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Type-1 Interferon to Prevent Leukemia Relapse after Allogeneic Transplantation

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Abstract:
A potent graft-versus-leukemia (GVL) response is crucial in preventing relapse, the major impediment to successful allogeneic hematopoietic cell transplantation (HCT). In preclinical studies, Type-1 interferon (IFNα) enhanced cross-presentation of leukemia specific antigens by CD8α dendritic cells (DCs) and amplified GVL. This observation was translated into a proof-of-concept phase I/II clinical trial with long-acting IFNα (pegIFNα) in patients undergoing HCT for high-risk acute myeloid leukemia (AML). Patients with treatment resistant AML not in remission or poor risk leukemia were administered four dosages of pegIFNα every 14 days beginning at day -1 before HCT. Dose selection was established by adaptive design that continuously assessed the probability of dose limiting toxicities throughout the trial. Efficacy was evaluated by determining the six-month incidence of relapse at the maximum tolerated dose (MTD). Thirty-six patients (median age of 60 years) received pegIFNα treatment. Grade 3 or greater SAEs occurred in 25% of patients establishing 180mcg as the MTD. In phase II, the incidence of relapse was 39% at six-months, which was sustained through one-year post HCT. The incidence of transplant-related mortality was 13% and severe grade III-IV acute GVHD occurred in 11%. Paired blood samples from donors and recipients after HCT indicated elevated levels of Type-1 IFN with cellular response, persistence of cross-presenting DCs and circulating leukemia antigen specific T cells. These data suggest that prophylactic administration of pegIFNα is feasible in the peri-HCT period. In high-risk AML, increased toxicity was not observed with preliminary evidence for reduction in leukemia relapse after HCT.

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ABSTRACT

A potent graft-versus-leukemia (GVL) response is crucial in preventing relapse, the major impediment to successful allogeneic hematopoietic cell transplantation (HCT). In preclinical studies, Type-1 interferon (IFNα) enhanced cross-presentation of leukemia specific antigens by CD8α dendritic cells (DCs) and amplified GVL. This observation was translated into a proof-of-concept phase I/II clinical trial with long-acting IFNα (pegIFNα) in patients undergoing HCT for high-risk acute myeloid leukemia (AML). Patients with treatment resistant AML not in remission or poor risk leukemia were administered four dosages of pegIFNα every 14 days beginning at day -1 before HCT. Dose selection was established by adaptive design that continuously assessed the probability of dose limiting toxicities throughout the trial. Efficacy was evaluated by determining the six-month incidence of relapse at the maximum tolerated dose (MTD). Thirty-six patients (median age of 60 years) received pegIFNα treatment. Grade 3 or greater SAEs occurred in 25% of patients establishing 180mcg as the MTD. In phase II, the incidence of relapse was 39% at six-months, which was sustained through one-year post HCT. The incidence of transplant-related mortality was 13% and severe grade III-IV acute GVHD occurred in 11%. Paired blood samples from donors and recipients after HCT indicated elevated levels of Type-1 IFN with cellular response, persistence of cross-presenting DCs and circulating leukemia antigen specific T cells. These data suggest that prophylactic administration of pegIFNα is feasible in the peri-HCT period. In high-risk AML, increased toxicity was not observed with preliminary evidence for reduction in leukemia relapse after HCT. This trial was registered at www.clinicaltrials.gov as NCT02328755.

KEY POINTS:

- Augmenting early GVL response by prophylactic Type 1 IFN may potentially reduce rates of leukemic relapse after HCT in very high-risk AML.
- Reciprocal toxicities including acute GVHD and non-relapse mortality were not increased after Type 1 IFN treatment.

INTRODUCTION

Graft-versus-leukemia (GVL) responses underlie the curative potential of allogeneic hematopoietic stem cell transplantation (HCT). Despite this effect relapse is the most frequent cause of mortality after HCT in Acute Myeloid Leukemia (AML)[1]. Increasing HCT conditioning intensity, administering donor lymphocyte infusions (DLI) or maintenance chemotherapies (5-azacitidine) have not improved leukemia-free survival (LFS) due to limited impacts on relapse or additional toxicity[2-5]. Given shared immunobiology, enhancing GVL is often associated with GVHD-related mortality.

GVL requires T cells to respond to both host allo-antigens and leukemia-specific antigens[6,7]. Dendritic cells (DCs) are necessary for priming T cells against viruses or tumors[8]. One mechanism, termed cross-presentation, involves presenting exogenous tumor antigens on MHC class I. In humans, BDCA-3$^+$ (CD141$^+$) DCs specialize in cross-presentation that promotes development of antigen specific CD8$^+$ T cells[9,10]. Pre-clinical studies demonstrate murine CD8α DCs (BDCA-3$^+$ homologs) generate leukemia specific T cells and GVL *in vivo* without aggravating GVHD[11]. Cross-presentation on CD8α DCs can be enhanced by Toll Like Receptor 3 agonists (poly:IC) or by Type-1 IFNs[12,13]. Type-1 IFNs also support CD8+ T cell function by expansion of effector and memory populations[13-15]. Pegylated IFNα (pegIFNα), a long-acting formulation with greater bioavailability relative to first generation preparations, is commercially available

for treatment of hepatitis B/C and myeloproliferative neoplasms[16-18], however, its role in preventing relapse is not known.

Patients entering HCT with refractory AML often attain remission, but approximately 60% will relapse within six months[19-23]. This suggests leukemia relapse may be reduced by augmenting early GVL. Herein, we hypothesize that enhancing GVL with pegIFNα will reduce relapse without increasing the severity of GVHD. We conducted a phase I/II clinical trial to evaluate the safety and efficacy of pegIFNα in high-risk AML, i.e. principally patients not in remission at HCT. We found that early administration of Type-1 IFN may limit relapse after HCT without increasing toxicity or rates of severe acute GVHD.

METHODS

Patients and Donors

Patients: A phase I/II, single-center, open-label, prospective clinical trial of Type-1 IFN was performed in patients with high-risk AML (clinicaltrials.gov NCT02328755). Study procedures involved screening all high risk AML patients proceeding to HCT at our center for eligibility from December 2014 to January 2019. Reasons for not enrolling include declining participation (n=8) or not open to accrual (n=5). Patients who did not participate (n=13) comprise a comparison cohort described in discussion. Eligibility consisted of one of the following criteria at time of HCT: a) persistent leukemia defined as ≥5% myeloblasts on aspirate, or if <5%, presence of leukemia by flow cytometry or cytogenetics (97% of enrollments) or b) leukemia in morphologic remission with preceding evidence of very poor risk cytogenetic / molecular features defined as

complex karyotype (≥4 clonal abnormalities), monosomal karyotype, inv(3), t(3;3), t(6;9) or FLT3-ITD mutation[24-26]. AML must have been resistant to two sequential rounds of induction chemotherapy, or if relapsed, one re-induction. The minimum age enrolled was eighteen years with no maximum age. An eligibility amendment was approved to allow one pediatric patient at 17-years to participate. Patients were required to have a minimum Karnofsky performance status of 70% and meet institutional requirements for organ function. This study (HUM0093471) is approved by Michigan IRB (IRBMED). All patients provided informed consent.

GVHD prophylaxis, conditioning and supportive care: Tacrolimus was initiated intravenously at day -3 combined with mini-dose methotrexate for matched unrelated donors. Patients were maintained on therapeutic levels of tacrolimus (5–15 ng/ml) for at least 90 days after HCT, unless evidence of toxicity or relapse. T-cell depleting sera (e.g. ATG) were prohibited for recipients of HLA matched HCT. Two recipients of HLA mismatched (haploidentical) donors received post-transplant cyclophosphamide on days 3 and day 4 as well as tacrolimus and mycophenolate mofetil beginning at day 5. All conditioning was required to be myeloablative per investigator which included: clofarabine (40 mg/m^2 days -5 to -2) with busulfan (3.2 mg/kg days -5 to -2), fludarabine (40 mg/m^2 days -5 to -2) with busulfan (3.2 mg/kg days -5 to -2) or fludarabine (30 mg/m^2 days -7 to -5) with TBI (200cGy twice daily days -4 to -2) for patients receiving haploidentical donors (n=2). No maintenance therapy was permitted including DLI or chemotherapy. After completing study treatment, two patients with FLT3-ITD mutations received Sorafinib according to institutional standards of care. Supportive care was according to clinical practice guidelines at the University of Michigan.

Donor selection and Post HCT Chimerism Analysis: Peripheral blood or bone marrow products were selected from the best available matched unrelated, matched related or first degree haplo-identical donors. High resolution matching was performed at HLA-A, B, C and DRB1. For chimerism studies, patients post HCT peripheral blood was analyzed for donor and recipient microsatellite markers by multiplex PCR and differential fluorescence. Full chimerism required establishment of ≥95% donor cells after HCT.

Study Design

Treatment: PegIFNα (PEGASYS, Genentech) was administered subcutaneously on day -1 before HCT and on days 14, 28, and 42 (± 7 days). Treatment was temporarily held for acute GVHD ≥ grade II and discontinued for grades III-IV.

Phase I: The primary endpoint was to determine a maximum tolerated dose (MTD) of pegIFNα for phase II. Three flat doses of pegIFNα were evaluated: 45mcg, 90mcg or 180mcg. The trial was initiated at 90mcg using the Bayesian modified toxicity probability interval (mTPI) design to allow continuous assessment and adjustment of dosage throughout the trial based on the occurrence of dose limiting toxicities (DLTs)[27].

Phase II: The primary efficacy endpoint was the cumulative incidence of relapse at six-months post HCT.

Assessment of AML Response: Remission was first assessed at day 30 (±7 days) after HCT. Complete remission (CR) status was established by achieving negativity of all of

the following six criteria: bone marrow myeloblasts < 5% without Auer rods, absence of circulating blasts, flow cytometry (sensitivity of approximately 0.01%), and cytogenetics. Where applicable, molecular analyses and assessment of extramedullary AML were incorporated into response assessments before and after HCT. As assessments were performed in the early post HCT period, full hematologic recovery was not a requirement for CR. Patients had to remain negative on all subsequent bone marrow assessments including day 180 to be considered in CR for the primary endpoint. Detection of AML by any method after HCT was treated as relapse (treatment failure).

Acute GVHD Assessments: GVHD was monitored at minimum weekly through day 100 after HCT and subsequently at regular clinical visits through day 180 using modified Glucksberg (Keystone) consensus criteria[28].

Statistics and Trial Endpoints

The estimated sample size was 35 subjects. This sample size was based on probability estimates that assumed at least 30 subjects would be treated at the MTD. For the primary relapse endpoint, at least 30 patients would provide 80% power, assuming a Type 1 error rate of 0.05, to show a difference between a promising six-month incidence of 40% versus a historical incidence of 60%[19-23]. The cumulative incidence for relapse was estimated using a proportional hazard model for the competing risk of non-relapse mortality (NRM)[29]. The cumulative incidence of NRM and GVHD was calculated using proportional hazard models. Overall survival (OS) and Leukemia-free survival (LFS) was estimated using Kaplan-Meier methods. OS was recorded from HCT (day 0) until death. LFS was from day 0 until death or relapse. Replacement enrollments were

allowed for patients experiencing DLTs that received ≤ one pegIFNα dose. All analyses were performed in R (Vienna, Austria).

Correlative Studies

Recipient plasma and peripheral blood mononuclear cells (PBMC) were collected and cryopreserved prior to conditioning and on days 28, 56, 100 and 180 post-HCT. After obtaining informed consent, a subset of donors had an aliquot of the stem cell product cryopreserved. Plasma cytokines and GVHD biomarkers were analyzed using Luminex (Invitrogen). pSTAT1 staining was assessed in $CD45^+$ PBMCs. We defined cross-presenting DC subsets as $CD45^+CD141^+CLEC9A^+$ populations[30]. Leukemia antigen specific T cells were analyzed in HLA-A*0201 positive patients using MHC I dextramers for Wilms' Tumor-1 (WT1) (Immunodex). Leukemia specific T cells were defined as $WT1^+CD8^+$ T cells[31]. Additional details are provided in supplementary methods.

RESULTS

Patient Characteristics and Dose Determination

A flow diagram for the phase I and phase II components of the study is shown in Figure 1. Thirty-six patients were treated with pegINFα. The median patient age was sixty years (17-72) with 64% having an HCT-CI of ≥ 3. Ninety-seven percent had detectible AML on pre-HCT disease assessments. Patients had a median risk score by Duval of 3 used for prognosis in AML not in remission[32]. The remaining patient characteristics are provided in Table 1.

No DLTs occurred in the first three patients treated at 90mcg, therefore the dosage was increased to 180mcg. An engraftment failure was observed at 180mcg in a recipient of a second allogeneic HCT meeting criteria for a DLT. However, because no additional DLTs were experienced, 180mcg was determined to be the MTD in phase I. Overall, 87% of peg-INFα dosages were administered. The most common cause for a dosage hold was acute GVHD (7%).

Engraftment and Severe Adverse Events

Neutrophil and platelet engraftment occurred at a median of 12 and 16 days, respectively. Post engraftment analysis for lineage specific chimerism was available in thirty patients by day 100. In the myeloid (CD33) compartment, twenty-nine patients (97%) established full donor chimerism. Grade 3 or greater non-hematologic severe adverse events (SAEs) during pegINFα treatment were graded according to common toxicity criteria (CTC) version 4.0 (National Cancer Institute, Bethesda, MD) are provided in Table 2. In total, 25% of patients experienced one or more SAEs. The most common toxicity was rash in 11% of patients which was classified as cutaneous GVHD. Two pulmonary complications were observed during treatment. One patient developed pulmonary edema related to fluid overload and another developed pneumonitis related to respiratory syncytial virus. Both pulmonary events resolved with standard supportive treatment.

Relapse and Leukemia Response

The primary endpoint of phase II was six-month leukemia relapse in recipients of HLA-matched HCT receiving pegINFα at the MTD (n=31). The cumulative incidence of

relapse was 39% (95% CI 24,58) which was sustained at one-year (Figure 2). For the entire study (n=36), inclusive of phase I and phase II cohorts, the six-month incidence of relapse was 42% (95% CI 27,60). At day 30 after HCT, 34 of 36 patients (94%) had confirmed morphologic remission on bone marrow examination including absence of any flow cytometric, cytogenetic, extra medullary or molecular aberrations that were present prior to HCT.

Non-Relapse Mortality and Graft-versus-Host Disease

Toxicity resulting in NRM was 13% (95% CI 5,31) and 25% (95% CI 12,46) at six-months and two-years, respectively (Figure 3). Early NRM (six-months) was due to GVHD (n=2), infection (n=2), and graft failure (n=1). The incidence of grade II-IV and grade III-IV acute GVHD was 39% (95% CI 24,58) and 13% (95% CI 5,31). For the entire study population, inclusive of phase I and phase II, grade II-IV acute GVHD was 36% (95% CI 23,54) and grade III-IV was 11% (95% CI 4,27) (Figure 4). GVHD characteristics for the study are given in Table 3.

Infection

Documented infections were recorded through day 180 by type and severity (Table 4). Overall, 72% of enrolled patients experienced one or more infections. Severe (grade 3) infections occurred in 9% of patients. Asymptomatic viral reactivation accounted for 53% of infections commonly involving CMV viremia. Among patients with CMV re-activation, 58% required anti-viral treatment and one developed viral enteritis. Bacterial infections occurred in 42% of patients, predominantly *staphylococcal* or *enterococcus*

bacteremia before engraftment. There were no grade 3 bacterial infections. Full infection data is listed in supplemental Table 1.

Survival

In phase II, OS was 55% (95% CI 40,75) and 33% (95% CI 19,58) at six-months and two-years, respectively (Figure 5A). LFS was 48% (95% CI 34,70) and 28% (95% CI 15,52) at six-months and two-years (Figure 5B). For the entire study, OS was 53% (95% CI 39, 72) and 32% (95% CI 19,54) at six-months and two-years, respectively. There were no differences in OS or LFS by age, conditioning therapy, donor type, cytogenetic risk, pre-HCT blast percentage or HCT-CI.

Correlative Studies

Plasma Type-1 Interferon, pharmacodynamics, and inflammatory Markers

Paired plasma and PBMCs samples were analyzed in a subset of patients before conditioning, at day 28 and at day 56 reflecting time points immediately prior, during and after completing pegINFα. Plasma levels of IFNα significantly increased at day 28 and day 56 compared to measurements before treatment ($p<0.01$) (Figure 6A). Levels of other Type 1 IFNs (IFN-β) and Type 2 IFNs (IFN-γ) did not change (supplemental Figure 1). We then evaluated PBMCs for IFNα signaling by STAT-1 phosphorylation (pSTAT1). The median frequency of CD45+ cells expressing pSTAT1 rose at day 28 compared to baseline levels before infusion ($p<0.05$) (Figure 6B). Cytokines, cytolytic granules and GVHD biomarkers were assessed as corresponding markers of inflammation. Plasma TNF-α, granzyme A and ST-2 increased compared to baseline, however, other measured cytokines (IL-1β, IL-6, 1L-15) did not change (supplemental Figure 1).

DC subsets and leukemia-specific T cells

We first confirmed whether $CD141^+$DCs, a rare population involved in antigen cross-presentation, were present in paired donor and recipient samples[9]. $CD141^+CLEC9A^+$ DCs detected in the donor inoculum fell in number but persisted on day 28 and 56 after HCT (Figure 6C). Having confirmed their presence, we then explored the function of cross-presentation by measuring leukemia specific antigen CD8 T cells. We analyzed MHC I dextramers loaded with WT1, an antigen commonly overexpressed in AML, in patients with HLA-A*0201 alleles. $WT1^+$ $CD8^+$ T cells were detected at low frequencies in donors and at day 28 in recipients, but showed a trend towards sustained persistence at day 180 (Figure 6D).

DISCUSSION

In this phase I/II clinical trial we evaluated the safety and efficacy of augmenting GVL responses after HCT. The data suggest that administration of exogenous IFNα does not alter toxicity, as measured by rates of AEs, acute GVHD or NRM. Importantly, the incidence of relapse was lower than hypothesized meeting the study's primary endpoint. This suggests Type-1 IFNs are potentially effective in reducing relapse in high-risk AML, and may increase OS, findings that will require confirmation in larger controlled trials.

Increasing GVL can be accomplished experimentally by enhancing cross-presentation, which enables expression of exogenous antigens within MHC class I molecules presented on DCs[11,13,14]. This results in priming of $CD8^+$ T cells towards tumors or leukemia antigens. In pre-clinical models, targeting cross-presentation with PolyI:C or Type-1 IFN improved LFS without aggravating GVHD[11,13]. Our phase I data suggests

pegIFNα is safe when administered every two weeks beginning on day -1 before stem cell infusion. DLTs were infrequent, occurring in one patient (engraftment failure). The remaining patients displayed normal hematologic engraftment, suggesting this was an isolated event in an at-risk subject (prior allogeneic HCT). The frequency of other SAEs were generally acceptable with rash (11%) and pulmonary events (6%) being most common. Consequently, 180mcg was selected as the phase II dose. Nonetheless, considering the limited sample size, HCT complications will require further monitoring in larger studies.

Previous case series using short acting Type-1 IFNs for treating post-HCT relapse indicate half of patients experience SAEs or acute GVHD[33,34]. Several potential reasons may account for the low rate of SAEs in our study. First, in contrast to previous studies that were administered for treatment after relapse, our study was performed in the setting of prevention. Second, the treatment schedule was brief, and the dosages were moderate when compared to previous studies. Third, pegIFNα in our study was administered in conjunction with calcineurin inhibitors for GVHD prophylaxis. This contrasts with previous studies where IFNα was given with reduced immune suppression or additional immunotherapies (IL-2, DLI)[35]. Another key safety finding of our study was the 11% incidence of severe acute GVHD and 25% NRM, suggesting pegIFNα does not significantly amplify toxicity or alloreactivity towards host tissues, similar to experimental studies. Prospective studies have reported GVHD rates of 38-50% and NRM of 25-36% in patients entering HCT with persistent AML[20], while retrospective analyses suggest NRM greater than 40%[36,37].

To determine if Type-1 IFN is effective in reducing relapse, we selected an exceptionally high-risk population, namely patients entering HCT with detectable AML. Although other factors such as mutational status of AML influence prognosis, entering HCT not in remission is the best known predictor for relapse and mortality[38,39]. At day 30 after HCT, 34 of 36 patients had confirmed CR, however, this may reflect transient leukemia clearance similar to other studies using myeloablative regimens[20,36]. Of greater importance, relapse incidence was 39% at six-months and at one-year suggesting, consistent with our hypothesis, early administration of pegIFNα results in sustained remissions. It is therefore plausible, as has been shown in preclinical studies, that Type-1 IFN promotes licensing of CD141+ DCs specialized in cross-presentation, thereby enhancing early GVL[6,10,11,13,40]. While our study did not distinguish effects on host versus donor DCs, it does show CD141+ DCs present in the donor inoculum persist early after HCT. Furthermore, new epigenetic and mutational modifiers such as FLT3 and IDH1 inhibitors reflect promising strategies for reduction of relapse post-HCT, however, their direct effects on immune cells and immune mediated GVL responses are understudied and will require ongoing assessments in experimental and clinical studies.

The net effect of elevated NRM and relapse results in poor survival. The two-year OS and LFS from the study was 33% and 28%, although not optimal, suggest a tangible improvement in survival compared to other HCT studies. Previous prospective and retrospective studies of refractory AML with morphologic or MRD positive disease at time of HCT have revealed a relapse incidence of 55-65% after HCT with OS ranging from 14-26%[20,21,23,32]. Similarly, although an imperfect comparison, contemporaneous AML patients with similar eligibility who did not participate in our study had a two-year

OS of 15% (unpublished, see supplemental Figure 2). Thus, Type-1 IFN may control relapse without reciprocal increases in toxicity and improved OS. Given our trial involved heterogeneous donor types, variance in leukemia subtypes (≥5% vs. <5% blasts, various cytogenetic risk) and a limited sample size, improvements in relapse and survival outcomes will require confirmation in a larger randomized trial.

To describe levels of Type-1 IFN during the study, paired blood samples were examined in a subset of donors and recipients. Significant elevations in plasma IFNα were detected after HCT along with increased pSTAT1 in PBMCs. However, without untreated controls these elevations may reflect endogenous IFNα release due to the inflammatory conditions of HCT. Nonetheless, recent experimental data suggest lack of cross-presentation and T cell exhaustion diminish GVL[40]. Our trial shows that $CD141^{+}CLEC9A^{+}$ DCs contained in the donor inoculum persist after conditioning, and that leukemia specific T cells were identified six-months after HCT. These findings, together with lower-than-expected rates of relapse, indicate the possibility of a sustained anti-leukemic T cell response. The low numbers of circulating cells, lack of controls and small trial size make these data descriptive in nature, thus precluding any direct correlation with pegIFNα dosing or clinical response. Furthermore, because most immune cells express Type-I IFN receptors, our study did not distinguish cross-presentation versus direct effects on other cell types (NK cells). Well controlled clinical studies will facilitate further assessment of the key immunologic targets of Type-I IFN in humans.

In summary, we provide evidence that exogenous Type-1 IFN is a potentially safe and feasible strategy to limit post HCT relapse in high-risk AML. The use of long acting IFNα

for prevention may augment the anti-leukemia response and potentially improve OS. These data require validation in a prospective randomized trial.

DATA SHARING STATEMENT

Data released to clinicaltrials.gov. Readers may contact the corresponding author for additional data inquiries: johnmage@med.umich.edu.

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AUTHOR CONTRIBUTIONS

JMM, AP, MR, SA, MG, MT, JM, BP, GY, SC, and PR participated in the recruitment of patients, collection, assembly, analysis and interpretation of clinical data. DP, JMM, BP and PR designed, performed and/or analyzed correlative data for immune subsets. TB performed statistical analysis and contributed to the statistical design of the trial. All authors participated in manuscript writing, review and provided final approval of the manuscript. JMM and PR designed the study.

CONFLICT OF INTEREST

All other authors have no conflict of interest to disclose. The authors are solely responsible for the design, data collection, analysis, and decision to publish this trial.

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Table 1: Patient Characteristics for Phase I/II Cohorts

Variable	**No.**	**%**
Total Treated	36	100
Age		
Median years (range)	60 (17-72)	
Time to HCT from AML Diagnosis		
Median days (range)	142 (55-2051)	
Gender		
Female	14	39
Male	22	61

HCT-CI, median (range)	3 (0-7)	
HCT-CI ≥3	23	64
HCT-CI <3	13	36
Disease Status at HCT		
Not in remission	35	97
Remission	1	3
% Blasts at HCT		
≥5%	20	56
<5%*	16	34
Cytogenetic risk		
Poor	18	50
Intermediate	17	47
Unknown	1	3
FLT3-ITD / NPM1 Mutation		
+/+	1	3
+/-	2	6
-/+	2	6
-/-	17	47
unknown	14	38
Disease Risk Score†		
≥ 3	21	58
2	12	33
1	3	9
Conditioning Regimen		
CloBu	17	47
FluBu	17	47
FluTBI	2	6
HLA Match / Donor Type		
Matched / Unrelated	20	56
Matched / Related	14	38
Haploidentical / Related	2	6
Donor Source		
PBSC	31	86

* Persistence of AML by cytogenetics (N=10), flow cytometry (N=4), myeloid sarcoma (N=1) or not detected (N=1).

† Duval et al. J Clin Oncol. 2010;28(23):3730-8

Abbreviations: HCT-CT = Hematopoietic Cell Transplantation-Comorbidity Index

HLA = Human Leukocyte Antigen

PBSC = Peripheral Blood Stem Cells

Table 2: Listing of ≥ grade 3 SAE*

Adverse Event	N	%
Rash[1]	4	11
Pulmonary[2]	2	6
Arthritis[3]	1	3
Graft Failure[4]	1	3
LFT elevation	1	3
Hypertension	1	3
Hypotension	1	3
Acute Kidney Injury	1	3

* any CTCAE 4.0 while on treatment with pegIFNα irrespective of attribution. Nine patients (25%) experienced a total of 12 SAE listed above.

(1) clinical features consistent with acute GVHD
(2) Pulmonary SAE include: pneumonitis (ARDS) related to infection (n=1) and acute hypoxemia (n=1)

(3) clinical features consistent with gouty arthritis

(4) met criteria for dose limiting toxicity (DLT)

Abbreviations: ARDS = acute respiratory distress syndrome SAE = severe adverse avent; LFT = liver function tests (AST/ALT)

ARDS = acute respiratory distress syndrome

Table 3: GVHD Characteristics

Acute GVHD Severity	N	%
Grade 1	4	11
Grade 2	9	25
Grade 3	2	6
Grade 4	2	6

Acute GVHD Organ Involvement		
Skin	9	25
GI tract	4	11
GI tract + Skin	2	6
GI tract + Liver ± Skin	2	6
Chronic GVHD Severity*		
Mild	2	6
Moderate	9	25
Severe	1	3

*minimum follow-up of 100 days post HCT

Table 4: Infections by Day 180 post HCT (all grades)

Class of Infection	Number	% Total
Viral Infection	25	53%
Bacterial Infection	20	43%
Fungal Infection	2	4%
Total Infections	47*	100%

*A total of 47 recorded infections occurred in 26 (72%) patients

FIGURE LEGENDS

Figure 1. Study flow diagram for phase I and phase II.

Figure 2. Relapse. The cumulative incidence of relapse for patients (n=31).

Figure 3. Non-Relapse Mortality (NRM). The cumulative incidence of NRM for patients receiving the phase II dosage of pegIFNα (n=31).

Figure 4. Acute Graft-versus-Host Disease (GVHD). The cumulative incidence of acute GVHD by day 180 after HCT. Grade II-IV (dotted line) and grade III-IV (solid line). Data inclusive of phase I and phase II cohorts (n=36).

Figure 5. Survival. (A) OS and (B) LFS for patients receiving the phase II dosage of pegIFNα (n=31).

Figure 6. Type-1 IFN levels, pharmacodynamic response and cellular immune subsets. (A) Paired plasma levels of IFN-α (n=12) at baseline (pre-conditioning), day 28 and day 56 in recipients were analyzed by Luminex array (B) frequency of phosphorylated STAT-1 protein (n=8) within CD45+ cells measured by FACS in paired samples from donors and then recipients at day 28 and at day 56 (C) numbers of $CD141^{+}CLEC9A^{+}$ DCs (n=3) was measured by FACS from donors and recipients at day 28 and at day 56 (D) numbers of $WT1^{+}CD8^{+}$ T cells by WT1 specific dextramers (n=3) in patients with HLA-A*0201. Analysis performed by FACS in paired donors and recipients at day 28, 56, 100 and 180 after HCT. Samples obtained after relapse or high dose corticosteroids for GVHD were excluded. Data reflect mean values with error bars represent the standard error of the mean (SEM). Paired t-tests were used for comparisons. * denotes $p<0.05$, ** denotes $p<0.01$

FIGURE 1

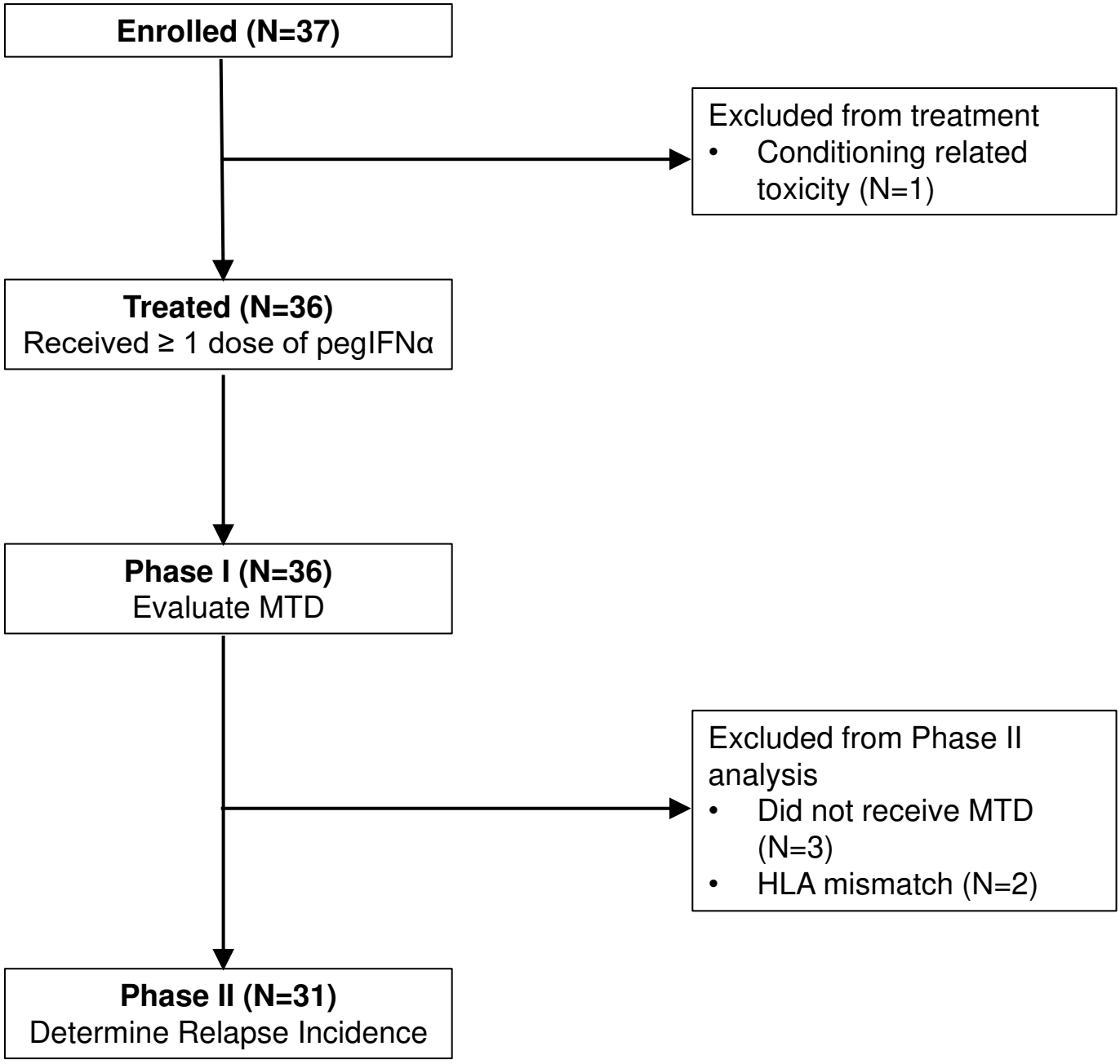


FIGURE 2

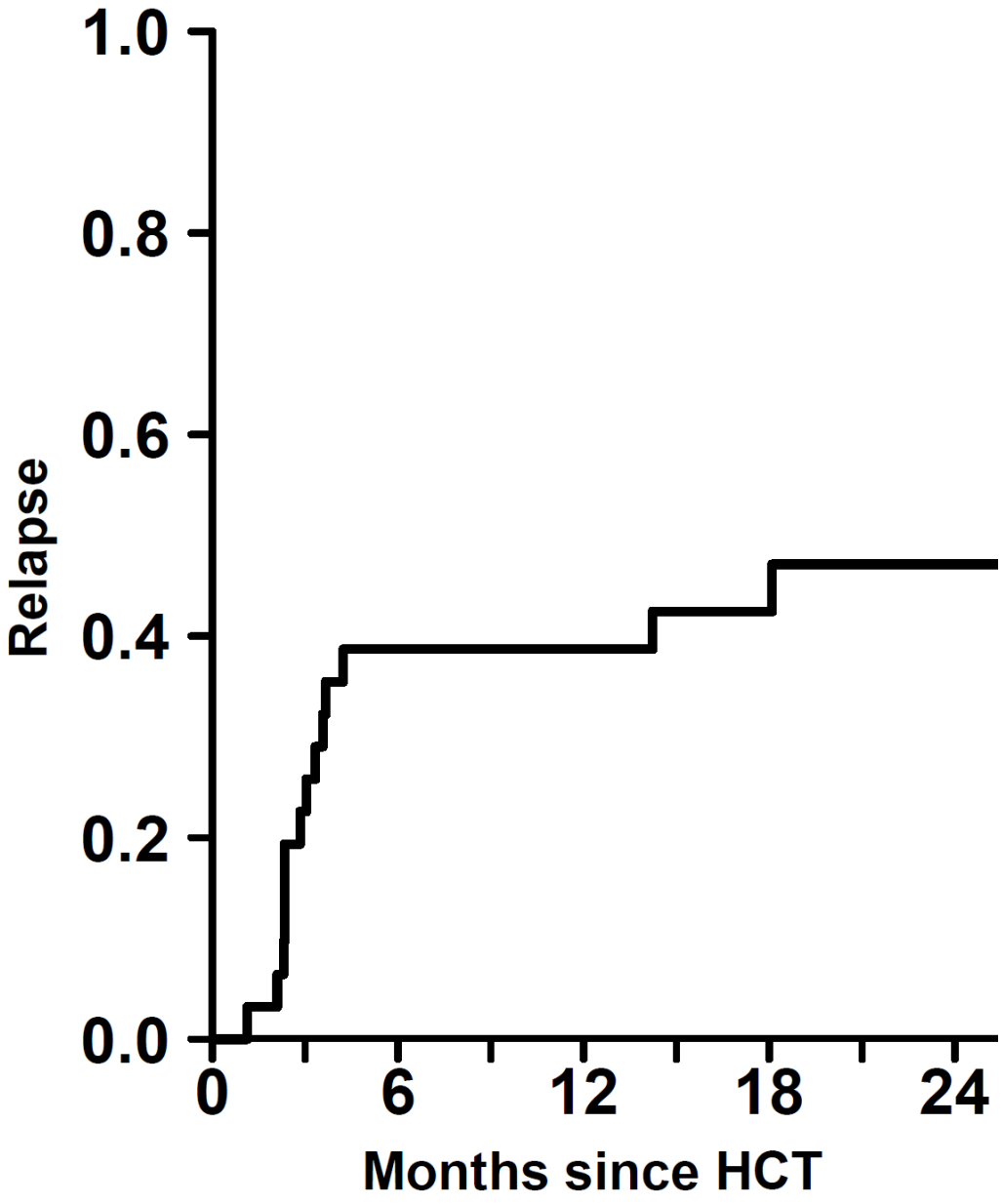


FIGURE 3

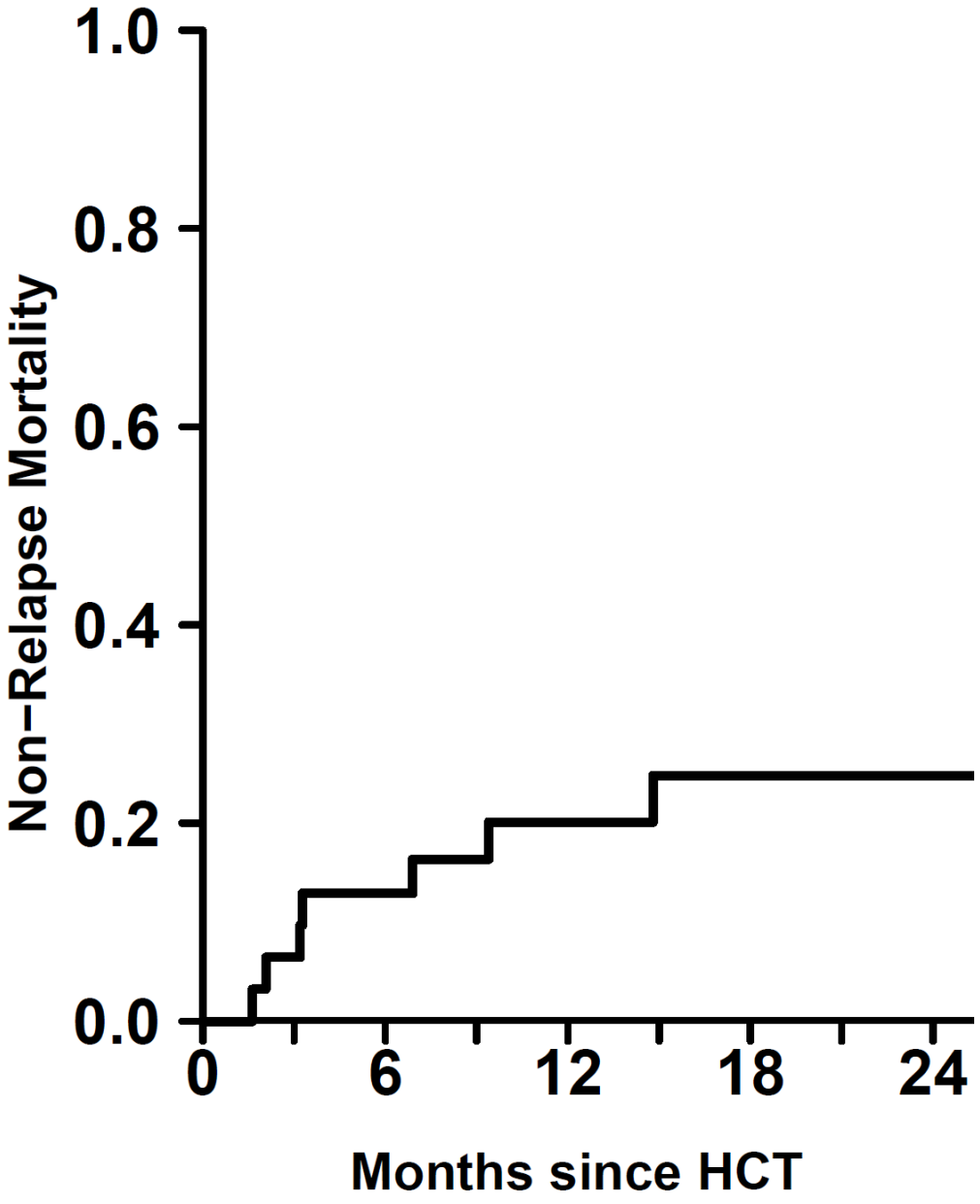


FIGURE 4

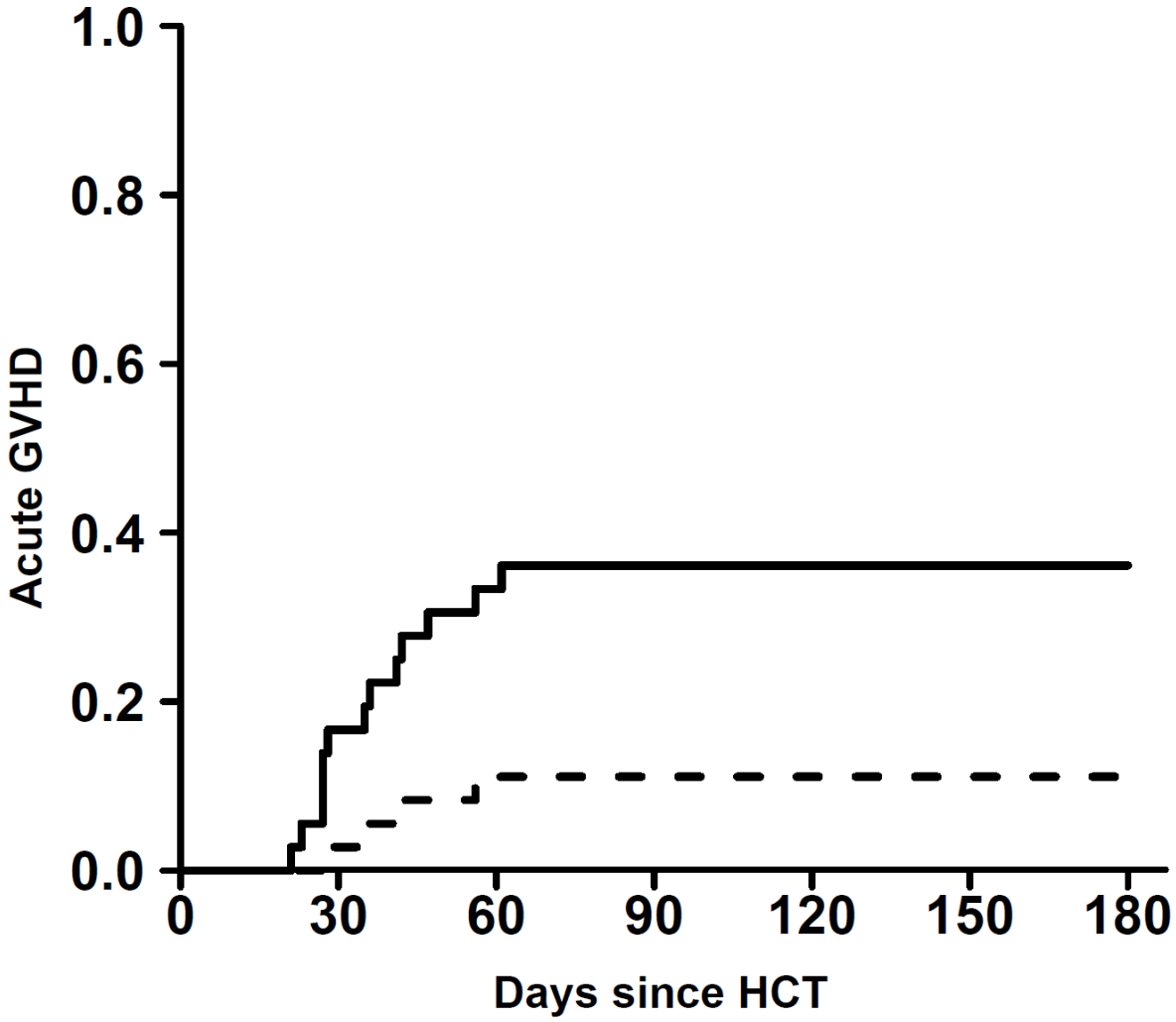
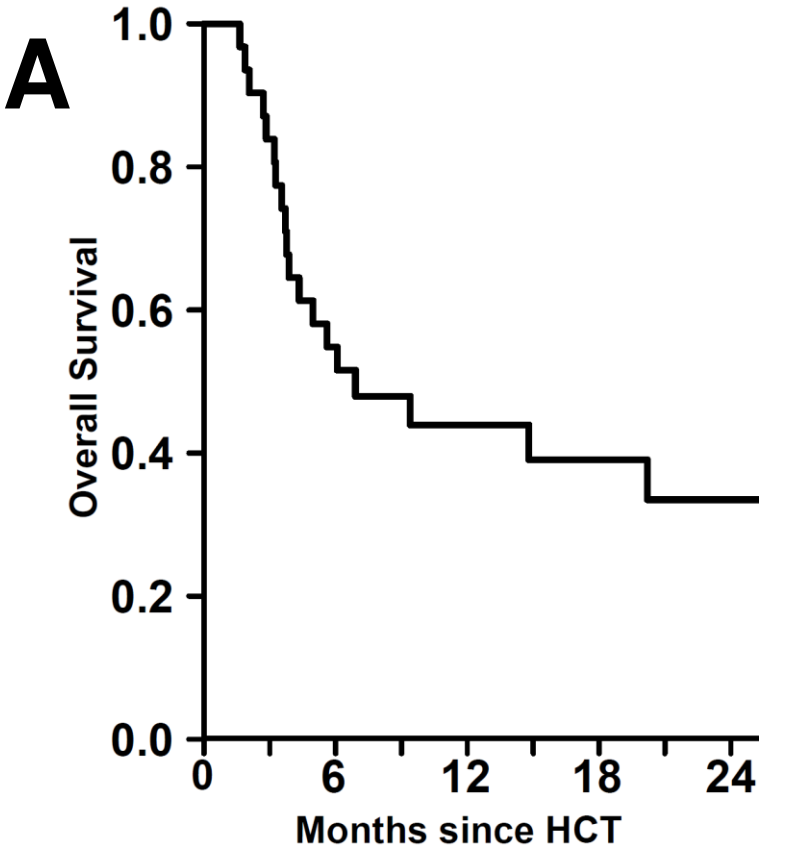


FIGURE 5



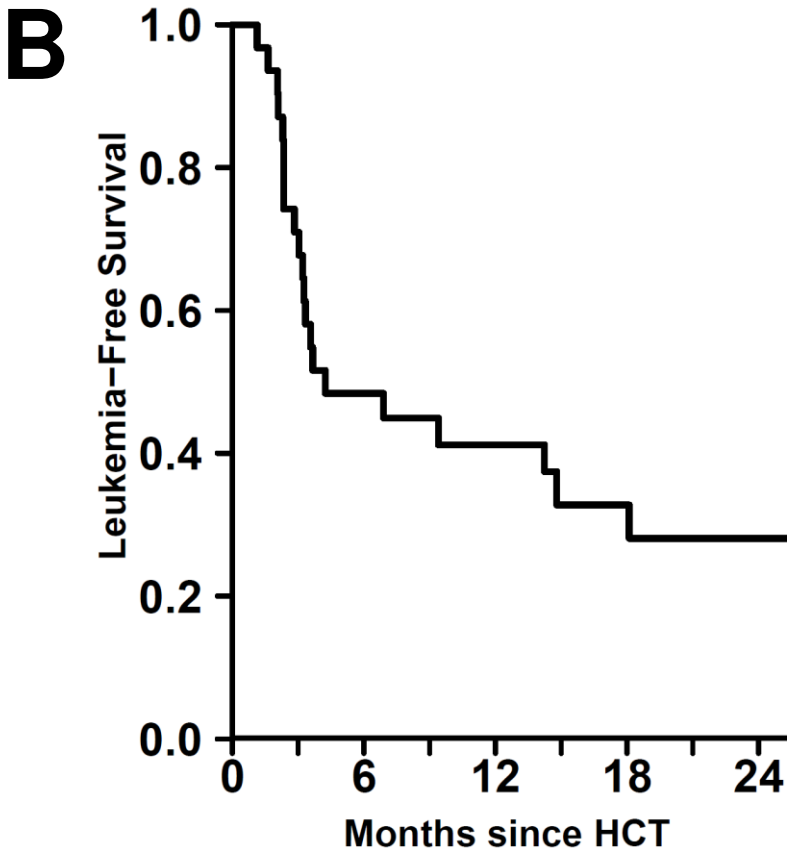


FIGURE 6