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Maintenance Therapy with Decitabine after Allogeneic Stem Cell Transplantation for Acute Myelogenous Leukemia and Myelodysplastic Syndrome

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Abstract

Decitabine is a hypomethylating agent that irreversibly inhibits DNA methyltransferase I inducing leukemic differentiation and re-expression of epigenetically silenced putative tumor antigens. We assessed safety and efficacy of decitabine maintenance after allogeneic transplantation for acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Decitabine maintenance may help eradicate minimal residual disease, decrease the incidence of graft-versus-host disease (GVHD) and facilitate a graft-versus-leukemia effect by enhancing the effect of T-regulatory lymphocytes. Patients with AML/MDS in complete remission (CR) after allotransplant started decitabine between day +50 and +100. We investigated 4 decitabine doses in cohorts of 4 patients: 5, 7.5, 10 and 15 mg/m²/day x 5 days every 6 weeks, for maximum 8 cycles. The Maximum Tolerated Dose (MTD) was defined as the maximum dose at which 25% of people experience dose limiting toxicities (DLT) during the 1st cycle of treatment. Twenty-four patients were enrolled and 22 were evaluable. All 4 dose-levels were completed and no MTD was reached. Overall, decitabine maintenance was well tolerated. Grade 3/4 hematological toxicities were experienced by 75% of patients, including all patients treated at the highest dose-level. Nine patients completed all 8 cycles and 8 of them remain in CR. Nine patients have died due to: relapse (n=4), infectious complications (n=3) and GVHD (n=2). Most occurrences of acute GVHD were mild and resolved without interruption of treatment; one patient died of acute gut GVHD. Decitabine maintenance did not clearly impact the rate of chronic GVHD. Although there was a trend of increased FOXP3 expression, results were not statistically significant. In conclusion, decitabine maintenance is associated with acceptable toxicities when given in post-allotransplant

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setting. Although MTD was not reached, the dose of 10 mg/m² for 5 days every 6 weeks appeared to be the optimal dose rather than 15 mg/m², where most hematological toxicities occurred.

Keywords

decitabine; stem cell transplantation; maintenance; myelodysplastic syndrome; acute leukemia

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a potentially curative therapy for patients with high-risk acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Over the past decade we have witnessed significant advances in therapeutic approaches for alloHSCT including the use of alternative stem cell sources, less toxic conditioning regimens and better supportive care, resulting in improved overall survival.(1–5) However, disease relapse remains the principal cause of treatment failure for these patients.(6–9) The risk of relapse varies from 20–60% depending on the diagnosis and stage of the disease at the time of transplantation, and outcomes of salvage treatments are poor.(10–12) Median time to relapse after nonmyeloablative alloHSCT is 3–6 months and somewhat longer after myeloablative alloHSCT. Therefore, early maintenance therapy, directed at eliminating minimal residual disease and promoting a graft-versus-leukemia (GVL) effect, could be an effective method to improve outcomes after alloHSCT.

The concept of post-transplant maintenance therapy with hypomethylating agents in AML and MDS has been initially studied by investigators at MD Anderson Cancer Center.(13–15) They showed that azacitidine (AZA) can be safely administered to heavily pretreated post-transplant patients and may prolong event-free and overall survival. In addition, recent studies have shown that AZA, followed by DLI, is well tolerated when administered for early AML/MDS relapse after alloHSCT.(16–18) Decitabine (5-aza-2'-deoxycytidine) is a hypomethylating agent that irreversibly inhibits DNA methyltransferase I (DNMT-1) leading to genome-wide global DNA hypomethylation. While AZA incorporates primarily into RNA and to the lesser extent DNA, decitabine is more selective, reducing only DNA methylation. In addition, decitabine is approximately 5-fold more potent inhibitor of DNA methyltransferase I than AZA. Decitabine induces leukemic differentiation and re-expression of tumor-associated genes that had been epigenetically silenced.(19) At high doses, cells die from apoptosis triggered by DNA synthesis arrest, and at low doses, cells survive but change their gene expression profile to favor differentiation, reduced proliferation and increased apoptosis. In addition, maximum effects of DNA hypomethylation have been observed at low doses, and with less side-effects.(20, 21) Decitabine has demonstrated activity in a variety of hematological malignancies including AML, MDS and blast phase CML.(22–30) It is generally well tolerated with the primary toxicity being prolonged myelosuppression. Our group has demonstrated that decitabine enhances FOXP3 expression and can convert CD4⁺CD25⁻FOXP3⁻ T cells into CD4⁺CD25⁺FOXP3⁺ T regulatory cells (Tregs).(31, 32) Through their immunoregulatory properties Tregs are thought to play an important role in modulating graft-versus-host disease (GVHD) without sacrificing the beneficial GVL effect.(33, 34) In addition, several

other groups have demonstrated effects of DNA hypomethylating agents on T cell-mediated anti-tumor activity.(35–39) These include increasing tumor-specific CD8 T cell responses by up-regulating tumor antigen expression on malignant cells and inducing expression of killer-cell immunoglobulin-like receptor in T cells, thereby enhancing cytotoxic effector function of T cells against tumors. In human studies, patients noted to have a higher relative frequency of Tregs after HSCT had lower rate and severity of GVHD, lower rate of non-relapse mortality, and equivalent relapse mortality.(34)

Taken together, these studies provide a rationale for the administration of decitabine after alloHSCT for AML and MDS. We hypothesize that decitabine maintenance may provide direct antileukemic effect both to eradicate minimal residual disease and provide disease control by facilitating a GVL effect. In addition, decitabine may decrease the incidence of GVHD by enhancing the effect of Treg lymphocytes.

Patients and Methods

This was a single-institution, open-label, prospective, dose finding study of low-dose decitabine as a maintenance therapy after alloHSCT. The trial was approved by the Institutional Review Board at the Washington University School of Medicine and informed consent was obtained in accordance with the Declaration of Helsinki. The trial was registered at www.clinicaltrials.gov (NCT00986804). Eisai Inc. provided decitabine for all enrolled patients.

Eligibility Criteria and Enrollment

Adults 18 years of age or greater, with AML or MDS, who achieved a complete remission (CR) after alloHSCT were enrolled in the study between day +50 and +100 after alloHSCT. Exceptions were made for 3 patients who were enrolled shortly after day +100: for 2 patients day +100 fell during the weekend/holiday and one patient, who was already consented, lived far away and had transportation problems. CR was defined as: <5% blasts in the bone marrow with a count of at least 200 nucleated cells, no blasts with Auer rods, no extramedullary disease, absolute neutrophil count (ANC) $\geq 1,500/\mu\text{L}$, platelet count $\geq 50,000/\mu\text{L}$ and no blasts in the peripheral blood. The higher threshold for ANC, than that recommended by IWG, was chosen in anticipation of neutropenia secondary to decitabine and lower platelet threshold was allowed to facilitate enrollment. No platelet transfusion within 7 days of enrollment or growth factor support was allowed to meet those criteria. Other major eligibility criteria included Eastern Cooperative Oncology Group performance status ≤ 2 , no grade 3–4 acute GVHD, no uncontrolled infection, creatinine $<1.5 \times$ upper limit of normal (ULN), bilirubin $\leq 1.5 \times$ ULN, and hepatic enzymes $\leq 2.5 \times$ ULN. Both myeloablative and non-myeloablative conditioning regimens were allowed, and both related and unrelated donors were permitted using either peripheral blood or bone marrow as a source of graft. Donors could be mismatched at a single antigen at HLA-A, -B or -DR locus, plus a single antigen mismatch at HLA-C; two-antigen mismatch at a single locus was not allowed. Acute GVHD prophylaxis was according to the treating physician.

Treatment Plan

Decitabine was administered as an intravenous infusion for 5 consecutive days every 6 weeks, for up to 8 cycles. The study consisted of 5 escalating dose-levels only one of which was open for accrual at a given time. The first cohort of 4 patients started decitabine at 5 mg/m²/day. In subsequent cohorts the dose was escalated to 7.5, 10 and 15 mg/m²/day according to the dose limiting toxicity (DLT) experienced at the previous dose level. Decitabine dose could be deescalated to 2.5 mg/m²/day if DLT was observed in the first cohort. There was an observation period of 42 days between enrolling each subsequent cohort.

The primary goal was to determine the maximum tolerated dose (MTD) of decitabine, defined as the maximum dose at which 25% of patients experience DLT during the first cycle of treatment. DLT was defined as: (1) ANC <500/ μ L and/or platelet count <30,000/ μ L sustained for two consecutive weeks without platelet transfusions, (2) inability to achieve ANC 1000/ μ L and platelet count 50,000/ μ L after a delay of the second cycle by a maximum 2 weeks, and (3) grade 3–4 non-hematological toxicities related to decitabine. Patients who met criteria for DLT started second cycle at one level dose reduction. Patients with inadequate counts 6 weeks after a previous cycle had their next cycle delayed by maximum of 2 weeks. Each cohort could contain 4 or 8 evaluable patients until MTD or a dose of 15 mg/m²/day were reached. Patients with documented progressive disease were removed from the study.

Dose adjustments:

- 0/4 DLT observed: current dose is acceptable, proceed with dose escalation.
- 1/4 DLT observed: enroll additional 4 patients at the same dose-level. If there is 1/8 DLT observed then the current dose is acceptable and escalated; if there are 2/8 DLTs then the current dose is deemed over-toxicity and the previous dose is considered MTD.
- 2/4 DLT observed: current dose is deemed over-toxicity and the previous dose level is considered MTD.

Evaluation of Response

All patients completing the first cycle of decitabine were included in the safety and efficacy assessment. History, physical exam, complete blood count and complete metabolic panel were performed at baseline and every 2 weeks thereafter. Bone marrow biopsy was performed at baseline, at cycle 3 day 1 and cycle 8 day 42 (or at end of study for any reason). Acute and chronic GVHD evaluation was performed every 2 cycles or sooner if clinically indicated. Chronic GVHD was diagnosed and graded according to the NIH Criteria.(40) Determination of relapse was based on the peripheral blood, bone marrow biopsy, or evidence of new extramedullary disease. Patients are being followed for survival and relapse for 5 years.

Toxicity Assessment

All patients receiving at least one dose of decitabine were included in the toxicity assessments. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), Version 3.0. Serious Adverse Event (SAE) was defined as any toxicity that met any of the following conditions: resulted in death, was life-threatening, required hospitalization, resulted in significant disability or incapacity. Toxicity assessment was performed at the beginning of each cycle.

Correlative Studies

Peripheral blood samples to examine levels of lymphocyte populations were obtained at baseline, immediately prior to administration of decitabine on Cycle 1 Day 1 (C1D1) and Cycle 3 Day 1 (C3D1), immediately following administration of decitabine on Cycle 1 Day 5 (C1D5) and Cycle 3 Day 5 (C3D5), and at the end of study (EOS). Flow cytometry of peripheral blood was performed to examine lymphocyte populations including CD3+CD4+ T cells, CD3+CD8+ T cells, CD4+FOXP3+ Tregs, CD3-CD19+ B cells, and CD3-CD56+ NK cells. The following antibodies were used: CD3 PerCP-Cy5.5 (OKT3, eBioscience, San Diego, CA), CD4 PE (RPA-T4, BD Biosciences, Franklin Lakes, NJ), CD4 APC-eFluor780 (RPA-T4, eBiosciences), CD8 APC (RPA-T8, BD Biosciences), CD19 APC (HIB19, BD Biosciences), CD56 PE (B159, BD Biosciences), CD45 FITC (HI30, BD Biosciences), and FOXP3 PE (236A/E7, eBiosciences). To determine absolute numbers of these cells, Sphero Accucount Fluorescent Particles (Spherotech, Inc., Lake Forest, IL) were used. To measure DNA methylation in the FOXP3 locus, CD4+ T cells were isolated from the cohort treated with the highest dose of decitabine (15mg/m²/day) using a Sony SY3200 cell sorter (Sony Biotechnology, San Jose, CA) operated by the Siteman Cancer Center Flow Cytometry shared resource. The purities of the cells were 90% or higher. Direct bisulfite modification from cells and pyrosequencing analysis were performed by EpigenDx, Inc (Hopkinton, MA). A total of 16 CpGs were analyzed: 5 CpGs in 5' UTR region (-6278 to -6216 from ATG, Ensembl Transcript ID ENST00000376207), 2 CpGs in the human Treg specific demethylated region (TSDR) (-2376 to -2371 from ATG), and 9 CpGs in the TSDR (-2330 to -2263 from ATG).(41-43)

Statistical Analysis

Demographic and clinical characteristics of patients, as well as outcomes and length of follow-up, were listed for each patient. Preliminary efficacy was also assessed from pooled data of all cohorts. The endpoints for efficacy included relapse of the hematological malignancy, incidence and severity of GVHD, as well as overall survival (OS) and disease-free survival (DFS). OS was defined as the time from transplantation to death due to any cause, and survivors were censored at the date of last contact. DFS was defined as time from transplantation to relapse or death, whichever occurred first. Those patients alive and relapse-free were censored at date of last contact. Probabilities of OS and DFS were estimated using the Kaplan-Meier product-limit method. The cumulative incidence of relapse was estimated using Gray's sub-distribution method to account for the presence of competing risk due to non-relapse mortality (NRM).(44) Statistical analyses were performed

using statistical packages cmprsk (<http://biowww.dfci.harvard.edu/~gray>) for competing risk analysis and SAS 9.2 (SAS Institutes, Cary, NC) for all other analyses.

Results

Patient Characteristics

Between May 2010 and April 2014, 24 patients were enrolled and treated on this study. Two patients failed to complete the first cycle of treatment due to early relapse; they were replaced and excluded from all analyses other than toxicity. The characteristics of 22 evaluable patients are shown in Table 1. There were 15 males. Median age was 59 years (range, 21–68). Seventeen patients had AML and 5 MDS. Twenty patients received myeloablative conditioning (2 TBI-based) and 2 patients non-myeloablative conditioning. Six patients received alloHSCT from 10/10 HLA-matched siblings, 1 from 8/10 mismatched sibling, 11 from 10/10 matched unrelated donors and 4 from mismatched unrelated donors. All patients received mobilized peripheral blood stem cells as the graft source, and methotrexate and tacrolimus as GVHD prophylaxis. All patients were in CR at the time of enrollment. The median time from alloHSCT to start of decitabine was 95 days (range 62–115).

Dosing of Decitabine

Four patients were initially enrolled in cohort 1 (5 mg/m²/day), Figure 1. One patient experienced a hematological DLT and therefore 4 additional patients were added to expand that cohort. None of the additional patients experienced a DLT. One patient was replaced as he failed to receive all cycle 1 doses due to non-compliance, however, this patient received subsequent cycles and remains evaluable for all assessments besides MTD. Four patients were enrolled in cohort 2 (7.5 mg/m²/day). One patient relapsed before completing cycle 1 and was replaced. No DLTs were observed in that cohort, therefore 4 patients were enrolled in cohort 3 (10 mg/m²/day). None of those patients experienced a DLT and 4 patients were enrolled in cohort 4 (15 mg/m²/day). There were no DLTs at this dose-level, however 2 patients were replaced. One patient was replaced do to the early relapse, before completing cycle 1. The other replaced patient was deemed non-evaluable for DLT because he missed several laboratory data points and received growth-factors for neutropenia during the first cycle; his decitabine dose was reduced to 10 mg/m²/day and he completed the remaining cycles at that dose. In summary, as only 1 patient met the criteria for DLT, MTD was not established.

Response and Mortality

The median number of cycles completed was 5 (range 1–8). The causes for early discontinuation were: toxicity (n=5), relapse (n=3), new-onset severe gut GVHD (n=1), physician discretion (n =1), non-compliance (n=2) and consent withdrawal (n=1), Table 2. Nine of 22 patients (41%) completed 8 cycles of decitabine and all of them are alive: 8 patients are in CR and 1 patient developed central nervous system (CNS) relapse and leukemia cutis one year after completing decitabine maintenance and underwent 2nd alloHSCT. The remaining 13 patients discontinued decitabine before cycle 8 and only 4 are alive: 3 patients are in CR and 1 patient developed CNS relapse and underwent 2nd

alloHSCT. Overall, 11 of 22 evaluable patients are alive in CR with full donor chimerism (50%) and additional 2 patients are alive after 2nd alloHSCT for CNS/extramedullary relapse.

After a median follow up of 26.7 months (range 3.4–49.1) 6 patients have relapsed and 9 have died. All of the 6 patients that relapsed had high-risk disease (3 were transplanted with persistent disease, 1 had MLL rearrangement, 1 had FLT3 mutation, and one had ETV6 gene amplification with persistent dysplasia after induction chemotherapy). Only one of the relapsed patients had evidence of mild acute GVHD. The 2-year OS and DFS were 56% (95% CI 38–83%) and 48% (95% CI 30–75%), respectively, Figure 2. After adjusting for competing risk of non-relapse mortality, the 2-year cumulative incidence of relapse was 28% (95% CI 8–48%). Causes of death included: relapse (n=4), infectious complications (n=3) and GVHD (n=2; one from acute gut GVHD, one from chronic lung GVHD). Among 6 patients who relapsed: 2 patients are alive after second alloHSCT and 4 died from their disease. Only one of the relapsed patients completed all 8 cycles of decitabine, others received 1–4 cycles. Pre-transplant conditioning chemotherapy did not appear to impact the results.

Acute and Chronic GVHD

Five patients had resolving grade I-II classic acute GVHD at the time of starting on the study, and all of those completely resolved while on decitabine, with taper of steroids. One patient developed stage I gut GVHD during the second cycle of decitabine that completely resolved after a brief course of high-dose steroids with rapid taper, and another patient developed stage IV gut GVHD coinciding with the first cycle of decitabine, requiring high dose steroids, that also completely resolved while on decitabine. One patient developed stage IV gut GVHD immediately after the first cycle, was taken off the study and eventually died from progressive acute gut GVHD. One patient had late-onset acute GI GVHD that resolved.

Among 9 patients who completed all cycles of decitabine, 5 have developed chronic GVHD: 2 have severe and 3 patients have moderate chronic GVHD, Table 3. Among 13 patients that discontinued decitabine earlier, 10 are at risk for chronic GVHD (2 patients died of early relapse and 1 patient died from acute gut GVHD). Seven of those 10 patients have developed chronic GVHD: 5 have severe chronic GVHD (1 with features of overlap syndrome) and 2 have moderate.

Toxicity Assessment

All 24 patients enrolled on the study were assessed for toxicity. Generally, study treatments were well tolerated, Table 4. Grade 3/4 hematological toxicities were experienced by 18 of 24 patients (75%), including all 6 patients treated at the 15 mg/m²/day dose level. However, typically hematological toxicities resolved quickly between treatment cycles and accounted for very few treatment delays. The only frequently occurring grade 3/4 non-hematological toxicities were infections which occurred in 8 of 24 patients (33%). Five patients discontinued study due to the toxicities: two of them for infection, one for neutropenia, one for bowel obstruction and one for fatigue. Among 3 patients who discontinued decitabine because of infections, only one was neutropenic when infection developed.

Correlative Studies

We examined the effect of decitabine on lymphocyte subpopulations, such as T cells, B cells, NK cells, and Tregs, in all the patient samples, using flow cytometry (Figures 3 and 4). Although we observed a trend toward increase of Tregs after decitabine treatment, the difference was not statistically significant. In addition, a total of 16 CpG sites of the FOXP3 locus in CD4+ T cells isolated from the cohort treated with the highest dose of decitabine (15mg/m²/day) were analyzed, as described in Methods. The gene structure of the FOXP3 locus is presented in Supplemental Figure 1. As shown in Figure 5 we found that there is no statistically significant difference in the degree of DNA methylation in the FOXP3 locus before (C1D1 and C3D1) and after (C1D5 and C3D5) treatment and end-of-study (EOS) even though there was a trend toward decrease in DNA methylation in Cycle 1. C1D5 sample of patient S101 shows decrease in DNA methylation when compared with C1D1 sample of the same patient. Accordingly, its FOXP3 expression is higher than that of C1D1 sample (15.7% vs 11.8% FOXP3+ Tregs among CD4+ T cells). All of these data indicate that our DNA methylation data is consistent with the FOXP3 protein expression data. Interestingly, we found that NK cells and CD8 T cells are more sensitive to decitabine treatment in vivo with a consistent quantitative decrease in these subsets after treatment while CD4 T cells were relatively resistant to decitabine treatment (Figures 3 and 4). The CD4 T cell number at EOS in the 5 mg/m²/day cohort is higher than at C3D1. The explanation for this observation is unknown and may simply represent a more rapid recovery of CD4 T cells compared to other T and NK cells after treatment.

Discussion

Disease recurrence after alloHSCT is the major cause of treatment failure for patients with AML and MDS. Treatment options for those patients are limited, responses poor and prognosis dismal. Since most of relapses occur in the first year after alloHSCT, preventive maintenance therapy should be considered early in the post-transplant course. De Lima et al. first described that AZA maintenance after reduced-intensity alloHSCT for high-risk AML and MDS is well tolerated and may prolong event-free and OS.(13)

In this study we examined the toxicity and responses to low-dose decitabine as a maintenance therapy after alloHSCT for patients with AML and MDS. To our knowledge, this is the first report of decitabine use in this setting. We have demonstrated that decitabine can be given safely in the out-patient setting to this group of post-alloHSCT patients. Approximately 67% of our patients were able to receive at least 4 cycles of decitabine and 41% received all 8 cycles. Main dose-limiting toxicity associated with decitabine use is myelosuppression.(45) The interval between cycles of 6 weeks rather than 4 weeks, as commonly used for treatment of AML and MDS, was chosen to facilitate count recovery. Several patients experienced neutropenia and one of these qualified as DLT. Interestingly, that patient relapsed a month later and it is possible that the neutropenia in this patient was due to the early relapse rather than a direct effect of decitabine. All patients treated at the highest dose-level (15 mg/m²/day) experienced grade 3/4 hematological toxicities, none qualifying for DLT. However, one patient from the last cohort was discontinued after 4 cycles due to neutropenia and the other required growth factor support. Therefore, although

no formal MTD was reached, 10 mg/m²/day may be a more optimal and better tolerated dose for decitabine maintenance in post-alloHSCT setting.

Three patients were discontinued from the study due to the relapse. All of them had high-risk disease and 2, in retrospect, already had early signs of relapse at the time of enrollment (manifested as mixed chimerism and evidence of multilineage dysplasia). Three additional patients, also with high risk disease, relapsed later, after being off decitabine. Interestingly, only one patient who completed all 8 cycles of decitabine relapsed. Longer administration of decitabine was associated with prolonged OS and DFS.

Although decitabine could have been started between day +50 and +100 after transplantation, majority of patients started close to day +100. Study required bone marrow biopsy within 2 weeks of starting decitabine. In some instances treating physicians were waiting for the “standard-of-care” 3-month post-alloHSCT biopsy, rather than obtaining “study” biopsy earlier. Many of those patients had blood counts adequate to start decitabine closer to day +50 and, in retrospect, should have been biopsied and, if eligible, enrolled.

Since most of our patients started decitabine around day +100 after alloHSCT it is difficult to determine the effect of decitabine on incidence of acute GVHD. Several patients had grade I-II acute GVHD at the time of starting decitabine which completely resolved and one patient had biopsy proven grade IV gut GVHD that started during the first week of decitabine therapy and completely resolved. However, one additional patient died from grade IV gut GVHD developing after the first cycle of decitabine. Decitabine maintenance did not clearly impact the rate of chronic GVHD, however, lack of comparison in this early phase study precludes any further conclusion. Although the severity of chronic GVHD appears to be lower in the group that completed all 8 cycles of decitabine, this is merely the observation; there were too few patients to suggest correlation between decitabine and chronic GVHD severity. Earlier post-transplant initiation of decitabine might have greater impact on the incidence of GVHD. This is another reason to support, for future studies, the use of lower decitabine dose of 10 mg/m² that should be better tolerated and likely result in less hematological toxicities and infectious complications.

Goodyear et al. used AZA maintenance after alemtuzumab-containing reduced-intensity alloHSCT confirming the tolerability of AZA in post-transplant setting.⁽³⁵⁾ They observed increased number of Tregs within first 3 months after transplantation and relatively low incidence of acute and chronic GVHD. However, important differences between their study and ours are that they started AZA earlier in post-transplant course and their patients all received alemtuzumab-containing reduced-intensity conditioning. Although we observed a trend of increased FOXP3 expression, results were not statistically significant. There are several possible explanations why we have not observed an increase in FOXP3 expression. First, we only examined peripheral blood. Considering that most alloreactive T cells traffic to GVHD-target organs, it might be possible that those alloreactive T cells converted into FOXP3 expressing T cells might be differentially localized in the GVHD-target organs instead of the peripheral blood. Thus, it might have been more informative to examine Tregs and T cell subsets from GVHD target organs (skin, liver and GI tract) and not the peripheral blood. Second, decitabine needs to incorporate into replicating DNA to block DNMT-1.

Therefore, unless alloreactive T cells are actively proliferating, the degree of incorporation of decitabine into DNA would be limited, thereby minimizing conversion of alloreactive T cells into suppressive FOXP3 expressing Tregs. Since decitabine maintenance was not performed immediately after T cell infusion (stem cell transplantation) and was performed in the context of standard GVHD prophylaxis (calcineurin inhibitors) that also limit T cell proliferation and viability, it is possible that the optimal effect of decitabine on GVHD and FOXP3 expression was not realized in this study. Finally, it is also possible that the doses of decitabine tested in this study are not optimal to convert alloreactive T cells in vivo into Tregs although they might be optimal for reduction of GVHD and maintenance of GVL. Decitabine does appear to reduce the numbers of NK cells and CD8 T cells while maintaining a relatively low rate of leukemia relapse suggesting possible direct anti-leukemia effect of decitabine in vivo and increase of tumor-specific CD8 T cells responses.

In conclusion, low-dose decitabine maintenance is associated with acceptable toxicities when given in post-alloHSCT setting. Although MTD was not reached the dose of 10 mg/m² for 5 days every 6 weeks appears to be the optimal dose rather than 15 mg/m². Starting decitabine closer to the stem cell and T cell infusion, when T cells are rapidly expanding in allogeneic stem cell transplant recipients, could potentially be more efficacious in limiting GVHD. This study provides essential data for subsequent studies of decitabine maintenance. The availability of oral hypomethylating agents might make this approach of post-transplant maintenance even more attractive.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Decitabine maintenance after allogeneic transplantation is associated with acceptable toxicities.
- Although the Maximum Tolerated Dose was not reached, the dose of 10 mg/m² for 5 days every 6 weeks appears to be a more optimal and better tolerated dose than 15 mg/m².
- Decitabine maintenance didn't clearly impact the rate of chronic GVHD.

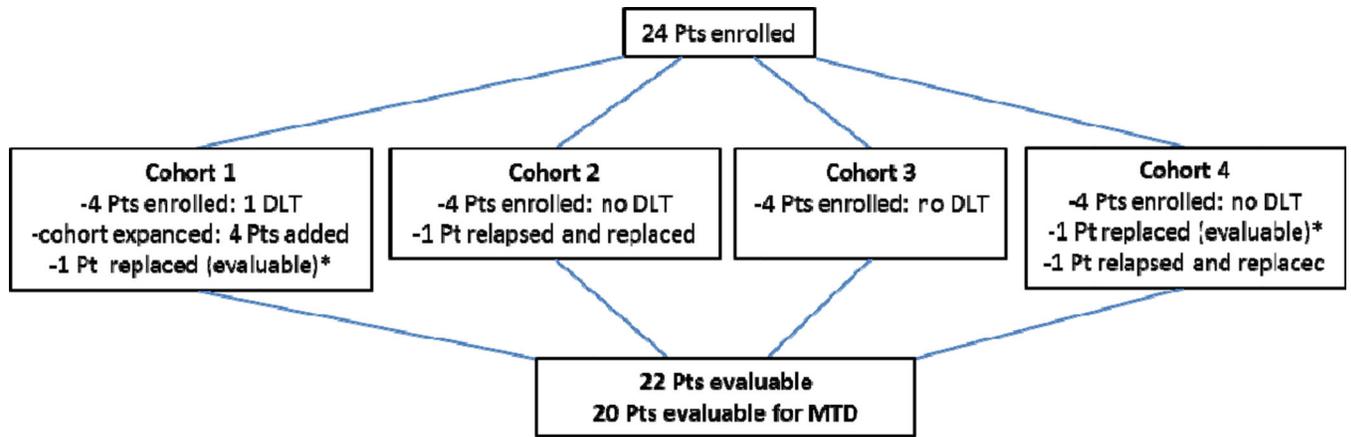


Figure 1.

Cohorts characteristics

*Evaluable for all the assessments except MTD

Abbreviations: DLT-dose limiting toxicity; MTD- maximal tolerated dose; Pts- patients

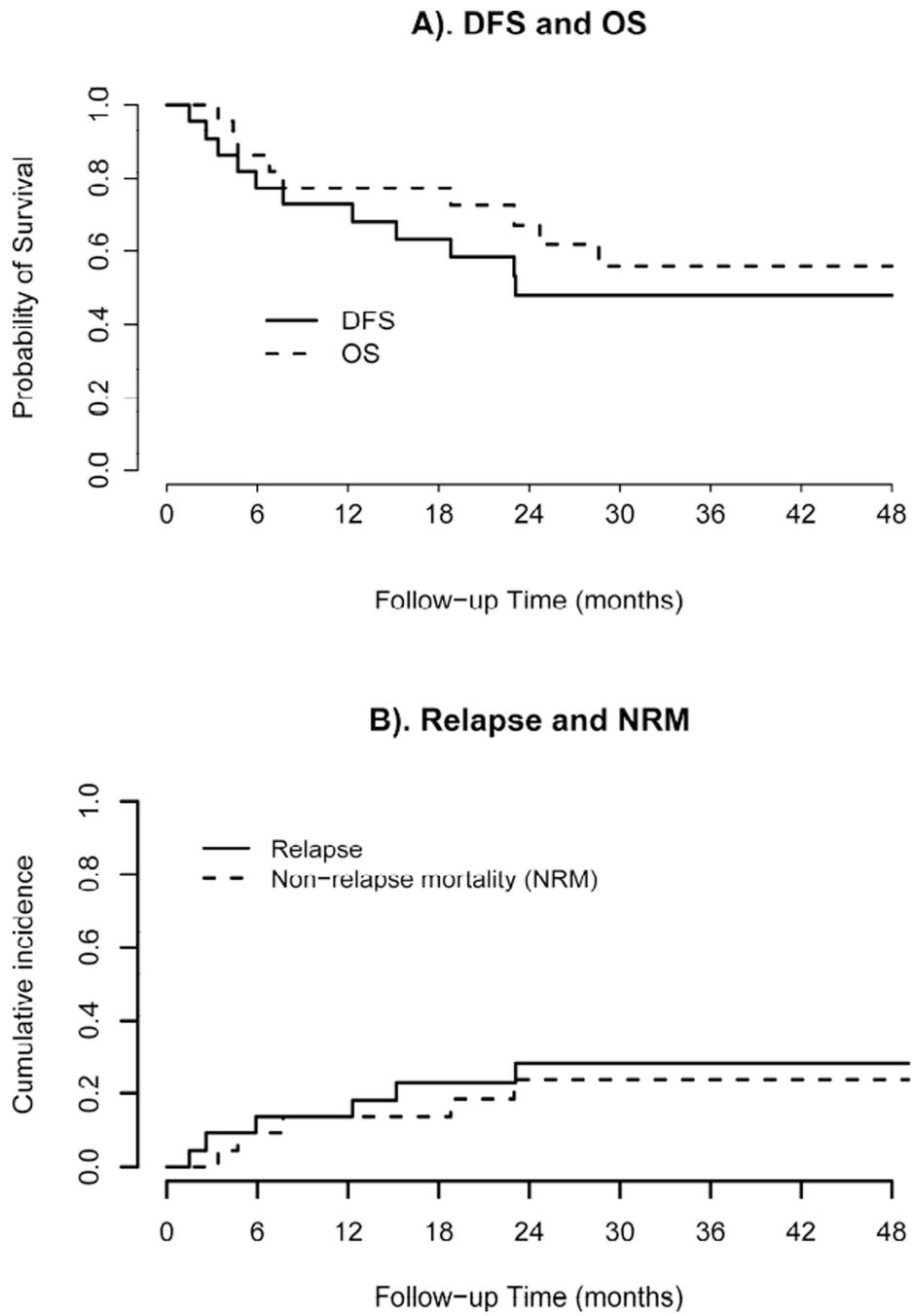


Figure 2.

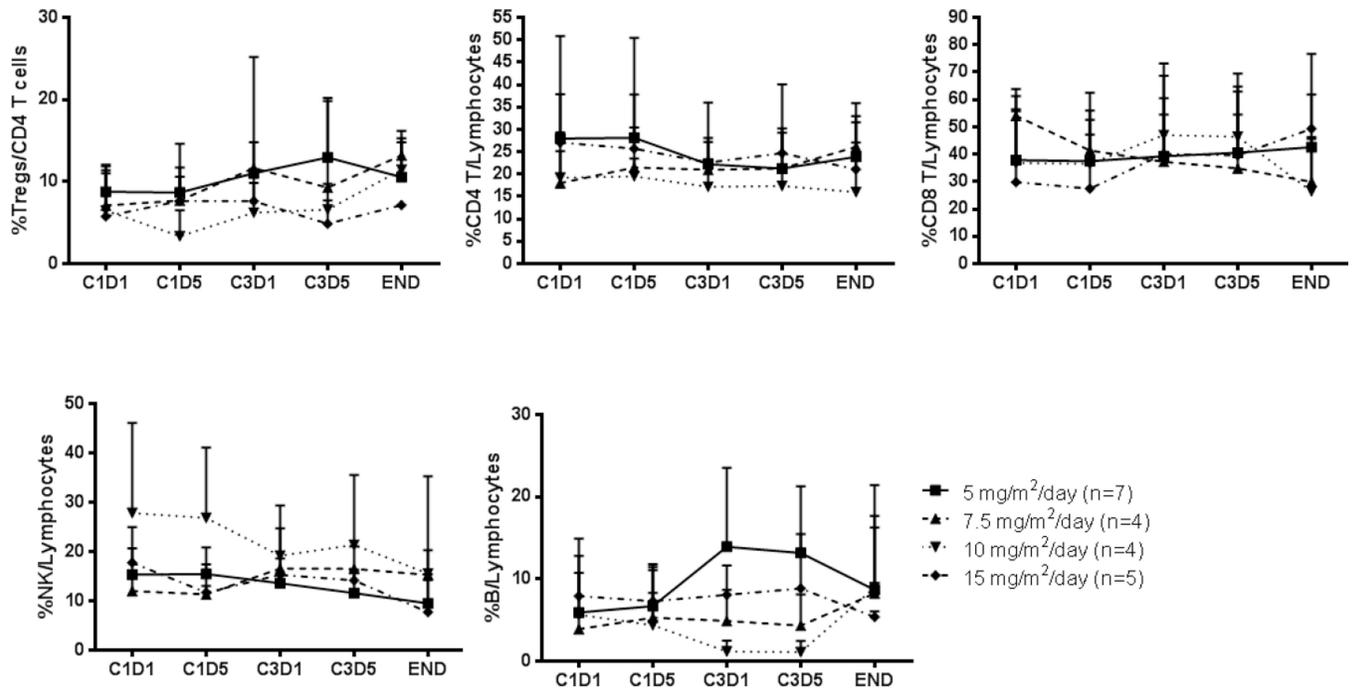


Figure 3.

Effect of decitabine on the frequency of Tregs, CD4 and CD8 T cells, B cells and NK cells in the peripheral blood at stated time points (C1D1: cycle 1 day 1; C1D5: cycle 1 day 5; C3D1: cycle 3 day 1; C3D5: cycle 3 day 5; END: end of study)

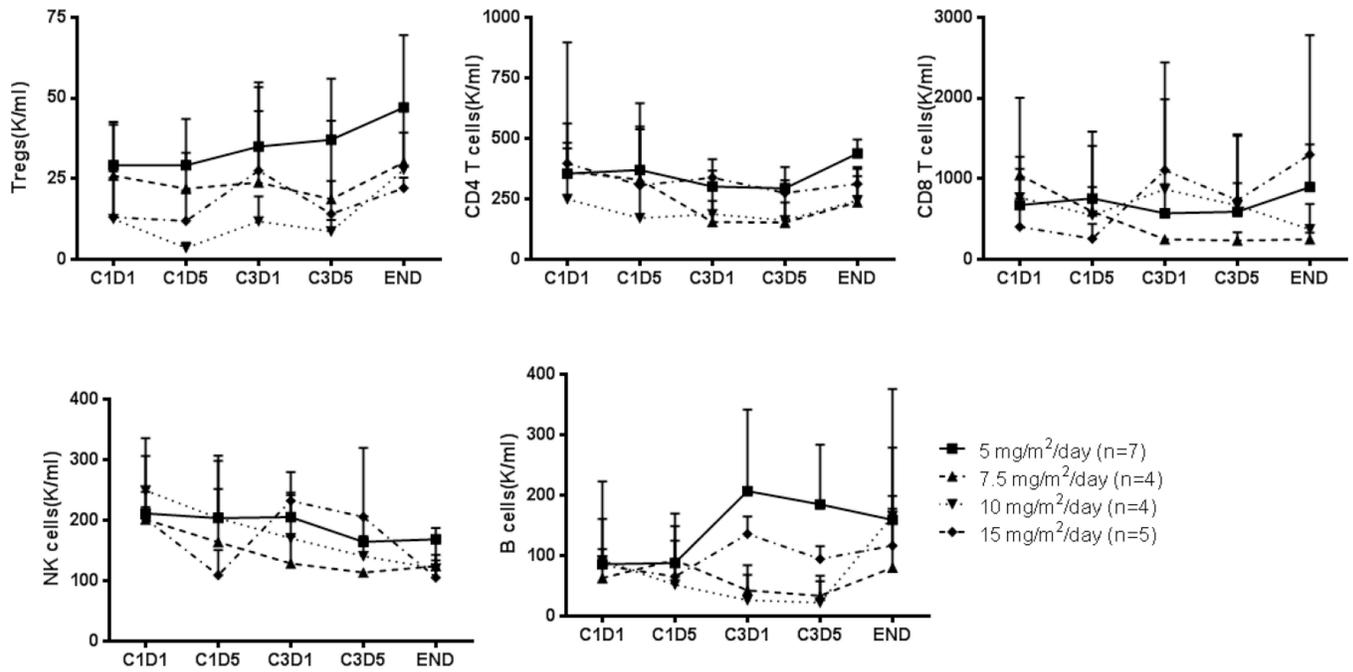


Figure 4.

Effect of decitabine on the absolute number of Tregs, CD4 and CD8 T cells, B cells and NK cells in the peripheral blood and stated time points (C1D1: cycle 1 day 1; C1D5: cycle 1 day 5; C3D1: cycle 3 day 1; C3D5: cycle 3 day 5; END: end of study)

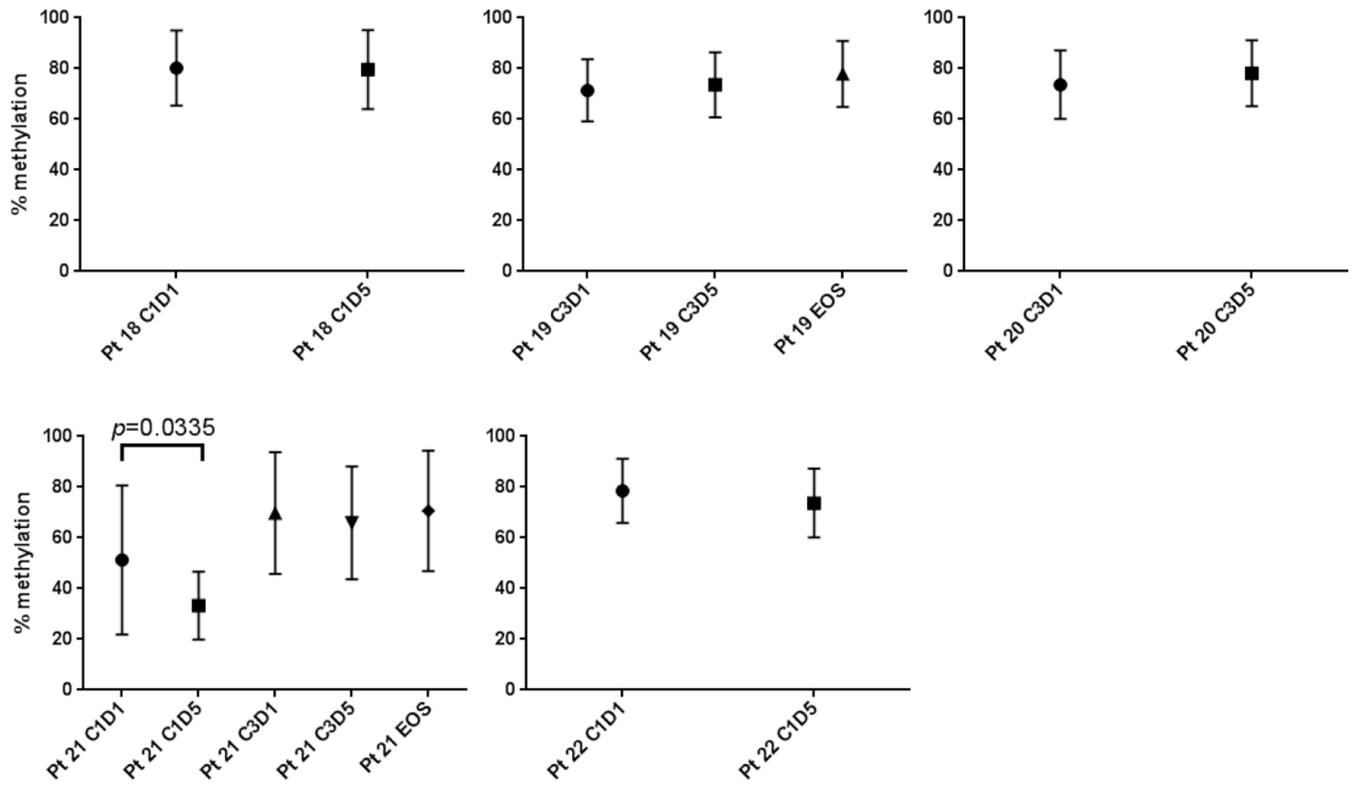


Figure 5.

DNA methylation status at the FOXP3 locus of cohort 4 (15 mg/m²/days)

Legend: Shown are means and standard deviations of DNA methylation status of 16 CpGs of each patient in cohort 4 (patients 18–22). Pt indicates patient; C, cycle; D, day.

Table 1

Patients Characteristics

Cohort (Dose-Level)	Pt #	Sex/ Age, y	Diagnosis-status at alloHSCT	Disease Risk	Donor type	Conditioning Regimen	Days from alloHSCT to Dec	Chimerism at enrollment, %
1 (5 mg/m ²)	1	M/62	AML-PD	Ph+ AML	mMUD	BU/CY	95	100
	2	F/59	AML-PD	Prior MF, trisomy 8, FLT3-ITD +	MRD	BU/CY	62	Mixed
	3	M/37	AML-CR1	Monosomy 7	MUD	BU/CY	75	100
	4	M/62	AML-PD	Prior MDS, trisomy 19	MUD	TBI/CY	95	Mixed
	5	M/57	MDS	Trisomy 8, 5q-	MUD	BU/CY	76	100
2 (7.5 mg/m ²)	6	M/59	AML-CR2	t (8,21), CR2	MUD	BU/CY	77	100
	7	M/42	AML-CR1	Del 12/ETV6	mMUD	BU/CY	115	100
	8	M/52	MDS	Del 5, Del 6	MUD	BU/CY	90	100
	9	M/58	AML-CR1	Complex cytogenetics	MRD	BU/CY	98	100
	10	F/68	AML-CR1	Complex cytogenetics	MRD	FLU/BU	96	100
3 (10 mg/m ²)	11	F/65	AML-CR1	FLT 3-ITD +	MUD	TBI/CY	96	100
	12	F/45	AML-CR1	Therapy related AML	mMRD	BU/CY	98	100
	13	M/63	AML-CR1	Prior MDS	mMUD	BU/CY	103	100
	14	M/21	AML-CR1	FLT3-ITD +	MUD	BU/CY	97	100
	15	F/56	AML-CR1	Complex cytogenetics	MUD	BU/CY	87	100
4 (15 mg/m ²)	16	M/62	MDS	High-grade MDS	MUD	BU/CY	95	100
	17	M/63	AML-CR1	Prior MDS	MRD	BU/CY	102	100
	18	F/54	AML-CR2	CR2	MRD	BU/CY	95	100
	19	F/51	AML-CR1	MLL rearrangement	MUD	BU/CY	82	100
	20	M/57	MDS	Del 7	mMUD	BU/CY	86	100
21	M/67	AML-CR1	Del 17p (p53)	MUD	FLU/BU	74	100	
22	M/66	MDS	High-grade MDS	MRD	BU/CY	100	100	

AlloHSCT indicates allogeneic hematopoietic stem cell transplantation; AML, acute myelogenous leukemia; BU, busulfan; CR, complete remission; CY, cyclophosphamide; Dec, decitabine; Del, deletion; Flu, fludarabine; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; MF, myelofibrosis; mMRD, mismatched related donor; mMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; PD, persistent disease; Ph, Philadelphia chromosome; Pt, patient; TBI, total body irradiation.

Table 2

Summary of Outcomes

Cohort (Dose-Level)	Pt #	No. of cycles	DLT	Reason for Discontinuation	Relapse	DLI	PFS (mo)	OS (mo)	Acute GVHD	Chronic GVHD, grade	Cause of death
1 (5 mg/m ²)	1	4	No	Relapse	After 4 cycles	No	5.9	28.6	No	Yes, severe	Relapse
	2	2	Yes	Relapse	After 2 cycles	1 mo after last dose	2.6	4.4	No	N/A	Relapse
	3	6	No	Non-Compliance	No	No	23.0	23.0	Yes	Yes, severe	Infection
	4	1	No	Relapse	After 1 cycle	3 mo after last dose	1.5	6.8	No	N/A	Relapse
	5	8	No	Completed	No	No	49.1+	49.1+	No	No	Alive
	6	8	No	Completed	No	No	48.8+	48.8+	No	No	Alive
	7	2	N/A	Non-Compliance	1 yr after 2 nd cycle	19 mo after last dose	12.3	24.7	No	Yes, severe	Relapse
	8	8	No	Completed	No	No	47.7+	47.7+	No	Yes, moderate	Alive
2 (7.5 mg/m ²)	9	1	No	Patient withdrew	No	No	44.9+	44.9+	No	Yes, severe	Alive
	10	3	No	Toxicity: Infection	No	No	7.7	7.7	No	No	Infection
	11	8	No	Completed	1 yr after 8 th cycle	No, 2 nd AlloHSCT	23.1+	40.4+	Yes	No	Alive
	12	2	No	PI Decision	No	No	35.2+	35.2+	No	No	Alive
3 (10 mg/m ²)	13	8	No	Completed	No	No	35.5+	35.5+	No	Yes, severe	Alive
	14	8	No	Completed	No	No	34.1+	34.1+	Yes	Yes, moderate	Alive
	15	8	No	Completed	No	No	33.9+	33.9+	Yes	Yes, severe	Alive
	16	8	No	Completed	No	No	31.6+	31.6+	Yes	Yes, moderate	Alive
4 (15 mg/m ²)	17	2	No	Toxicity: Ileus, Infection	No	No	18.8	18.8	Yes	Yes, severe	Lung GVHD
	18	2	No	Toxicity: Infection	No	No	4.7	4.7	Yes	No	Infection
	19	4	No	Toxicity: Neutropenia	1 yr after 4 th cycle	No, 2 nd AlloHSCT	15.2+	21.2+	No	Yes, moderate	Alive
	20	6	No	Toxicity: Fatigue	No	No	19.7+	19.7+	No	Yes, moderate	Alive
	21	8	N/A	Completed	No	No	13.1+	13.1+	Yes	No	Alive
	22	1	No	Acute gut GVHD	No	No	3.4	3.4	Yes	N/A	Acute gut GVHD

AlloHSCT indicates allogeneic hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; PFS, progression free survival; OS, overall survival; PI, principal investigator; Pt, patient.

Table 3

GVHD Rates

GVHD type	No. of Pts with GVHD/ No. of Pts at risk
Classic acute GVHD	
Grade I-II	6/22
Grade III-IV GI	2/22
Late-onset acute GVHD	1/22
Chronic GVHD	
Moderate	
Pt completed 8 cycles	3/9
Pt completed < 8 cycles	2/10
Severe	
Pt completed 8 cycles	2/9
Pt completed < 8 cycles	5/10*

* One of these 5 patients had overlap features of GVHD

GVHD indicates graft-versus-host disease; GI, gastrointestinal; Pt, patient.

Table 4

Grade 3–4 Adverse Events

Event	Grade 3–4
Anemia	3
Lymphopenia	2
Neutropenia	11
Thrombocytopenia	13
Hypotension	1
Pain, not specified	2
Diarrhea	1
SGOT (AST)	1
SGPT (ALT)	2
Infection/Sepsis	8
Hypokalemia	1
Hyponatremia	1
Neuropathy	2
Dyspnea	1
Renal Failure	1

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