co, Inc.) have largely replaced short acting preparations in the clinic. In studies of patients with viral hepatitis and myeloproliferative neoplasms, adverse events are typically

constitutional in nature, mild to moderate in severity, and do not result in discontinuation (48, 49, 57). Pertinent for HCT, bone marrow suppression did occur but was uncommon, and predominantly affected neutrophils. Serious adverse events were reported in 10% of chronic hepatitis C patients receiving long term therapy, but only 3% of patients receiving short term administration (24 weeks). The proposed duration of treatment for the current study is 6 weeks.

3.7 Rationale and use of pegylated IFN-α in AML not in remission at HCT

We propose administering commercially available pegylated IFN-α2a or PEGASYS® (herein referred to as peg-IFN-α) as a strategy to prevent relapse after HCT. AML not in remission is the population to be studied, which is a frequently encountered clinical problem associated with very poor outcomes, primarily due to high rates of relapse. While HCT is the only known curative therapy, additional strategies that improve disease control in this setting are urgently needed. New insights into the underlying immune biology of HCT suggest sustained exogenous administration of IFN-α will promote cross presentation by DCs, thereby increasing GVL. We hypothesize that peg-IFN-α will limit early relapse after HCT for AML not in remission. Peg-IFN-α, administered at approved dosages over short durations has been associated with good safety, tolerability, and the sustained bioavailibility necessary to best assess IFN mediated DC cross-presentation and prevention of relapse.

Thusfar, in this clinical trial IFN-α, has been escalated to and remains at the maximum planed dosage (180mcg), with ongoing assessments for DLT by mTPI design (section 16.2). During an interim analysis for safety (section 16.3) at 24 patients, no stopping rules were met for excessive engraftment failure, non-relapse mortality or acute GVHD.

3.8. Magnetic resonance imaging (MRI) of bone marrow with parametric response mapping (PRM).

MRI is sensitive to the fat and water composition of bone marrow using quantitative methods such as multi-echo Dixon sequences that separate signals from fat and water with relaxation correction. These methods allow computational analyses for percentages and volumes of fat and water within each volume element of an image. Normal hematopoietic bone marrow in adults is composed primarily of fat signal as detected by MRI with the remainder being hematopoietic and stromal cells (water signal). Increases in percent fat indicate a loss of cellularity and vice versa. We propose that quantitative changes in bone marrow fat can measure spatial and temporal dynamics of bone marrow ablation and repopulation after HCT for AML.

MRI also provides quantitative measurements of mobility of water (diffusion) and heterogeneity of tissue composition (susceptibility and cross relaxation transfer techniques). Mobility of water in living tissue is affected by factors including cell membranes, intercellular interactions, cell size, and extracellular matrix. Our group and others have shown that quantitative changes in mobility of water in a tissue or tumor

predict response to treatments similar to conditioning regimens used prior to HCT (58-60). Susceptibility and cross relaxation transfer methods for MRI, such as susceptibility weighted imaging, T2* decay, and chemical exchange saturation transfer, detect heterogeneity of anatomic environments. As examples, these MRI methods have been used to measure trabecular bone structure and extracellular matrix, which we propose will provide a non-invasive assessment of the bone marrow microenvironment in AML and HCT.

MRI data typically are analyzed based on mean values for an entire tissue of interest, such as bone marrow within an entire bone. While simple to implement, these whole tissue analyses inherently lose information about heterogeneous composition of tissues and non-uniform responses to treatment. To capture the spatial localization of disease extent and treatment responses, we will use an image analysis technique, parametric response mapping (PRM) that precisely maps each imaging volume element (voxel) to the same site over sequential imaging studies (61). By co-registering identical voxels from MRI studies performed before and during therapy, we can determine changes in imaging parameters, such as diffusion and proton density fat fraction (PDFF), with much finer spatial and temporal resolution. In the context of cancer therapy, this voxel-wise image analysis method substantially improves accuracy for identifying response to therapy early in the course of treatment. We propose that this voxel-wise image analysis technique also will improve the ability to use MRI to quantify the extent of bone marrow ablation after pre-HCT conditioning, dynamics of bone marrow engraftment and repopulation after HCT, and potentially relapse of AML.

4.0 STUDY POPULATION

4.1 INCLUSION CRITERIA

4.1.1 Disease criteria and status:

To be eligible for this protocol the patient must have AML not in remission or at very high risk for post HCT relapse. This will generally be defined as greater than ≥ 5% myeloblasts by aspirate morphology as determined by a bone marrow aspirate and biopsy obtained within 2 weeks of study registration. The following scenarios indicate exceptions to the requirement for ≥ 5% myeloblasts:

a) In the event induction treatment results in a hypoplastic bone marrow status (< 10% cellularity), precluding accurate enumeration of blast percentages, the patient is still eligible if the preceding bone marrow aspirate contained ≥ 5% myeloblasts. To meet this condition, prior induction therapy must have been completed a minimum of 21 days prior to this result.

b) In the event < 5% myeloblasts by aspirate are enumerated but persistence of low level AML can be documented the patient is eligible. This can be defined as clear (probable or definite) flow cytometric persistence of the leukemia associated clone defined at time of diagnosis. Alternatively, persistence of cytogenetic abnormalities by karyotype satisfies this requirement.

c) The patient has evidence at diagnosis of poor cytogenetic or molecular risk associated with very high risk for relapse after HCT. Adverse cytogenetic risk is defined as complex karyotype with ≥ 4 clonal abnormalities, inv(3) / t(3;3), and t(6;9). Monosomal karyotype is defined as the presence of two or more autosomal chromosome monosomies or one single autosomal monosomy in the presence of one or more structural chromosomal abnormalities, excluding marker and ring chromosomes. Poor molecular risk is defined as the presence of FLT3-ITD mutation.

4.1.2 Prior Treatment (one of the following scenarios): To be eligible the patient must have received previous therapy in one of the following scenarios:

1) **Primary refractory:** for newly diagnosed AML, patients must have achieved two consecutive induction attempts without achieving complete remission.

2) **Relapsed / refractory:** for patients initially in complete remission whose AML relapses > 6 months after preceding remission, one re-induction must be attempted to be eligible

3) **Relapsed / untreated:** for AML patients with early relapse, in whom the preceding remission is shorter than 6 months duration, no re-induction regimen is necessary to be eligible.

4) **Patients with antecedent MDS (or CMML) who progress to AML:** may have therapies rendered during both phases counted towards these requirements. For example, a patient with MDS (RAEB-II) receives hypomethylating agent or other induction therapy that progresses to AML. In such cases regimens administered prior to transformation can be counted.
5)
6) **Patients with poor cytogenetic or molecular risk:** associated with very high risk for relapse after HCT (Section 4.1.1 C) may proceed without provisions for prior treatment as listed above. However, they must have received at least one induction attempt. Note: Hypoplastic bone marrow status (section 4.1.1 A) and persistence of low level AML (section 4.1.1 B) do not constitute achieving complete remission.

4.1.3 Donors and Stem Cell Source:

1) Availability of an 8/8 matched related or unrelated donor at A, B, C, and DR loci. Note: Mismatchs at HLA DQ and/or HLA DPB1 are permissible.

2) Availability of a HLA-haploidentical donor from a first degree relative (parents, children or sibling). In this context, HLA matching at only one haplotype is acceptable according to usual BMT program practice guildelines.

3) Peripheral blood or bone marrow stem cells are acceptable.

4.1.4 Age: Patients must be ≥ 18 years of age and considered a candidate for HCT. There is no defined upper age limit so long as patient meets institutional criteria for myeloablative HCT (see exception of haplo-identical donors in section 5.4).

4.1.5 Performance status: Karnofsky ≥ 70% (see **APPENDIX A**)

4.1.6 Organ function: Patients must meet acceptable organ function criteria for allogeneic transplant per institutional guidelines. A summary of these guidelines are shown in Table 1 below and may also be found at the following URL: http://www.med.umich.edu/i/cancer/guidelines/bmtsoc.htm

TABLE 1. ORGAN FUNCTION CRITERIA FOR PROCEEDING ON STUDY

Total bilirubin	≤2.5 mg% (unless from Gilbert's disease or disease-related)
AST(SGOT)/ALT(SGPT)	<5.0 X institutional upper limit of normal
Estimated or actual GFR*	>40 mL/min/1.73 m2 for patients with creatinine levels above institutional normal *GFR should be corrected for BSA
Pulmonary Function Tests	DLCO, FEV1, FVC > 50% DLCO should be corrected for hemoglobin
Ejection Fraction	>50%

4.1.7 Consent: All patients must be informed of the investigational nature of this study and given written informed consent in accordance with institutional and federal guidelines.

4.1.8 The effects of Peg-IFN-α on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial (e.g., tacrolimus and methotrexate) are known to be teratogenic, women and men of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior

to the study, for the duration of study participation, and 3 months after completion of Peg-IFN-α.

4.2 EXCLUSION CRITERIA

4.2.1 Prior chemotherapy treatment for AML within 21 days from the initiation of HCT conditioning. Note, use of hydroxyurea or other low intensity treatment not intended to induce remission but rather stabilize disease are acceptable.

4.2.2 Patients may NOT have evidence or symptoms of CNS disease at the time of enrollment.

4.2.3 HIV or HTLV1 / HTLV2 (seropositivity and/or PCR positivity)

4.2.4 Patients less than 18 years of age.

4.2.5 Pregnant and nursing mothers are excluded from this study

4.2.5 Patients with untreated or uncontrolled neuropsychiatric illness.

4.2.6 Any physical or psychological condition that, in the opinion of the investigator, would pose unacceptable risk to the patient.

4.2.7 Uncontrolled infections. Patients still under therapy for presumed or proven infection are eligible provided there is clear evidence (radiologic, clinical and/or culture) that the infection is well controlled.

4.2.8 Unrelated donor with HLA mismatch at A, B, C, and DR loci.

5.0 STUDY DESIGN

This is a prospective, non-randomized, phase I/II study of commercially available peg-IFN-α (peginterferon alfa-2a; PEGASYS®, Genentech, Inc.) for prevention of relapse in AML patients not in remission undergoing HCT. The pre transplant chemotherapy will consist of myeloablative conditioning per investigator. HCT will be administered on day 0. GVHD prophylaxis will consist of calcineurin inhibitor plus methotrexate per institutional guidelines. **An overview of the study is shown below in FIGURE 5.**

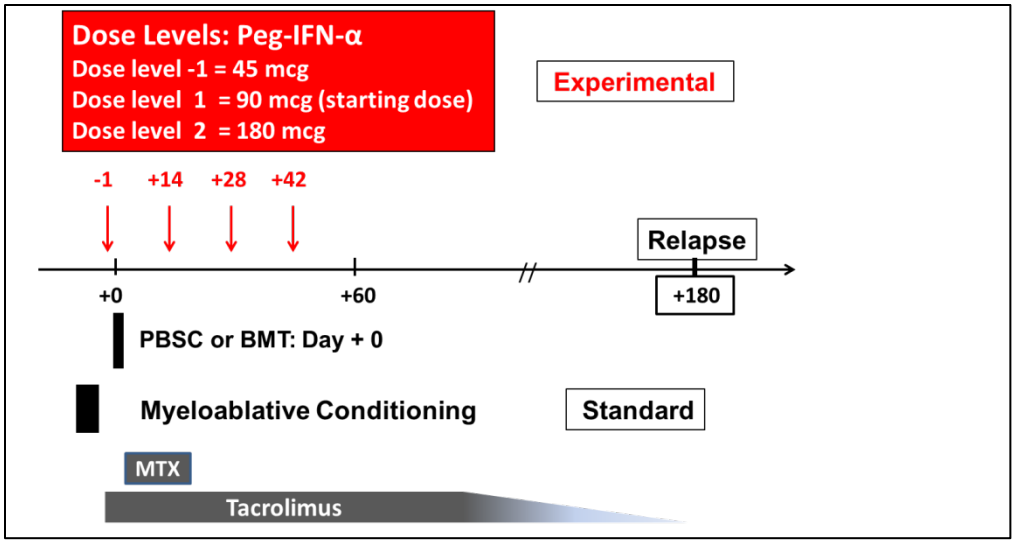

Figure 5: Study schema for peg-IFN-α in prevention of relapse for AML not in remission at HCT

5.1 Patient Evaluations

An IRB-approved informed consent must be obtained from patients (or legal guardians) prior to the initiation of treatment on this protocol. Patient demographics, including underlying disease, donor type, and eligibility criteria will be recorded at entry into the study. All screening evaluations will be completed as part of the standard work up for HCT.

5.2 peg-IFN-α: Is the study agent and will be administered prior to HCT (donor stem cell infusion) and at three subsequent time points post HCT. (Maximum of 4 doses)

5.2.1 Timing of dosing: peg-IFN-α will be administered by subcutaneous injection every 14 days as shown in **Table 2**.

TABLE 2. SCHEDULE OF ADMINISTRATION FOR PEG-IFN-α

Dose	Day of administration	Last day to administer (if delay)
1	-1 (Pre HCT)	+6
2	+14	+21
3	+28	+35
4	+42	+49

A maximum of four doses will be administered. If dosing is postponed for any reason on its scheduled day of administration, it can be given for up to 7 days. Subsequent doses should be administered as per the above schedule. There should always be a minimum of 7 days between doses. No doses will be administered beyond day + 49.

5.2.2 Determination of dose level: The first patient will be assigned to dose level 1 (90 mcg) and subsequent dose assignments for future patients will be determined using the modified toxicity probability interval (mTPI), based on the occurrence of dose limiting toxicities (DLTs). Briefly, if minimal toxicity is encountered, dose level 2 will be employed (180mcg), which is the FDA approved starting dose of peg-IFN-α. If excessive toxicity, we will de-escalate dose level -1 (45mcg). If excessive toxicity occurs at dose level -1, the study will be halted to determine if the trial should be amended or closed. The study is also subject to termination according to stopping rules listed in section 16. Finally, the study principle investigator with input from BMT data safety monitoring board may elect to defer dose escalation or continue at lower dosage if deemed to be in the best interest of patient safety. Note, specifics of this design and dose assignment are described in the statistical section of this protocol (section 16).

5.3 Allogeneic Stem Cell Collection and Donor Mobilization

Stem cells are to be collected and infused on day 0 as per the institutional Clinical Practice Guidelines. Recommended stem cell dosages are per clinical practice guidelines.

5.4 Conditioning Regimen

Eligible patients will receive a ***myeloablative*** conditioning regimen considered suitable for patients undergoing an allogeneic HCT according to the current institutional BMT program clinical practice guidelines. The specific agents used for myeloablative conditioning will not be mandated, and will be determined by the patient's primary physician and BMT team at weekly clinical consensus meetings. **Reduced intensity conditioning regimens are prohibited with the exception of recipients of haplo-identical donor HCT. In haplo-identical contexts, conditioning intensity and regimen will be selected the patient's investigator. 5.5 Seizure Prophylaxis**

Seizure prophylaxis per institutional guidelines is recommended for recipients of busulfan containing preparative regimens.

5.6 Graft versus Host Disease (GVHD) Prophylaxis

Eligible patients will receive a standard GVHD prevention regimen according to the current institutional BMT program clinical practice guidelines. This will include a calcineurin-inhibitor (tacrolimus or cyclosporine) based regimen. Calcineurin-inhibitor will typically be combined with mini-dose methotrexate (MTX). Combination with mycophenolate mofetil (MMF) is an alternative option. The preferred calcineurin-inhibitor is tacrolimus, but cyclosporine can be substituted if patients cannot tolerate tacrolimus. In patients who are unable to tolerate tacrolimus or cyclosporine, sirolimus may be substituted.

5.6.1 Tacrolimus

Tacrolimus begins on day -3 and is dosed IV or orally according to BMT program clinical practice guidelines, with a desired trough level of 5-15 ng/ml. When cyclosporine is substituted for tacrolimus a desired trough level is 200-300 ng/ml. It is suggested that tacrolimus be tapered below therapeutic range starting around day 90 post-transplant, per current institutional practice, provided the patient has no evidence of GVHD. The recommended taper is approximately 20% of the daily tacrolimus dose at the time of initiating taper. In the absence of GVHD, tapers are suggested every 14 - 28 days with goal of stopping tacrolimus at day + 180.

5.6.2 Methotrexate

If methotrexate is administered as part of GVHD prophylaxis it will be given as an intravenous infusion at 5-15 mg/m^2 on day +1, and 5-10mg/m^2 +3, + 6, and + 11 post transplant. Standard criteria for administration will be followed as outlined in the institutional BMT program clinical practice guidelines.

5.6.3 Mycophenolate mofetil (MMF or CellCept)

If mycophenolate is administered as part of GVHD prophylaxis it will be given as 1 gram PO every eight hours starting on day 1 and ending on day 28 post HCT. Mycophenolate can also be given intravenously at a dose of 10 mg/kg/dose maximum 1 gram/dose), administered every 8 hours if the patient is unable to tolerate oral medications. The conversion is 100% of the oral dose.

5.7 Post-transplant Supportive Care

Post-transplant supportive care, including infection prophylaxis will generally be conducted as per the BMT program Clinical Practice Guidelines.

Micafungin 50 mg IV once daily is recommended for fungal prophylaxis from admission until day +5 post transplant to minimize potential interaction with hepatic function, especially if using clofarabine x busulfan based conditioning (ie. CloBu4). Anti-fungal coverage with mold activity (Voriconazole or Pozaconazole) are recommended starting at day + 5.

Granulocyte colony stimulating factor (G-CSF, Neupogen) will be administered post - transplant per institutional Clinical Practice Guidelines.

6.0 MANAGING ACUTE GVHD

6.1 Assessment and treatment: The absence or presence of clinical Acute GVHD (aGVHD), including severity, should be clinically evaluated within 72 hrs of study drug administration. aGVHD will be diagnosed, treated, and recorded by using the standard

BMT program clinical practice guidelines, which including clinical grading once weekly. aGVHD severity will be determined clinically (see **APPENDIX B**). Biopsies of affected organs are strongly encouraged whenever possible.

6.2 Dose modification of peg-IFN-α: Although pre-clinical and clinical data suggest peg-IFN-α will not significantly increase aGVHD, this remains a potential risk of enhancing post HCT immune responses. Thus, the parameters in **Table 3** are designed to enable patients with very high risk for relapse to remain on study therapy, but without excessive exposure to risk.

TABLE 3: DOSE MODIFICATION ACCORDING TO ACUTE GVHD

GVHD Grade	Dose Modification of peg-IFN-α
Grade I	continue without modification
Grade II	Hold further doses (1)
Grade III – IV	Stop further doses (2)

(1) Grade II aGVHD: peg-IFN-α should be temporally held pending observation of clinical response. If the patient is responding well to GVHD directed therapy, has been tapered to a corticosteroid dose of ≤ 0.5 mg/kg, and remains within the study treatment period (e.g. < 50 days post HCT), peg-IFN-α can be resumed at discretion of primary BMT physician. For example, a patient develops grade II aGVHD of the skin with 60% body surface area (BSA) involvement, and after two weeks of treatment has < 25% BSA involvement. Co-enrollment on acute GVHD treatment trials is permitted.

(2) Grade III-IV aGVHD: no further doses of peg-IFN-α will be administered, The patient will be removed from further study treatment. In cases of grade III-IV or steroid refractory GVHD, in addition to treatment with standard of care, co-enrollment on acute GVHD treatment trials is permitted.

7.0 ASSESSMENT AND MANAGING AML RELAPSE

7.1 Scheduled disease assessments: are per standard BMT clinical practice guidelines for AML not in remission. Bone marrow biopsy and aspirate will be obtained pre-transplant (within 14 days of study registration) and post-transplant on day 28 (±7), day 100 (± 14), and day 180 (± 28). Additional assessments of relapse can be performed at any time there is clinical suspicion for relapse.

7.2 Definitions:

7.2.1 Remission:

For the purposes of this study, we define remission as achieving a morphologic leukemia free state by achieving all of the following criteria: bone marrow

myeloblasts < 5% by morphologic assessment; **AND** absence of circulating blasts with phenotypic or morphologic features of leukemia (e.g. Auer rods) **AND** no evidence of extramedulary disease (62). Note, since these assessments are occurring in the early post HCT period where numerous factors can affect blood counts, full hematologic recovery is not a required component for remission.

7.2.2 Relapse or Progression:

We define relapse by meeting one of following criteria: Bone marrow myeloblasts ≥ 5% by morphologic assessment not attributed to other causes (e.g., bone marrow regeneration, G-CSF administration within previous 7 days) **OR** reappearance or persistence of circulating blasts with phenotypic or morphologic features of leukemia **OR** the development or persistence of extramedullary leukemia. Note, due to the large leukemic burden of patients at time of HCT, suspicion for low level disease or minimal residual disease (MRD) by cytogenetics, flow cytometric abberency, or molecular methods will not be counted as a relapse event, but will be recorded. The presence of these features will be later correlated with occurrence of hematologic relapse.

7.3 Dosing of peg-IFN-α after relapse:

Patients with documented relapse or disease persistence as defined in section 7.2.2 may continue to receive peg-IFN-α at the discretion of their primary BMT attending. Rapid tapering and / or withdrawal of immunosuppression to illicit aGVHD is a standard practice in the setting of relapse and is allowed. However, if additional leukemia directed treatment is initiated (e.g. re-induction chemotherapy, donor lymphocyte infusion, or clinical trial) the patient will be removed from this study.

8.0 DOSE MODIFICATIONS BASED ON LABORATORY VALUES

8.1 Hematologic parameters

The following hematologic dose modifications pertains to post – engraftment doses of peg-IFN-α (i.e. doses administered on or after day + 28)

If platelet < 20,000 will hold dose, may resume once platelet >20,000 without transfusion in the preceding 72 hrs.

If ANC < 0.5 ul / mm^2 will hold dose, may resume when ANC > 0.5 ul / mm^2. Use of G-CSF is allowed.

8.2 Renal function

If GFR is less than 30 mL/min and patient is assigned to dose level 2 (180 mcg), the patient should have their dose reduced to 90 mcg

8.3 Hepatic function

Patients receiving HCT with active AML and myeloablative conditioning may have transient elevations in LFTs and total bilirubin. Peg-IFN-α has been safely administered to patients with active hepatitis and cirrhosis. However, if ALT or AST is greater than 10 times above the upper limit of normal **OR** total bilirubin ≥ 8 mg/dl, treatment will be temporarily suspended. If ALT or AST fall to < 5 x ULN and total bilirubin < 8 mg/dl, peg-IFN-α can be resumed.

8.4 Fevers and infection

Fevers are expected after HCT and are commonly encountered during interferon therapy. Infectious causes of high or persistent fever must be ruled out, particularly in patients with neutropenia. There are no specific dose modifications for fever.

9.0 REQUIRED OBSERVATIONS (STUDY CALANDER):

In general pre and post HCT care will follow BMT program clinical practice guidelines. For example, routine history, physical, organ function testing (PFT, Echocardogram), and laboratory evaluations (including serologic and/or PCR based viral studies) will follow institutional practice guidelines.The following studies will be obtained pre-therapy and post-therapy **(TABLE 4)**. These studies will be collected as required observations for study.

TABLE 4: STUDY CALANDER

	Pre	**Treatment**				**Follow-up**			
Observation Period (day)	**-28 to -7**	**-1**	**+14**	**+28**	**+42**	**+56**	**+100**	**+ 180**	**Relapse**
Informed Consent	x								
Pregnancy test	x								
KPS (Karnofsky)	x								
Medical examination	x	x	x	x	x				
Laboratory testing [1]	x	x	x	x	x				
peg-IFN-α [2] **(study agent)**		x	x	x	x				
Acute GVHD assessment [3]			x	x	x				
BM aspirate & biopsy[4]	x			x			x	x	x
Research samples	x			x		x	x	x	x
MRI	X	X		X					

[1] Includes CBC, serum chemistries, and standard viral pcr screening in pre-HCT period as part of institution practice guidelines (CPG). CBC with differential, serum creatinine, AST, ALT, and total bilirubin should be measured within 3 days of peg-IFN-α administration.

[2] Per protocol, peg-IFN-α may be administered for up to 7 days after treatment date indicated above (see section 5.2.1). In such instances, exam, labs, and aGVHD assessments can be deferred to the new treatment day.

[3] Per institution practice guidelines (CPG) including assessments for chronic GVHD.

[4] Pre - HCT marrow aspirate must be within two weeks of study registration.

NOTE: required observations: Patient's medical condition and scheduling issues may impact the timing and acquisition of post-HCT observations. The acceptable time frame for completing these observations is ± 10 days for observations prior to day 56 and ± 14 days for observations including and after day + 56. Failure to obtain research blood collections or MRI in accordance with the above schedule will not be considered a protocol deviation. The day -1 MRI, if delayed, must occur before infusion of donor cells on day 0 or will be omitted.

10.0 RESEARCH SAMPLES

10.1 Research blood samples

We will obtain peripheral blood samples in 5 heparinized tubes (green top), 1 Cell-Free DNA BCT® ("Streck") tubes, and 1 EDTA tube (purple top) (approximately 50 mL total)from each patient at 5 planned timepoints during the course of the study. Samples will be collected coincident with routine blood draws on the patients. An additional 5 mL of the bone marrow aspirate will be procured at the same time as pre-HCT and post HCT bone marrow examinations. Finally, one heparinized blood sample (approximately 2-3ml mL) will be taken from the donor stem cell grafts prior to patient administration. Note: Donors must sign and additional separate consent for this donor stem cell research specimens to be collected. Subjects participation in the collection of research samples is optional.

Samples will be obtained at baseline (day -7) prior to conditioning, study drug, and stem cell infusion and then on day 28 (after 2 doses of peg-IFN-α), day + 56 (after 4 doses peg-IFN-α), day + 100 and day +180. PBMCs and serum will be isolated and frozen for future analysis. All blood samples will be processed, frozen, and banked in the Immunology Core Lab at the University of Michigan with the exception of the Streck tube and EDTA tube, which will be processed, frozen, and banked in the laboratory of Dr. Brian Parkin, respectively. All urine samples will be processed, frozen, and stored in the laboratory of Dr. Muneesh Tewari.

10.1.1 Research urine samples

Recent studies have demonstrated that nucleic acid and other analytes in urine may serve as markers for systemic (i.e. non-urinary tract) disease status. This includes microRNA, cell-free (cf) trans-renal DNA fragments, potentially other diverse species of RNA, and other biomarker types. In this study, we plan to study blood and urine cfDNA and microRNA as potential biomarkers for GVHD in AML patients undergoing HCT, and to compare the concordance of biomarkers between blood and urine.

We will obtain urine specimens in a urine hat or urinal using the Simple Urine Collection subject instructions. (Please see Appendix A below for Subject Instructions for urine collection.) Ideally within 30 minutes of urination, the urine will be transferred to a collection container with EDTA solution. Samples will be collected coincident with routine blood draws or other routine patient care.

10.2 Correlative studies

The primary aim of the correlative studies is to assess DC mediated cross presentation by serial measurement of AML specific immune responses. Generation of T cells directed towards tumor associated antigens (TAA) commonly overexpressed by myeloid leukemias, such as WT1, Pr3, NE, MAGE-A3, PRAME, will be measured by responses to peptide libraries and HLA class I tetramer staining. T cells specific for TAA will be measured before HCT (donor cell graft) and serially after HCT following exposure to AML antigens. Tetramer staining will be restricted to HLA-A*0201 positive patients. Experiments involving peptide libraries and tetramer staining using de-identified patient samples will be directed by the laboratory of Dr. Pavan Reddy (Univ. of Michigan) ~~but in collaboration with Dr. John Barrett (National Institute of Heath) and /~~ or Dr. Katayoun Rezvani (MD Anderson Cancer Center) who have extensive expertise in these methods. Any human samples sent for such analysis by external collaborators will be deidentified to protect subject personal health information. All other experiments will be performed solely at the University of Michigan in the laboratory of Pavan Reddy. These experiments will include phenotyping of key immune subsets including numbers and activation status of BDC3A+ DCs, functional assays, pharmacodynamic assessment of IFNα signaling, and cytokine profiles.

Furthermore, the secondary aim of the correlative studies is to determine the dynamics of molecular minimal residual disease (MRD) during and after treatment and to characterize genomic and transcriptomic features of non-remission AML and any subsequent relapses after the experimental treatment. MRD will be measured in bone marrow and peripheral blood cell-based genomic DNA as well as plasma-based cell-free DNA using a combination of droplet digital PCR and third-generation DNA sequencing to detect mutations previously identified in the pretreatment specimen. Genomic and transcriptomic profiling of pre-treatment and post-relapse specimens will include whole exome sequencing, total mRNA sequencing, and determination of DNA copy number/LOH with genome-wide SNP arrays. These experiments will be performed at the University of Michigan in the laboratory of ~~Drs. Sami Malek and~~ Dr. Brian Parkin.

Pharmacodynamic (PD) targets of peg-IFN-α signaling will be assessed including, but not limited to, components of JAK, Tyk2, and STAT pathways. Serum measurements of inflammatory cytokines will be analyzed. Finally, the laboratory will perform *in vitro* assays to evaluate DC, tumor and viral specific T cell responses (proliferation, Elispot for cytokine production).

10.3 Correlative imaging studies.

Subjects in this study may opt to participate in MRI of bone marrow in the lumbar spine and pelvis with PRM analysis to quantify spatial and temporal dynamics of bone marrow ablation and repopulation prior to and following HCT, respectively. Subjects may withdraw from the MRI component of the protocol without withdrawing from the remainder of the protocol. Additional eligibility criteria for a subject to participate in the correlative MRI studies are: 1) no contraindications to MRI, such as a pacemaker; and 2) able to undergo MRI without general anesthesia and 3) medical condition sufficiently stable in the opinion of the treating physician to enable transportation and completion of the MRI sequences . This is particularly relevant to plan imaging on Day -1

MRI examinations of the spine, pelvis, and proximal femora will be performed with a 6-channel torso coil, body coil, or dedicated surface coil as determined by size of the patient. Survey scans will be performed for patient positioning and defining anatomic sites for subsequent scans. We will perform MRI sequences for quantitative fat and water imaging, cross relaxation transfer, T2* decay, susceptibility, and mobility of water. Specific sequence parameters will be adjusted within FDA-approved safety parameters for individual patients. No intravenous contrast material will be used. Anticipated duration of each MRI study is approximately 40 minutes.**10.4 Additional research samples**

Additional samples will be collected whenever a possible new diagnosis of suspected relapse is made. Attempts will be made to obtain samples in a timely fashion before treatment is initiated. However, treatment will not be delayed while awaiting sample procurement. Obtaining samples for research purposes are secondary, not primary objective of this study. As such, failure to have a research sample drawn at any time point will not be considered a protocol violation.

11.0 DRUG INFORMATION

11.1 Peginterferon alfa-2a (peg-IFN-α, PEGASYS®)

11.1.1 Formulation

The chemical structure for peg-IFN-α is made by conjugating a single branched polyethylene glycol chain (PEG) of approximate molecular weight of 40 kilodaltons (kD) to interferon alfa-2a (20 kD) via a stable amide bond. The combination of PEG and interferon alfa-2a forms an intact active molecule known as peginterferon alfa-2a, having an approximate molecular weight of 60 kD. Chemically, it is a bis-(N-monomethoxypolyethylene-glycol-urethanyl) lysyl interferon alfa-2a.

11.1.2 Availability

Peg-IFN-α is a sterile ready-to-use solution for subcutaneous injection. It is available as autoinjectors in two strengths, 135 mcg/0.5 mL and 180 mcg/0.5 mL, as prefilled syringes containing 180 mcg/0.5 mL, or as single-use vials containing 180 mcg/1.0 mL, per institutional standard. The solution is clear and colorless to light yellow. The strength of

peg-IFN-α is expressed as the amount of interferon alfa-2a, with excipients sodium chloride, benzyl alcohol, sodium acetate, acetic acid, polysorbate 80 and water for injections.

11.1.3 Storage and stability

The samples will be stored per the manufacturer's recommendation.

11.1.4 Pharmacokinetics

Time to Peak Concentration: The absorption of peg-IFN-α is sustained with peak serum concentrations reached 72 – 96 h after dosing. Serum concentrations are measurable within 3 – 6 h of a single subcutaneous injection of peg-IFN-α 180 mcg. Within 24 h, about 80% of the peak serum concentration is reached. The absolute bioavailability of peg-IFN-α is 84% and is similar to that seen with interferon alfa-2a

Peak Concentration: The peak concentrations (Cmax) after single administration of 180 mcg dose of peginterferon alfa-2a, was 14 ± 5 ng/ml.

Elimination half-life: A mean elimination half-life of 160 h (84 – 353 h) at primary elimination phase was observed in patients after subcutaneous (SC) administration of peg-IFN-α. The elimination half-life determined after SC administration may not only reflect the elimination phase of the compound, but may also reflect the sustained absorption of peg-IFN-α.

Distribution: Peg-IFN-α is found predominately in the bloodstream and extracellular fluid as seen by the volume of distribution at steady-state (Vss) of 6 – 14 L after intravenous (IV) dosing in humans. Based on studies in rats, peg-IFN-α is distributed to the liver, kidney, and bone marrow in addition to being highly concentrated in the blood.

Area Under the Curve: The mean area under the curve (AUC), after single administration of 180 mcg dose of peg-IFN-α, was 92 hrs ± 27.

Metabolism: The metabolic profile of peg-IFN-α is not fully characterized.

11.1.5 Administration

Peg-IFN-alpha-2a will be administered subcutaneously every two weeks to begin on day -1 for a maximum of 4 doses.

11.1.6 Adverse events

Use in hepatitis C and B: The principal adverse reactions listed below are primarily from clinical studies in patients who received peg-IFN-α at 180mcg once weekly for 24 - 48 weeks for treatment of chronic hepatitis B or C with or without evidence of compensated cirrhosis. (**adapted per PEGYSUS package insert, 2013 Genentech, Inc.)**

In general, the most common adverse reactions (incidence greater than 40%) are fatigue/asthenia, pyrexia, myalgia, and headache. The most common life-threatening or fatal events induced or aggravated by PEGASYS include depression, suicide, relapse of drug abuse/overdose, and bacterial infections, each occurring at a frequency of less than 1%. Hepatic decompensation occurred in 2% (10/574) of CHC/HIV subjects.

The most common serious adverse reactions (3% in CHC and 5% in CHC/HIV) was bacterial infection (e.g., sepsis, osteomyelitis, endocarditis, pyelonephritis, pneumonia). Other SAEs occurred at a frequency of less than 1% and included: suicide, suicidal ideation, aggression, anxiety, drug abuse and drug overdose, angina, hepatic dysfunction, fatty liver, cholangitis, arrhythmia, diabetes mellitus, autoimmune phenomena (e.g., hyperthyroidism, hypothyroidism, sarcoidosis, systemic lupus erythematosus, rheumatoid arthritis), peripheral neuropathy, aplastic anemia, peptic ulcer, gastrointestinal bleeding, pancreatitis, colitis, corneal ulcer, pulmonary embolism, coma, myositis, cerebral hemorrhage, thrombotic thrombocytopenic purpura, psychotic disorder, and hallucination.

In clinical trials, 98 to 99 percent of subjects experienced one or more adverse reactions. For hepatitis C subjects, the most commonly reported adverse reactions were psychiatric reactions, including depression (18%), insomnia (19%), irritability (19%), and flu-like symptoms such as fatigue (56%), pyrexia (37%), myalgia (37%), headache (54%), and rigors (35%). Other common reactions were anorexia (17%), nausea and vomiting (24%), diarrhea (16%), arthralgia (28%), injection site reactions (22%), alopecia (23%), and pruritus (12%).

Chronic hepatitis C monoinfected subjects treated for 24 weeks with PEGASYS and 800 mg COPEGUS (PEGASYS + Ribaviron) were observed to have lower incidence of serious adverse reactions (3% vs. 10%), Hgb less than 10 g/dL (3% vs.15%), dose modification of PEGASYS (30% vs. 36%) and of withdrawal from treatment (5% vs. 15%) compared to subjects treated for 48 weeks. The most common reasons for dose modification of PEGASYS in CHC and CHC/HIV subjects was for neutropenia (20%) and thrombocytopenia (4%).

The following serious conditions, some of which may become life threatening, have been reported have been observed in patients treated with PEGASYS. Hypertension, supraventricular arrhythmias, chest pain, and myocardial infarction. Development or exacerbation of autoimmune disorders including myositis, hepatitis, thrombotic thrombocytopenic purpura, idiopathic thrombocytopenic purpura, psoriasis, rheumatoid arthritis, interstitial nephritis, thyroiditis, and systemic lupus erythematosus have been reported in patients receiving alpha interferon. PEGASYS causes or aggravates hypothyroidism and hyperthyroidism. Hyperglycemia, hypoglycemia, and diabetes

mellitus have been observed to develop in patients treated with PEGASYS. Acute hypersensitivity reactions (e.g., urticaria, angioedema, bronchoconstriction, and anaphylaxis) have been observed during alpha interferon. Pancreatitis, sometimes fatal, has occurred during alpha interferon. Dyspnea, pulmonary infiltrates, pneumonia, bronchiolitis obliterans, interstitial pneumonitis, pulmonary hypertension and sarcoidosis, some resulting in respiratory failure and/or patient deaths, may be induced or aggravated by alpha interferon therapy

Use in myeloproliferative neoplasms: A phase II study of weekly PEGASYS in 79 patients was conducted in patients with advanced essential thrombosis or polycythemia vera exploring dosages ranging from 45mcg to 450mcg (48). Patients received therapy indefinitely as long as they deriving clinical benefit. At a median follow up was 22 months 79% of patients remained on therapy. Ninety-six percent of patients developed some toxicity, generally grade 1 or 2. The most frequent grade 3 or 4 toxicity was neutropenia, which occurred in 20%, others included elevated LFTs (6%), fatigue (4%), pain (3%), depression (3%). The toxicity profile of peg-IFN-α was significantly more benign in the 28 patients who started at 90mcg weekly. The 90mcg weekly schedule resulted in no grade 4 toxicities and a low rate of grade 3 toxicities, including neutropenia in two patients (7%) and infection, diarrhea, depression, and elevated liver function tests in one patient (4%) each.

Use of IFN after HCT in AML. Data in the post HCT setting is limited to multiple case series and reports of short acting IFN preparations for treatment of post – HCT relapse (63-67). IFN has been administered in combination with complete cessation of immunosuppression together with either chemotherapy, DLI, or additional cytokines therapies (e.g. IL-2) to illicit GVHD. All reports suggest that the use of IFN was feasible and tolerable, but assessment of adverse events are limited by confounding variables and progressive AML. Under these conditions aGVHD occurred in approximately 60% of patients. The use of IFN was not associated with more severe aGVHD or death. While the presence of GVHD may be associated with increased likelihood of achieving durable remission. In a small randomized study of acute lymphoblastic leukemia (ALL), human leukocyte IFN was administered prophylactically after HCT to prevent CMV reactivation. Compared to control patients CMV rates were similar, but relapse was lower in the IFN treated patients and there was no difference in the probability or severity of aGVHD (51).

11.2 Methotrexate

11.2.1 Formulation

Chemically methotrexate is N - [4 - [[(2, 4 – diamino – 6 – pteridinyl) methyl] methylaminolbenzoyl] – L - glutamic acid. Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, adult rheumatoid arthritis, and prevention of acute GVHD. Methotrexate inhibits dihydrofolic acid reductase. Dihydrofolates must be reduced to tetrahydrofolates by this enzyme before they can be utilized as carriers of one-carbon groups in the synthesis of purine nucleotides and

thymidylate. Therefore, methotrexate interferes with DNA synthesis, repair, and cellular replication.

11.2.2 Availability and administration

Methotrexate sodium for injection is available in 25mg/ml solution. The appropriate amount is drawn into a syringe for administration. Store at controlled room temperature, 20°-25° C (68°-77° F); excursions permitted to 15°-30° C (59°-86° F). Protect from light.

11.2.3 Potential Side Effects

The most frequently reported adverse reactions associated with methotrexate use as GVHD prophylaxis include ulcerative stomatitis, leucopenia and suppressed hematopoiesis, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, undue fatigue, chills and fever, dizziness and decreased resistance to infection. Methotrexate may be associated with increased rates of pulmonary complications after transplantation. The risk of infections is due to the suppression of hematopoiesis after transplantation.

11.2.4 Potential Drug Interactions

Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored. Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad-spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria. Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

11.3 Mycophenolate (CellCept)

11.3.1 Formulation and mechanism of action

Mycophenolate mofetil is the 2-morpholinoethyl ester of mycophenolic acid (MPA), animmunosuppressive agent; inosine monophosphate dehydrogenase (IMPDH) inhibitor. Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to formMPA, which is the active metabolite. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without

incorporation into DNA. Because T- and Blymphocytes are critically dependent for their proliferation on de novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes. MPA prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil did not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 and interleukin-2, but did block the coupling of these events to DNA synthesis and proliferation.

11.3.2 Availability

CellCept is available for intravenous or oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil. CellCept Intravenous is the hydrochloride salt of mycophenolate mofetil. CellCept Intravenous is available as a sterile white to off-white lyophilized powder in vials containing mycophenolate mofetil hydrochloride for administration by intravenous infusion only. Each vial of CellCept Intravenous contains the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt. The inactive ingredients are polysorbate 80, 25 mg, and citric acid, 5 mg. Sodium hydroxide may have been used in the manufacture of CellCept Intravenous to adjust the pH. Reconstitution and dilution with 5% dextrose injection USP yields a slightly yellow solution of mycophenolate mofetil, 6mg/mL.

11.3.3 Administration

The dosing for oral and intravenous mycophenolate are the same. For oral dosing, doses should be rounded to the nearest 250 mg. The oral solution (200 mg/ml) may be substituted for capsules or tablets at patient preference or physician discretion, in which case, the dose should be rounded to the nearest 100 mg (0.5 ml).

11.3.4 Adverse events

The principal adverse reactions associated with the administration of CellCept include diarrhea, leukopenia, sepsis, vomiting, and a higher frequency of infections, including opportunistic infection. The adverse event profile is similar regardless of the form administered.

11.4 Tacrolimus (Prograf, FK506)

11.4.1 Formulation

Tacrolimus, previously known as FK506, is the active ingredient in Prograf. Tacrolimus is a macrolide immunosuppressant produced by Streptomyces Tsukubaensis. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the generation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

11.4.2 Availability and administration

Prograf is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 1-mg capsule shell contains gelatin and titanium dioxide, and the 0.5 mg and 5-mg capsules shell contains gelatin, titanium dioxide and ferric oxide. Prograf is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v. Prograf injection must be diluted with 0.9% sodium chloride injection or 5% dextrose injection before use. Intravenous administration will be given by continuous infusion. Oral preparation will be administered on empty stomach every 12 hours.

11.4.3 Potential Side Effects

a. Increased susceptibility to infection and the possible development of lymphoma may result from immunosuppression.

b. Nephrotoxicity has been noted in 40% and 33% of liver transplantation patients receiving Prograf in the U.S. and European randomized trials, respectively. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. A lymphoproliferative disorder (LPD) related to Epstein - Barr virus (EBV) infection has been reported in immunosuppressed organ transplant recipients. The risk of LPD appears greatest in young children who are at risk for primary EBV infection while immunosuppressed or who are switched to Prograf following long-term immunosuppression therapy.

c. Mild to severe hyperkalemia has been noted in 44% and 10% of liver transplant recipients treated with Prograf in the U.S. and European randomized trials and may require treatment.

d. Neurotoxicity, including tremor, headache, and other changes in motor function, mental status, and sensory function were reported in approximately 55% of liver transplant

recipients in the two randomized studies. Tremor and headache have been associated with high whole-blood concentrations of tacrolimus and may respond to dosage adjustment. Seizures have occurred in adult and pediatric patients receiving Prograf. Coma and delirium also have been associated with high plasma concentrations of tacrolimus.

e. Hypertension is a common adverse effect of Prograf therapy. Mild or moderate hypertension is more frequently reported than severe hypertension. Antihypertensive therapy may be required; the control of blood pressure can be accomplished with any of the common antihypertensive agents. Since tacrolimus can cause hyperkalemia, potassium-sparing diuretics should be avoided. While calcium-channel blocking agents can be effective in treating Prograf-associated hypertension, care should be taken since interference with tacrolimus metabolism may require a dosage reduction.

f. Hyperglycemia was associated with the use of Prograf in 47% and 29% of liver transplant recipients in the U.S. and European randomized studies, respectively and may require treatment.

11.5 MRI of bone marrow

Participation in this study exposes subjects to three separate MRI examinations, each of which may last up to 60 minutes. The MRI sequences are entirely non-invasive and do not require intravenous contrast. MRI does not use ionizing radiation. MRI without intravenous contrast has an outstanding safety record, so the overall risk to each subject is very minimal.

12.0 STUDY DEFINITIONS

12.1 Definition of "screening": A patient is considered to be in the screening period from the time they signed consent until the date their eligibility criteria have been determined as either "eligible" or "ineligible (screen fail).

12.2 Definition of "Enrolled": A patient is considered to be enrolled onto the study once they have signed consent and have successfully met all screening criteria, as documented by the inclusion/exclusion document., and the eligibility criteria has been reviewed and accepted by either the P.I. or a Co-I. The date of enrollment will be documented as the date the P.I. or Co-I. has reviewed and approved eligibility.

12.3 Treatment Period: The study treatment period is defined as the first day of treatment with peg-IFN-α through the last day of treatment with peg-IFN-α. This period is expected to last from day -1 through day 56 (or two weeks after last dosage of peg-IFN-α, whichever occurs later). Thus, this period may be extended, due to a postponement of the patient's BMT procedure or peg-IFN-α dosing, without constituting a deviation. Patients who develop stage III-IV acute GVHD will be considered "off treatment" (see Section 6.2).

12.4 Follow Up Period: A patient is considered to be in the "follow up period" from the 14 days after they no longer receive peg-IFN-α therapy (~ day 56) through day +180 post

transplant (primary endpoint). After day 180 there are no additional study required protocol observations (sec. 9.0), and subjects will be only be monitored for routine clinical endpoints of GVHD, relapse, and survival for at least one year or until the study is terminated. More detailed outcome information after day 180 may be collected from clinical databases on other BMT research studies (HUM00043287, HUM00048885, if the subject has consented to these ancillary studies, and may be analyzed in relation to patients participating in this study (e.g for determination of long-term outcomes).

12.5 On Study: Patients are considered "On Study" from the time they are enrolled until they meet one of the "Off Study" criteria listed below.

12.6 Off Study: Patients are considered "Off study" if they meet one or more of the following criteria:

- Death
- Lost to follow up
- Entry onto a competing trial (e.g. AML therapeutic trial)
- Withdrawal of consent by patient or investigator for any further treatment and follow up observations
- Relapse of underlying malignancy and receiving AML directed therapy other than peg-IFN-α-2a
- Development of new malignancy
- Unacceptable or dose limiting toxicity or complication

Patients off study (who do not withdrawal consent)will still be followed for routine HCT clinical endpoints (e.g. GVHD, Relapse) and survival. Patients who develop AML relapse or GVHD may have collection of planned research samples. Patients have the right to withdraw from the study or refuse research samples at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of illness, adverse events, treatment failure, protocol violation, or other reasons. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation should be made at the time of the patient's withdrawal with an explanation of why the patient is withdrawing and every effort should be made to perform follow-up evaluations. The assessment and reporting period for all adverse events, reportable under this protocol, will occur from the first day the treatment with peg-IFN-α is administered until day +56 post transplant or until 14 days after the last dose of peg-IFN-α is administered, whichever comes last.

12.7 Definition of an "evaluable" patient: Patients removed from study who receive peg-IFN-α for at least one dose will be considered fully evaluable for toxicity. The number of patients removed from study prior to being fully evaluable will be monitored regularly by the study's DSMB in order to identify and address problems that may develop with respect to patient accrual. We estimate that < 5 subjects may need to be replaced.

12.8 Definition of engraftment and delayed engraftment:

12.8.1 Engraftment: Engraftment for neutrophils is defined as the first of three consecutive days in which the absolute neutrophil count (ANC) is > 500/uL. Engraftment for platelets is defined as the first of three consecutive days in which the platelet count is > 20,000/uL, without transfusion support.

12.8.2 Engraftment failure: will be defined as ongoing ANC <500/uL. Patients who engraft prior to day 28 and later have low ANC or platelet transfusion requirements, as frequently occurs in HCT patients due to infection and other complications will not be considered to have delayed engraftment.

13.0 ADVERSE EVENTS AND REPORTING CRITERIA

An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product. As such, the AEs will be followed and reported after the study subjects have been started on the investigational drug.

These events may be:

- *Definitely related:* clearly associated with study drug/treatment
- *Probably related*: likely associated with study drug/treatment
- *Possibly related*: may be associated with study drug or other treatment
- *Unlikely to be related*, or
- *Definitely not related* to the study drug/treatment

For reporting purposes, an AE should be regarded as definitely or probably related to the regimen if the investigator believes that at least one of following criteria are met:

- There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment.
- There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE.
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.
- A potential alternative cause does not exist.

Serious Adverse Drug Experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death,
- a life-threatening adverse drug experience,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

***Expected Event**:* - An event that is expected in that it has been addressed or described in one or more of the following: Informed consent document(s) for this study, IRB application for this study, grant application or study agreement, protocol or procedures for this study, investigators' brochure or equivalent (for FDA regulated drugs or devices), DSMB/DSC Reports, published literature, other documentation, or characteristics of the study population.

Unexpected adverse events are those that:

- those that do not fall in to the above criteria
- are not described in the package insert as far as peg-IFN-α is concerned.
- are not anticipated in the study informed consent or the BMT Program clinical transplant consent. This includes adverse events for which the specificity or severity is not consistent with
- the description in the informed consent.

The severity or grade of an adverse event may be measured using the following definitions:

Mild: Noticeable to the subject, but does not interfere with the subject's expected daily activities, usually does not require additional therapy or intervention, dose reduction, or discontinuation of the study. In the transplant setting CTCAE v. 4.0 grades 1 and 2.

Moderate: Interferes with the subject's expected daily activities, may require some additional therapy or intervention but does not require discontinuation of the study. In the transplant setting using the CTCAE v. 4.0 some grade 2 and most grade 3.

Severe: Extremely limits the subject's daily activities and may require discontinuation of study therapy, and/or additional treatment or intervention to resolve. In the transplant setting using the CTCAE v. 4.0 some grades 3 and all grade 4.

Therapy for hematological malignancies, with or without stem cell transplantation, is associated with significant toxicity. These toxicities are generally viewed as an anticipated consequence of therapy rather than an adverse event. Therefore, event reporting for HCT Protocols can be complicated and confusing to investigators, data managers, and regulatory oversight bodies because patients typically develop numerous complications such as infections, chemotherapy-related organ damage, medication side effects, etc. as part of the typical course of a bone marrow transplant and not related to the study therapy. Furthermore, transplant-related complications often occur both simultaneously and in series, as one complication leads to a series of additional downstream events, making

time-sensitive reporting of events difficult. Therefore, a well-conceived event reporting plan will separate complications that might be seen with any transplant, from study-related events that are relevant to subject safety. In order to achieve this goal, the DSM plan for this study will focus on rapid and specific identification of SAEs as follows:

a. Adverse events with severity grades 1, 2, 3, and all expected grade 4 toxicities will not be reported to the IRB, so long as they are expected for patients undergoing stem cell transplantation for hematological malignancies.
b. Grade 4 events which are serious and likely (probably or definitely) related to the investigational component of study therapy will be reported to the IRB.
c. Events occurring at unusual frequency or severity in study subjects compared to non-study subjects undergoing similar transplants will be reported to the IRB.
d. Events resulting in death (other than relapse or GVHD) regardless of attribution.

Therefore, we will not report as SAEs events that are expected and coincident with a typical transplant course unless they are either fatal or probably or definitely related to the investigational therapy.

Event grading: The NCI Common Terminology Criteria (Version 4.0) will be used to grade intensity of adverse events and assist in reporting adverse events.

Events not reported:
The large majority of patients undergoing unrelated stem cell transplant will frequently experience hematologic events (i.e. anemia, thrombocytopenia, leukopenia, neutropenia), infections, electrolyte abnormalities, and organ toxicities. Given the frequency with which these occur in transplant patients, we will not report events that coincide with events that typically occur during the pre-and post transplant related therapy regardless of severity unless they otherwise meet the criteria for reporting as detailed above and below. An event is regarded as typical if it is specified in the study consent or the BMT Program transplant consent and / or it occurs in more than 5% of transplant patients.

Events reported as adverse events in tabular format annually:
Events that are classified as severe (all CTCAE v.4 grade 4) will be reported to the Cancer Center DSMB with each routine report (quarterly) and in the annual IRB report so long as the events do not meet the criteria for expedited reporting as defined above. Events determined to be a CTCAEv4.0 grade 1 or 2 will not be captured as part of this protocol.

14.0 DATA SAFETY MONITORING PLAN (DSMP)

The following are the procedures for data and safety monitoring of this clinical trial to be conducted within the Blood and Marrow Transplant program. This is to insure the safety of participants, the validity of research data, and the appropriate termination of studies for

which significant benefits or risks have been uncovered or when it appears that the trial cannot be concluded successfully. This protocol will conduct a data and safety monitoring process as described in the plan below.

14.1 Trained and Certified Personnel

All of the research protocol personnel who will work with study subjects, study subject data or subjects' research samples have completed training in the protection of human research participants per guidelines issued by the U. S. Department of Health and Human Services, Office of Human Research Protections. The documentation of completion of the certification is maintained in the UMCCC Clinical Trials Office or the Blood and Marrow Transplantation Office. The investigator and designated associates have attended an IRB sponsored HIPAA research presentation in accordance with the policy of the study site. Each participant in this research trial will be listed by study specific numbers, without initials or date of birth; however, the date of transplant may be included when corresponding with the IRB or outside agencies.

Designation of Responsibilities: The Principal investigator(s) are solely responsible for the implementation and conduct of this trial. The principal investigator has however, designated associates to assist with the protocol implementation which includes but is not limited to the following:

1. BMT Physicians – have been designated to assist with participant education, informed consent process, study implementation and compliance, recording of primary source documentation, AE assessment and reporting, adherence to all regulations.
2. BMT Research Nurse(s) - have been designated to assist with participant education, informed consent process, study implementation and compliance, recording of primary source documentation and adherence to all regulations.
3. BMT Data Manager(s) - have been designated to assist with patient enrollment/eligibility, verification of protocol compliance, all data collection and recording from primary source, AE reporting, DSM reports and adherence to all regulations.
4. BMT Clinical Team - The members of the BMT clinical team that have been designated to assist the investigator in any aspect of this protocol will be listed on the protocol specific designation log.
5. The BMT internal Data and Safety Monitoring Committee (DSMC) will meet monthly to review the data, safety and monitoring reports and all SAEs that have been filed. The report will be submitted to the UMCCC DSMB and to the UM IRB at least monthly. Whenever an unanticipated data, safety and monitoring board meeting takes place or when a new development occurs the IRB will be notified of the occurrence.

14.2 Storage and Dissemination of Reports

The Clinical Trials Office (CTO) is responsible for collating all the Data and safety Monitoring Reports and providing the information to Data Safety Monitoring Board. The CTO will coordinate the reporting process between the Investigator and the IRBMED and

UM DSMB as well as other applicable reporting agencies (FDA, and study sponsors). Copies of all related correspondence and reporting documents will be maintained in a locked file by the CTO regulatory team and the research data file will be maintained in a file by the BMT data management team.

14.3 Clinical Monitoring Procedures

Clinical studies at the University of Michigan must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices and in compliance with other applicable regulatory requirements.

This study will be monitored by a CTO representative of the University of Michigan Cancer Center. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a site initiation meeting will be conducted by the CTO. The PI and the study staff should make every effort to attend the site initiation meeting. Study-related questions or issues identified during the site initiation meeting will be followed by the appropriate University of Michigan Cancer Center personnel unit they have been answered and resolved.

The first annual monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The initial annual visit is not justified unless there is at least one participant enrolled on a study. At a minimum, a routine monitoring visit will be done at least once every 12 months, or once during the course of the study if the study duration is less than 12 months. The purpose is to verify:

a. Adherence to the protocol
b. Completeness and accuracy of study data and samples collected
c. Proper storage, dispensing, and inventory of study medication
d. Compliance with regulations

Monitoring may be in the form of a site visit or a review of the documents at the CTO. During a monitoring, access to relevant hospital and clinical records must be given by the PI to the CTO representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The CTO expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified

during these visits will be communicated and are expected to be resolved in a time manner.

At close-out upon completion, termination, or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study will occur, and the PI will be informed of his / her ongoing responsibilities. In general, close-out is conducted during a site visit. However, a site close-out can occur without a site visit if all of the following apply:

a. No patient has signed the Informed Consent Form
b. Investigational agent (peg-IFN-α) has not been dispensed
c. All investigational agent (agent) and materials have been returned as defined for the study or destroyed and accounted for properly.

14.4 Quality Assurance and Audits

The Quality Assurance Review Committee (QARC) of the UMCCC performs quality assurance audits of investigator-initiated clinical trials. Audits provide assurance that trials are conducted in compliance with the protocol. Further, they ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements. A QARC audit of each clinical trial is conducted annually. Audits occur within the month of the study's initial IRB approval. All audit findings are reported by QARC to the UMCCC DSMB. The DSMB can also request QARC for a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues. A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Clinical Trials Office that such a request has been made.

15.0 REMOVAL FROM STUDY

Patients have the right to withdraw from the study at any time for any reason. The PI also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, treatment failure, protocol violation, or other reasons. Should a patient (or a patient's legally authorized guardian or representative) decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation should be made at the time of the patient's withdrawal with an explanation of why the patient is withdrawing and every effort should be made to perform follow-up evaluations. Patients may be removed from the study treatment if one or more of the following events occur:

- Significant protocol violation or noncompliance, either on the part of the patient or the PI.
- Refusal of the patient to continue treatment and / or observations.
- Unacceptable or dose-limiting toxicity
- Decision by the PI that removal from the study is in the patient's medical interest.
- Unrelated medical illness or complication.

- Lost to follow-up.
- Disease relapse or progression.

Patients removed from the study who have received one or no doses of peg-IFN-α will be evaluable for toxicity only and will therefore be replaced. The number of patients removed from the study prior to being fully evaluable will be monitored regularly by the study's DSMB in order to identify and address problems that may develop with respect to patient accrual. We estimate that <10% of subjects may need to be replaced because they encounter toxicity from relapsed AML prior to HCT or during conditioning therapy. Patients who die before day +14 will be counted toward the endpoints but additional patients will be enrolled to make up for the very short follow up on such patients.

16.0 STATISTICAL CONSIDERATIONS

16.1 Study Endpoints

16.1.1 Phase I: The primary endpoint of phase I is to determine a tolerable dose of peg-IFN-α for phase II study in patients with AML not in remission at HCT.

16.1.2 Phase II: The primary endpoint is to determine the cumulative incidence of relapse at six months post-HCT in patients receiving the phase II dose of peg-IFN-α. All patients who have persistent AML or relapse between day 0 and day 180 will be counted towards this endpoint.

16.2 Study Design and Sample Size Justification

16.2.1 Phase I: The aim of the dose finding portion of this study is to determine the maximum tolerated dose (MTD) of peg-IFN-α among two dose levels: 90 mcg and 180 mcg. Our targeted DLT rate is 0.30. The first patient will be assigned to 90 mcg and the dose assignment of each future patient will be determined using the modified toxicity probability interval (mTPI) design (68). If 90 mcg proves to be overly toxic, a lower dose of 45 mcg will be included as a "fall-back" dose and be assigned to the next patient to avoid closure of the study. If 45 mcg also proves to be overly toxic, the study will be halted and subject to either closure or amendment following DSMB review. At the end of the study, the dose assigned to the most patients will be selected as the MTD.

16.2.2 Definitions of dose limiting toxicity (DLT). The following events with be considered DLTs:

- Unexpected Grade 4 CTCAE v. 4, non-hematologic toxicity, considered probably or definitely related to the study drug.
- Any death not related to relapse of AML or GVHD.
- Graft failure defined as ANC < 500 by day + 28, not related to persistent AML.

Only DLTs encountered during the treatment period, prior to day 56 post HCT (or 14 days after final treatment, whichever comes later), will be counted. DLTs after the treatment period will be counted only if they reflect an ongoing toxicity that initiated in the treatment period.

16.2.3 Dose Assignment: Dose assignments for the mTPI are outlined in **FIGURE 6**. For example, if the first patient (who is assigned to 90 mcg) does not experience DLT, the next patient will be assigned to 180 mcg. However, if the first patient experiences DLT, the next patient will be assigned to 45 mcg.

Based upon simulations, with a sample size of 35 patients, if the true DLT rates of the three doses are 0.10, 0.30, and 0.50 (90 mcg is the MTD), we have a probability of 50% of selecting 90 mcg as the MTD and 50% of patients will be assigned to 90 mcg on average. If the true DLT rates of the three doses are 0.10, 0.20, and 0.30 (180 mcg is the MTD), we have a probability of 80% of selecting 180 mcg as the MTD and over 90% of patients will be assigned to 180 mcg on average.

16.2.4 Phase II

We will enroll a maximum of 35 patients to this study. This sample size was selected to have high probability that at least 30 patients would be assigned to 180 mcg during the study if 180 mcg is the MTD. We want to have 30 patients receiving the same dose because we will have 80% power, assuming a Type I error rate of 0.05, to show a difference between a promising six month relapse rate of 40% versus a relapse rate of 60%, which is the primary aim of the phase II portion of this study.

		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
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er assignment

Figure 6: Dose assignment grid for mTPI design

16.3 Stopping Rules

16.3.1 Stopping Rules for acute GVHD and Non-Relapse mortality:

Once 12 patients have been enrolled and have started treatment we will evaluate for stopping criteria once this 12th patient has completed 100 days of follow-up. At this point, the study will stop if 6 or more patients have developed Grades III-IV aGVHD that is refractory to corticosteroid therapy (methylprednisone 2mg/kg or equivalent) before day 100 post HCT (GVHD100). Independent from the occurrence of GVHD100, the study will also stop if 6 or more patients have died from causes other than relapse before day 100 post HCT (NRM100).

Although our stopping rules require 100 days of follow-up for each subject, we will not halt accrual to wait for full follow-up of previously-enrolled subjects unless there is potential for an ‘early stopping’ condition. For example, we will enroll the 13th subject as

soon as they are eligible, regardless of whether the first 12 subjects have all been followed for 100 days, unless 5 or more patients have already experienced GVHD100 or NRM100 event. As a result, there is a possibility of enrolling additional subjects on this study before we discover the 'stopping rule'/halt on accrual condition has been met.
If less than 6 patients have either these events, 12 more patients will be enrolled, for a total of 24 patients. At this point, the study will stop if 12 or more patients (out of 24) have developed GVHD100 or NRM100. If less than 12 out of 24 patients have either of these events, the study will continue to enroll the remaining subjects. (See **TABLE 5**) If the true rate of grades III-IV acute GVHD or non-relapse mortality within 100 days of transplant is 40%, 50%, 60%, or 70%, the study will stop with probability of 0.13, 0.44, 0.80, or 0.97, respectively.

16.3.2 Stopping Rule for Graft Failure

At the same time evaluation for possible stopping for excessive severe acute GVHD and non-relapse mortality, we will also assess for possible stopping due to excessive graft failure. The study will stop if 2 of the first 12 or 3 of the first 24 patients experience graft failure within 28 days of transplant (EF28). If the true rate of graft failure within 28 days of transplant is 5%, 10%, or 15%, the study will stop with probability of 0.17, 0.50, or 0.75, respectively.

TABLE 5 Stopping Rules for GVHD, Non-relapse mortality, and engraftment failure.

Number of Evaluable Patients	Number of Patients with GVHD100	Number of Patients with NRM100	Number of Patients with EF28
12	6	6	2
24	12	12	3

16.4 Data Analysis Methods

16.4.1 Primary Outcome:

The cumulative incidence for relapse will be estimated using proportional hazard model for the competing risk of non-relapse mortality (NRM)(69).

16.4.2 Secondary Outcomes:

The cumulative incidence of NRM and GVHD will be calculated using proportional hazard models methods. Overall survival (OS) will be calculated from the day of transplantation (day 0) until death. Event free survival (DFS) will calculated from the day of transplantation until death or post HCT detection of relapsed or persistent AML. OS and DFS will be estimated using Kaplan-Meier methods.

16.4.3 Biologic Outcomes:

The frequency and absolute numbers of tumor antigen specific T cells (peptide responsive or Tetramer + cells), BDC3A+ DCs, *ex vivo* PBMC responses and other cellular immune subsets measured serially will be used to generate kinetic curves that will be correlated with pharmacodynamic markers, serum inflammatory markers, and the primary clinical endpoint of relapse. **These secondary endpoint analyses are descriptive (e.g. means and standard errors) and preclude formal hypothesis testing**. Due to the small sample size, they will be used primarily for hypothesis generation and design of future studies.

The optional MRI component of the proposal is a pilot study to measure changes in bone marrow composition and environment during HCT for AML. As this is a pilot study, no formal sample size or power calculations will be performed, and there will be no formal testing of statistical hypotheses. We will compute summary statistics of data for groups of patients including PRM scatterplots, image overlays of PRM data, mean values, medians, range, quartiles and standard deviations. These data will be used primarily to generate hypotheses and design future studies.

16.5 Projected Accrual:

Based upon review of our programs clinical database over the past 10 years, we estimate approximately 10-12 patients will be enrolled per year and completion of the trial in 3 years. Previous protocols for AML not in remission at University of Michigan have accrued similar numbers in this timeframe (UMCC 2007.055). We will monitor enrollment rates closely in the first six months and make adjustments to eligibility as necessary including expansion to other centers. There are currently no competing protocols.

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APPENDIX A: KARNOFSKY PERFORMANCE SCALE

%	Description

100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, minor symptoms of disease
80	Normal activity with effort, some signs of symptoms of disease
70	Cares for self (consistent with age), unable to carry on normal activity or do active work/school/play
60	Requires occasional assistance (beyond age-appropriate care), but is able to care for most of their needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization is indicated although death is not imminent
20	Hospitalization is necessary, very sick, active support treatment is necessary
10	Moribund, fatal processes progressing rapidly

APPENDIX B: ACUTE GVHD ASSESSMENT

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dl	Adult: < 500 ml/day Child: < 10 ml/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dl	Adult: 500–999 ml/day Child: 10 -19.9 ml/kg/day Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.
2	Maculopapular rash 25 – 50% BSA	3.1-6 mg/dl	Adult: 1000-1500 ml/day Child: 20 – 30 ml/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dl	Adult: >1500 ml/day Child: > 30 ml/kg/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation > 5% BSA	>15 mg/dl	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

- For GI staging: The "adult" stool output values should be used for patients ≥ 50 kg in weight.
- Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is estimated to be 50% of total stool/urine mix.
- For stage 4 GI: the term "severe abdominal pain" will be defined as:
  (a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
  (b) Pain that significantly impacts performance status, as determined by the treating MD.
- If colon or rectal biopsy is +, but stool output is <500 ml/day (<10 ml/kg/day), then consider as GI stage 0.
- There is no modification of liver staging for other causes of hyperbilirubinemia (see appendix A).

Overall Clinical Grade:

Grade 0	No stage 1-4 of any organ
Grade I	Stage 1-2 rash and no liver or gut involvement
Grade II	Stage 3 rash, or Stage 1 liver involvement, or Stage 1 GI
Grade III	Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV	Stage 4 skin, liver or GI involvement

APPENDIX C: INSTRUCTIONS FOR COLLECTING A URINE SAMPLE

1. Read the instructions carefully, and follow each of the steps to ensure you collect the correct specimen for the study.

2. Use the urine hat or urinal provided to you for collection.

3. Lift toilet seat and place hat on top of the toilet rim, then lower toilet seat. Make sure the hat is in the correct orientation to collect urine, not stool. Urinate into the hat. If you need to have a bowel movement, collect the urine separately.

Important:

Do not allow bowel movement to fall into the hat.

Do not place toilet paper into the hat.

A study team member will receive the urine sample from you and provide additional instructions if needed.