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Title: Phase I/II study of the deacetylase inhibitor panobinostat after allogeneic stem cell transplantation in patients with high-risk MDS or AML

Short Running Title: Panobinostat maintenance in acute myeloid leukemia

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Key Points:

- Weekly and biweekly administration of panobinostat following allogeneic stem cell transplantation for high risk AML and MDS patients is feasible with excellent long-term outcome
- Combined treatment of panobinostat with donor lymphocyte infusions is feasible without evidence of enhanced GvHD
- These data provide the basis for a confirmatory prospective, randomized phase III trial in patients with advanced and high risk AML and MDS

Abstract

Relapse is the major cause of treatment failure following allogeneic hematopoietic stem cell transplantation (HSCT) for acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). Salvage treatment with discontinuation of immunosuppression and donor lymphocyte infusions is rarely effective and frequently induces severe graft-versus-host disease. The deacetylase inhibitor (DACi) panobinostat demonstrated moderate anti-leukemic activity in phase I/II trials and displays a broad range of immunomodulatory functions, prompting us to investigate its use as post-transplant prophylaxis. Primary objective of this phase I/II trial (n=42 patients) was to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of panobinostat given after HSCT by two different schedules. The MTDs were 20 mg TIW with weekly and 30 mg TIW with alternating week schedules, DLTs were gastrointestinal and constitutional. Alternating week administration was better tolerated with delivery of a significantly higher cumulative panobinostat dose. In these high risk patients, of whom 67% had active disease at the time of HSCT, survival probability at 2 years is 81% with a 20% cumulative rate of relapse. Panobinostat as post-HSCT maintenance therapy for high-risk AML and MDS is feasible with very good outcome. A prospective, randomized trial is needed to confirm the role of DACi maintenance after HSCT.

Introduction

Relapse is the major obstacle following an allogeneic hematopoietic stem cell transplantation (HSCT) for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) (1), (2), particularly in patients with high-risk features based on adverse cytogenetics or advanced disease. While sequential intensive cytoreductive therapy followed by a reduced intensity conditioning has improved outcome compared with standard conditioning (3), (4), (5), relapse rate remains substantial and ranges from 30 to 60% (6), (7), (8), (9). Moreover, these regimens rely on a graft-versus-leukemia (GvL) effect achieved by early discontinuation of immunosuppression and prophylactic donor lymphocyte infusions (DLI) which frequently induce significant graft-versus-host disease (GvHD) (10), (11), the principal cause of ensuing non-relapse mortality.

Salvage treatment for relapse after HSCT is rarely effective; in studies combining DLI with the hypomethylating agents azacitidine (12), (13), (14), (15), (16) or decitabine (17), 10-27% of patients achieved a CR and median OS is 10-15%. In contrast, azacitidine initiated pre-emptively upon evidence of impending relapse has been more effective in delaying or possibly preventing overt relapse, with 40% of patients alive at 16 months (18).

Evidence for anti-leukemic activity of the deacetylase inhibitor (DACi) panobinostat stems from phase I/II trials in which complete and partial responses were observed in a small subset of patients with relapsed or refractory AML and high-risk MDS (19), (20). In addition to direct antiproliferative and proapoptotic effects on leukemic cells (21), DACi exhibit diverse immunostimulatory and -inhibitory effects attributed to targeting of histones and non-histone proteins (22), (23)). In AML blasts, upregulation of major histocompatibility complex (MHC) class I and II molecules as well as costimulatory and adhesion molecules suggests that DACi may enhance leukemia-specific cytotoxicity (24), (25). A similar effect may be mediated by downregulation of the immune checkpoint programmed cell death protein 1 (PD1) on CD4 and CD8 positive T cells, as observed with panobinostat treatment for Hodgkin´s lymphoma (26). Conversely, DACi may reduce the number of antigen presenting cells, downregulate

secretion of proinflammatory cytokines, increase number and suppressor function of regulatory T cells and impair NK cell function (27), (28), (29), effects that could potentially impair GvL but also be beneficial by mitigating GvHD.

We hypothesized that on balance, panobinostat could reduce the relapse rate in patients with high-risk myeloid diseases while simultaneously reducing GvHD. Here, we present the results of the phase I/II PANOBEST trial using panobinostat as maintenance post-HSCT in high-risk AML and MDS patients.

Methods

Patients

Adult patients (aged ≥ 18 years) with high-risk AML (except acute promyelocytic leukemia, AML M3) or MDS intermediate-2 or high-risk according to the International Prognostic Scoring System (IPSS), or RAEB according to the World Health Organization (WHO) classification who who were in complete hematologic remission following hematopoietic stem cell transplant (HSCT) were eligible. High-risk features for AML were defined as one or more of the following criteria: (i) refractory to or relapsed after at least one cycle of standard chemotherapy; (ii) > 10% bone marrow blasts at day 15 of the first induction cycle; (iii) adverse risk cytogenetics including complex karyotype (≥3 abnormalities or abnormalities of chromosomes 3, 5 or 7) regardless of stage; and/or (iv) secondary to MDS or radio-/chemotherapy. All conditioning regimens except for >8 Gy total-body irradiation or >12 mg/kg total oral busulfan dose were permitted. Patients had to be enrolled between days 60 and 150 after HSCT.

Key inclusion criteria included Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2, ANC ≥1,000/µL, platelets ≥75,000/µL, adequate organ function and no active acute GvHD grades 2 - 4 or extensive chronic GvHD. Patients with clinical symptoms of central nervous system leukemia or impaired cardiac function (e.g. LVEF <45% by echocardiography) were excluded. Additionally, patients receiving a drug known to prolong

the QT interval that could not be terminated or who had received prior treatment with a DACi were not eligible.

The study protocol was reviewed by the independent ethics committee at each center, and written informed consent was obtained from each patient prior to any screening procedures. The study is registered at http://clinicaltrials.gov as NCT01451268.

Study Design

This was an open-label, oligocenter phase I/II study. The primary objective of phase I was determination of the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of panobinostat for two different administration schedules (Figure 1A). Secondary objectives included preliminary analyses of the safety and tolerability of the two regimens. Patients were enrolled in escalating dose cohorts in two sequential schedules using a classical 3+3 design. Panobinostat was administered orally thrice weekly (TIW), either every week (schedule A) or every other week (schedule B) and was scheduled for up to one year. The one week on/one week off regimen evaluated the ability to deliver higher individual and cumulative panobinostat doses by avoiding excess hematologic and gastrointestinal toxicity. DLT was determined separately in both arms during the first 28 days of panobinostat treatment only and defined as grade 4 (CTCAE version 3.0) hematologic toxicity (i.e. G4 neutropenia or thrombopenia of >7 consecutive days or febrile neutropenia), ≥G3 non-hematologic toxicity, acute GvHD ≥G3 scored with modified Gluckberg criteria (30) or moderate of severe chronic GvHD as assessed according to the National Institutes of Health consensus criteria (31).

In schedule A, panobinostat was started at a dose of 10 mg TIW and escalated to 30 mg TIW; in schedule B, dose escalation started at the MTD determined for weekly panobinostat (schedule A) and was given at doses of 20-40 mg TIW. Dose reductions or interruptions were permitted in case of any newly developed G3 non-hematologic AEs considered clinically relevant to the investigator or any G4 non-hematologic AEs, with a minimum panobinostat dose of 10 mg. Upon determination of the MTD, up to 42 patients were to be enrolled in the phase 2 portion and randomly assigned in a 1:1 ratio to receive panobinostat

at the MTD either weekly or every other week on a treatment schedule identical to that from phase I. Secondary objectives of phase 2 were to generate preliminary efficacy data of panobinostat at the MTD as measured by the probability of overall and relapse-free survival, cumulative incidence (CI) of hematologic relapse and death as well as acute and chronic GvHD at one year after HSCT. To take into account that patient enrollment was permitted until day 150 after HSCT, outcome measures reported actually refer to the first day of panobinostat dosing. Monitoring of immune cell reconstitution during panobinostat therapy was an exploratory objective.

Safety and Efficacy Assessments

Patients were monitored for safety throughout the trial and up to 28 days after the last dose of study treatment. Adverse events (AEs) were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Safety evaluations included monitoring of hematology, blood chemistry, thyroid function test and urine, and regular assessment of vital signs, physical condition, body weight, ECOG PS, and cardiac monitoring.

Flow cytometric analysis for quantification of leukocyte subsets

Flow cytometric analysis was performed to determine cellular immune reconstitution post SCT in patients receiving panobinostat. Peripheral blood EDTA samples were analyzed within a maximum of 24 hours after collection using a FC500 5-color/ one laser flow cytometer (Beckman Coulter, Krefeld, Germany). Absolute cell count was calculated from the percentage values using a dual-platform approach. Monoclonal IgG1 and [2]IgG2a antibodies against CD45, CD3, CD4, CD8, CD19, CD56, [2]CD14, CD25[#] and CD127 ([#] from Becton Dickinson (BD), all others from Beckman Coulter, Immunotech, Marseille, France) were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-Texas-Red (ECD), phycoerythrin-cyanine-5 (PC5) and phycoerythrin-cyanine-7 (PC7) for staining. An automated lyse/no wash procedure with a fixation step followed by the ImmunoPrep®

reagent using TQ-Prep™ Workstation (Beckman Coulter, Krefeld, Germany) was performed. The first and second tube examined the quantity of leukocytes, lymphocytes, $CD14^+$ monocytes, $CD3^+$ T cells, $CD3^+CD4^+$ helper T cells, $CD3^+CD8^+$ cytotoxic T cells, $CD56^+CD3^-$ NK cells and $CD19^+$ B cells. The third panel allowed the measurement of the $CD4^+CD25^{++}CD127^{dim/neg}$ regulatory T cell amount.

The optical alignment, fluidic stability and accuracy of the flow cytometer were tested with Flow Check Pro Fluorospheres (Beckman Coulter) and Immuno-Trol™ Cells daily, before measurements. Flow set Fluorospheres (Beckman Coulter) served to set up the photomultiplier tube values, weekly. Peripheral blood of healthy donors and stained Cyto-Comp cells (Beckman Coulter) were used to compensate the fluorescence overlap.

Statistical analysis

Patients were evaluated on an intent-to-treat basis, all patients who received at least one dose of study drug were included in the analysis. DLT was defined as prolonged grade 4 (G4) hematologic toxicity or any non-hematologic toxicity ≥G3 unrelated to disease progression or intercurrent illness within 28 days of the first panobinostat dose.

Overall survival was estimated by the Kaplan-Meier method starting from the day of the first dose of panobinostat to either death or up to 30 months of follow up, whichever occurred first. Events for relapse-free survival were death or relapse. Cumulative incidence of relapse, non-relapse mortality (NRM) and chronic GvHD were estimated by the proportional hazards method with relapse and NRM as competing events for each other, and relapse and NRM as competing events for chronic GvHD. IBM SPSS statistics version 23 and R software were used as statistical software.

Longitudinal analysis of cellular reconstitution was performed using the R software for statistical computing, version 3.2.1 and the *Linear and Nonlinear Mixed Effects Models* (nlme) package (32). First, cells counts were logarithmically transformed in order to attain

residuals with normal distribution. Afterwards, mixed spline linear regression was fitted for each cell population and for the percentage of T_{reg} (in % of helper T cells). The applied statistical method considers dependencies in the data due to repeated measures. P-values were considered significant for $p<0.05$ (*) and $p<0.01$ (**).

Results

Patients

A total of 42 patients (37 AML, 5 MDS) with a median age of 52 (21-71) years and ECOG PS of either 0 (57%) or 1 (43%) were enrolled between January 2011 and January 2015. Data cutoff for this analysis is November 13, 2015. The majority of patients (n=28, 67%) were transplanted with active disease (BM blasts 0-80%, median 21%, 1 patient with isolated extramedullary AML), 9 in CR1 (21%) and 5 in CR2 (12%). Patient and transplant characteristics were equally distributed between schedules A and B and are summarized in table 1.

Determination of maximal tolerated dose and dose limiting toxicity

Patient disposition is outlined in figure 1B. All 12 patients in the phase 1 part of schedule A (10 mg, n = 3; 20 mg, n = 6; 30 mg, n = 3) and 11 of 12 patients in phase 1 of schedule B (20 mg, n = 3; 30 mg, n = 6; 40 mg, n = 3) were evaluable for determination of MTD. Overall, 5 DLTs were observed, three in schedule A (fatigue G3 at 20 mg, colitis and nausea/emesis G3 at 30 mg in one patient each) and two in schedule B (diarrhea and headache G3 at 40 mg in one patient each). Four patients continued treatment after dose de-escalation. One patient was taken off study by investigator´s decision because of ECG alterations G1 after three doses of panobinostat; this patient was not evaluable for DLT and was replaced. The MTD for schedules A (weekly) and B (every other week) was determined to be 20 mg TIW and 30 mg TIW, respectively.

Safety

All enrolled patients who received at least one dose of panobinostat were included in the safety analysis. Most patients (35 out of 42, 83%) experienced at least one G3/4 AE, which was judged to be panobinostat-related in 22 patients (52%) with no significant difference between schedules (schedule A: n=12, 57%; schedule B: n=10, 48%). However, panobinostat-related G4 hematologic toxicity and G1-3 constitutional symptoms were only observed in schedule A. All panobinostat-related G3/4 AEs are listed in table 2. G1/2 AEs were included only if they constituted a DLT or resulted in a modification of the panobinostat dose. Panobinostat-related AEs were fully and rapidly reversible after interrupting panobinostat, there were no study-related deaths and no patient died on treatment or within 28 days of the last panobinostat dose.

Thrombocytopenia is a well-recognized class effect of DACi. Platelet counts decreased significantly from baseline levels already by 8, i.e. after 3 doses of panobinostat in both schedules A and B (170±12 vs. 127±9/nL, n=42, mean ± SEM, $p < 0.001$, paired samples t-test). Thrombocytopenia was more pronounced with schedule A, increasing progressively until day 57 (146±15 vs. 71±7/nL, n=15, $p < 0.001$) (figure 2). In schedule B, platelet counts recovered to baseline values by day 15 (195±22 vs. 195±19/nL, n=19, p=ns) and up to 40 mg panobinostat was not associated with cumulative hematologic toxicity in schedule B.

The most common G3/4 AEs irrespective of causality to study drug are listed in supplementary table 1. Twelve patients (29%) developed AEs that led to permanent discontinuation of the study drug after a median of 30 days (range, 7-293), including acute GvHD G3 (n=2), fatigue G3, relapse of lung cancer, pulmonary fibrosis, elevation of lipase G3 and ECG abnormalities (ST deviations).

Dose intensity and treatment duration

Panobinostat was started a median of 96 days (range, 60-147) after HSCT. Twenty-two patients (52%) received panobinostat for one year as scheduled (A: 10/21 patients, 52% vs. B: 12/21, 57%). Besides AEs (n=12), reasons for prematurely discontinuating the study were relapse (n=5), patient decision (n=2) or prohibited comedication (n=1). Of the 27 patients treated at the MTD, 15 patients (55%) dropped out early after a median of 47 days (range, 11-172) and additional 4 patients (15%) required reduction of the panobinostat dose. Median duration of treatment at the MTD was 52 days (range, 11-368) in schedule A and 228 days (16-365) in schedule B.

Outcome

To date, median OS and DFS have not been reached after a median follow up of 22 months (range, 6-57) (Figure 3). At two years after the first panobinostat dose, the CI of relapse and non-relapse mortality were 20% (95% CI, 7-33%) and 5% (95% CI, 0-11%, figure 4A) resulting in probabilities for 2-year OS and DFS of 81±7% and 75±7%, respectively. Ten patients died from relapse (n=6), sepsis (n=1), severe chronic GvHD (n=1), relapse of pre-existing lung cancer (n=1) and sudden death (n=1; 3.5 months after study discontinuation), respectively.

Concurrent donor lymphocyte infusions and panobinostat

Only three out of 42 patients developed acute GvHD while on study (G1, n=1; G3, n=2). Donor lymphocyte infusions were not mandated by the study protocol but could be given optionally at the discretion of the treating physician. During panobinostat treatment, 18 patients (43%) received a median of two DLI (range, 1-6) because of falling donor chimerism (n=6), detection of MRD (n=2) or prophylacticly (n=10). Prophylactic DLI were initiated at a median of 88 days (range, 49-317) after the first panobinostat dose and contained a median of $0.2x10^6$ CD^{3+} cells/kg body weight, whereas preemptive DLIs were administered slightly

later (104 days, range, 50-231) at a higher starting dose of $0.9x10^6$ CD^{3+} cells/kg. DLI were equally distributed between patients in both treatment schedules (43% of patients each). CI of moderate (n=10) or severe (n=2) chronic GvHD was 29% (95 CI, 16-42%) at two years after the first panobinostat dose and did not differ between both arms (Figure 4B).

Reconstitution of immune cells

Previous data have shown that the immune system is a critical component of the anti-tumor effects of DACi (33). Thus, peripheral blood samples were collected for prospective assessment of lymphocyte subpopulations from 26 out of 42 patients (62%) during study treatment. As detailed in figure 5A and B, $CD4^+$ and $CD8^+$ T cell counts improved over time and did not differ between study arms. The frequency of regulatory T cells, however, showed statistically significant differences for at least five months of panobinostat therapy with a higher proportion in schedule B ($p<0.01$, Figure 5D). The absolute cell count of regulatory T cells was also higher in schedule B, but not statistically significant (Figure 5C). NK cell recovery does not seem to be affected by panobinostat treatment with absolute NK cell values reaching the normal ranges of adult patients (103-760 cells/µl, Figure 5E). Importantly, B cells numbers increased significantly faster in schedule B during the first three months of panobinostat ($p=0.001$, Figure 5F). After one year, B cell values fell well within normal ranges (81-760 cells/µl).

Discussion

Maintenance therapy after allogeneic HSCT has become an area of evolving research activity, fostered by the dismal outcome of therapy for overt relapse after transplant (1). To date, few clinical trials have explored post-transplant intervention in AML, reflecting the still limited number of available agents combining anti-leukemic potential with a favourable toxicity profile. These properties are essential prerequisites for post-transplant maintenance therapy and are not met by conventional cytotoxic agents.

Our trial is one of only two trials examining a DAC inhibitor after HSCT (34) and the first completed prospective study assessing feasibility of prolonged post-transplant administration of a DACi as prophylaxis after HSCT for AML or any acute leukemia. The study hypothesis, that panobinostat would have a beneficial impact on post-transplant outcome by combining direct and immune-mediated antileukemic effects with suppression of GvHD, was based on results of early phase clinical trials in the non-transplant setting and on preclinical data (22). Phase I/II trials for relapsed or refractory AML or high-risk MDS suggested a dose-dependent response, but the MTD and RP2D determined in these trials subsequently proved to be incompatible with prolonged administration (20), (35), (36), (37). Moreover, tolerability and hematologic adverse events, most notably thrombocytopenia, differed by schedule of panobinostat administration (20). We therefore evaluated weekly and every other week schedules during dose-escalation to identify a panobinostat dose and schedule compatible with long-term administration. The MTDs defined as 20 mg TIW and 30 mg TIW with weekly and alternating week schedules, respectively, are in accordance with those identified in non-transplanted patients requiring prolonged therapy, such as myeloma, for which panobinostat was recently approved as salvage therapy (38), as well as primary myelofibrosis or post-polycythaemia vera / post- essential thrombocythaemia myelofibrosis (39).

The degree of thrombocytopenia, fatigue and gastrointestinal toxicity that was observed favored the alternating week administration and resulted in a significantly higher cumulative dose delivered at the MTD by this schedule. Both of these AE categories are dose-dependent class effects of DACi that have been observed in all disease entities studied and are not unique to the post-transplant setting. The early decline in platelets is likely due to defective maturation of megakaryoctes rather than long-term cytotoxic effects on megakaryopoiesis (40), (41), and was not associated with severe bleeding episodes with either panobinostat schedule in our study. Nevertheless, the alternating week schedule is likely to provide a bigger margin of safety in patients who often have low starting levels of platelets after HSCT. Hematotoxicity has also been a consistent finding with post-transplant administration of hypomethylating agents (42), (43), (44), although no direct comparison

between DACi and azacitidine or decitabine in terms of tolerability and toxicity after HSCT is available.

Preliminary evidence for efficacy in our trial is very encouraging, with survival probability of 81% at 2 years after starting panobinostat and a 20% cumulative rate of relapse at 2 years. These outcome data are even more remarkable considering that all AML patients were high risk by commonly accepted criteria (45) and the majority (67%) had active disease at the time of HSCT. Survival rates reported for such patient cohorts are extremely poor, with cumulative probability of relapse exceeding 30-60% at 2-3 years after HSCT (7), (6), (9). In view of our remarkably good outcome data, we have to consider the possibility of selection bias due to inclusion criteria requiring peripheral blood count recovery, adequate organ function and absence of clinically relevant acute or chronic GvHD. These criteria are typical of phase I trials but necessarily limit the risk for transplant-related mortality, which may have contributed to the very good survival. In addition, we permitted patient enrolment only between days 60 and 150 after HSCT, and not at the time of transplant because of the phase I/II nature of the trial. Although relapse risk was analysed as a time dependent variable, we cannot exclude that patients with extremely rapid disease kinetics are underrepresented, as the median time to relapse after HSCT is 4 to 6 months for high-risk AML (3), (46). Nevertheless, median time to enrollment after HSCT in our study was 3 months, which is less than the median time to relapse of 8 months reported in the RICAZA trial (43).

Notably, the low relapse rate observed in the PANOBEST trial was not counterbalanced by a higher than expected incidence of clinically significant chronic GvHD even among patients receiving additional DLI, suggesting that panobinostat does not impair development of peripheral tolerance and may actually mitigate GvHD.

Our data are in agreement with the only other phase I/II study of peri-transplant DACi that has been published to date, which investigated whether vorinostat reduces the risk of acute GvHD when administered together with standard immunosuppressive therapy during and up to 100 days after reduced-intensity conditioning (34). The incidence of grade 2-4 acute GvHD

was significantly lower compared to a historical patient cohort, and translational studies confirmed a vorinostat-mediated increase of regulatory T cells and *FOXP3* and *IDO* expression, as previously described for pan-DACi (47), (48). Preclinical data indicate that these immunomodulatory effects of pan-DACi on Tregs may be dose-dependent (49). Whereas pan-DACi such as panobinostat at low doses primarily inhibit class I HDAC and exert regulatory T cells suppressive effects, higher doses predominantly target class II HDAC and promote regulatory T cells. Our study indeed demonstrates that the proportion of regulatory T cells decreases with schedule A, while remaining stable with the higher doses delivered during alternating week panobinostat in schedule B. Thus, in addition to determining tolerability, dosing of panDACi such as panobinostat may have profound clinical implications due to the dose-dependence of its effects on regulatory T cells. Schedule B also seemed to be superior in promoting B cell recovery after HSCT, a potentially relevant finding considering recently reported B cell-mediated anti-tumor effects (50). In both our study and the trial of post-transplant vorinostat, immunomodulatory effects were not associated with a higher relapse rate, while possibly mitigating GvHD. In the setting of allogeneic HSCT, pan-DACi may thus ameliorate alloreactivity while preserving an beneficial GvL effect. This concept is currently being evaluated in the setting of unrelated donor HSCT (NCT01790568). As regulatory T cell number and function are known to have opposing effects on GvL and GvHD, attention to dosing will be critical in future studies of post-HSCT use of pan-DACi.

Conceptually, our study is most closely related to clinical trials investigating the role of hypomethylating agents in the post-transplant setting. Both drug classes represent epigenetic therapies and have well-recognized immunomodulatory properties, although no direct or comprehensive comparison of their activities has yet been conducted. To date, two prospective phase I/II trials have examined post-transplant maintenance with azacitidine in patients with AML or MDS (42) or AML only (RICAZA trial) (51), (43) one trial studied prophylactic decitabine in AML or MDS (44). Eligibility criteria and patient characteristics in the study performed by de Lima et al. were similar to our study, but no DLI were given. Median EFS and OS of the 45 patients who received azacitidine were encouraging, with 18.2

months and 30.8 months, respectively. In the RICAZA trial, 2-year OS and RFS probabilities were 81% (95 CI, 69-95%) and 49% (95% CI, 35-68%), respectively and median time to relapse was 8 months. In contrast to our study, most patients (32/37; 86%) were transplanted in CR rather than with active disease (43). The only study of decitabine maintenance after HSCT reported 2-year OS and EFS rates of 56% (95% CI, 38-83%) and 48% (95% CI, 30-75%), respectively in 22 patients with HR-AML or MDS. As in the RICAZA study, most patients (64%) were in CR at the time of HSCT (44). In these three studies, only 20-43% patients received all scheduled cycles of HMAs, highlighting the difficulty of delivering maintenance therapy in the post-transplant setting.

Taken together, the results of our study and available data from clinical trials of post-transplant HMA support a prophylactic versus a pre-emptive strategy, particularly given the limited availability of highly sensitive and clinically validated MRD markers. We demonstrate that panobinostat as post-HSCT maintenance therapy for high-risk AML and MDS is feasible and associated with excellent survival. Longer follow-up and a prospective, randomized trial are needed to confirm the role of DACi maintenance after HSCT for high risk myeloid malignancies.

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Figure legends

Figure 1A. Study design. In phase 1, patients received escalating doses of panobinostat, initially administered three times per week (TIW) every week (Schedule A), a subsequent patient cohort received panobinostat in alternating weeks (Schedule B). In phase 2, patients were randomized to receive treatment with panobinostat either according to Schedule A or Schedule B, at the respective maximum tolerated dose (MTD) determined for each schedule. Time window for enrollment was 60 to 150 days after transplant. Cond, conditioning; HSCT, hematopoietic stem cell transplantation

Figure 1B: Patient allocation to administration schedule and dose levels. The first group of 12 patients (cohorts 1, 2 and 3) received panobinostat weekly at dose levels of 10 mg, 20 mg and 30 mg TIW. Patients in cohorts 4, 5 and 6 received panobinostat during alternating weeks at dose levels of 20 mg, 30 mg and 40 mg TIW. In the expansion cohorts 7A and 7B, an additional 9 patients in each schedule received panobinostat at the respective MTD.

Figure 2: Platelet counts during study. Curves represent mean platelet counts for all patients treated in Schedules A and B, respectively, irrespective of dose level and starting with baseline counts. Error bars denote standard error of the mean (SEM).

Figure 3. **Survival analysis.** Kaplan-Meier curves are shown for all patients enrolled, irrespective of treatment schedule or dose level and calculated from the first dose of panobinostat. **Panel A:** Overall survival; **Panel B:** Relapse-free survival. Symbols represent censoring times. Median follow up is 22 months (range, 6-57 months)

Figure 4. **Panel A: Cumulative incidence (CI) of relapse and non-relapse mortality.** Of all 42 patients enrolled, 9 relapsed and 5 died in complete remission (CR). At two years after starting panobinostat, the CI of relapse and non-relapse mortality were 20% (95% CI, 7-33%) and 5% (95% CI, 0-11%) respectively. **Panel B:** The CI of moderate (n=10) or severe (n=2) chronic GvHD was 29% (95 CI, 16-42%) at two years after the first panobinostat dose and did not differ between both arms (Figure 4B).

Figure 5: **Reconstitution of immune cells**

Peripheral blood (PB) samples were prospectively collected at 6 timepoints (pre-treatment, 8, 30, 90, 150 and 365 days after starting panobinostat) during the one year of study treatment for assessment of lymphocyte subpopulations. Data is available for 26 of 42 patients; the number of patient samples available for each timepoint is indicated by schedule. $CD4^+$ T cell counts **(Panel 5A)** and $CD8^+$ T cell counts (**Panel 5B)** did not differ between study arms. The absolute number of regulatory T cells was not statistically significantly different between schedules **(Figure 5C),** whereas the frequency of regulatory T cells (Treg) was significantly higher in patients treated according to Schedule B **(Figure 5D)**. NK and B cell recovery are depicted in **Figures 5E** and **5F**, respectively.

Table 1: Patient, disease and HSCT characteristics

	Schedule A (N=21)	**Schedule B (N=21)**	**P value**
Median age, years (range)	58 (21-68)	50 (30-71)	.351
Sex			.212
male / female	10 (48%) / 11 (52%)	14 (67%) / (33%)	
Disease			.634
AML / MDS	19 (90%) / 2 (10%)	18 (86%) / 3 (14%)	
de novo	15 (72%)	16 (76%)	.890
secondary to MDS	3 (14%)	2 (10%)	
therapy-associated	3 (14%)	3 (14%)	
Karyotype (ELN criteria), AML only (n=37)			.859
good	2 (10%)	3 (17%)	
intermediate-1/-2	10 (53%)	9 (50%)	
adverse	7 (37%)	6 (33%)	
Stage prior to HSCT			.404
CR1/CR2	6 (28%)	8 (38%)	
primary refractory	10 (48%)	7 (33%)	
relapse	2 (10%)	5 (24%)	
untreated	3 (14%)	1 (5%)	
Median BM blasts at start of conditioning	n=15	n=13	.207
(range), n=28	20 (8-63)	23 (0-80%)	
Donor			.877
MRD / MMRD	4 (19%) / 2 (10%)	5 (24%) / 1 (5%)	
MUD / MMUD	13 (61%) / 2 (10%)	12 (57%) / 3 (14%)	
Conditioning regimens			
Fludarabine/Melphalan +/- BCNU	7	7	33%
Flamsa-RIC	5	3	19%
Fludarabine/Melphalan/TBI 2-8 Gy	3	3	14%
Fludarabine/TBI 2-8 Gy	2	3	12%
Fludarabine/Busulfan	1	2	7%
Other	3	3	14%

Abbreviations: ELN, European Leukemia Net; HSCT, hematopoietic stem cell transplantation; BM, bone marrow; MRD, matched related donor; MMRD, mismatched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; Flamsa-RIC, fludarabine, amsacrine, AraC, reduced-intensity conditioning; TBI, total body irradiation; Gy, Gray;

Table 2: Adverse events considered related to panobinostat by treatment schedule and initial dose cohort

Panobinostat-related toxicity	Arm A, n (%)			Arm B, n (%)		
	G 1-2	G 3	G 4	G 1-2	G 3	G 4
Blood /Bone marrow	0	6 (29)	2 (10)	0	7 (33)	0
Cardiac	1 (5)	0	0	1 (5)	0	0
Constitutional symptoms						
Fatigue	2 (10)	4 (19)	0	0	0	0
Weight loss	1 (5)	0	0	0	0	0
Gastrointestinal symptoms						
Nausea/Vomiting	4 (19)	1 (5)	0	3 (14)	0	0
Diarrhea	3 (14)	1 (5)	0	1 (5)	2 (10)	0
Colitis	0	1 (5)	0	0	0	0
Anorexia	1 (5)	0	0	0	0	0
Oral mucositis	0	0	0	1 (5)	0	0
Taste alteration	0	0	0	1 (5)	0	0
Pain	1 (5)	1 (5)	0	1 (5)	0	0
Headache	0	0	0	0	1 (5)	0
Renal failure	0	1 (5)	0	0	0	0
Rash	0	0	0	1 (5)	0	0
Sensory neuropathy	0	0	0	0	1 (5)	0
Metabolic/Laboratory						
Elevated liver function tests	0	0	0	2 (10)	2 (10)	0
Creatinine increased	1 (5)	0	0	0	0	0
Hyperuricemia	0	1 (5)	0	0	0	0

Figure 1A

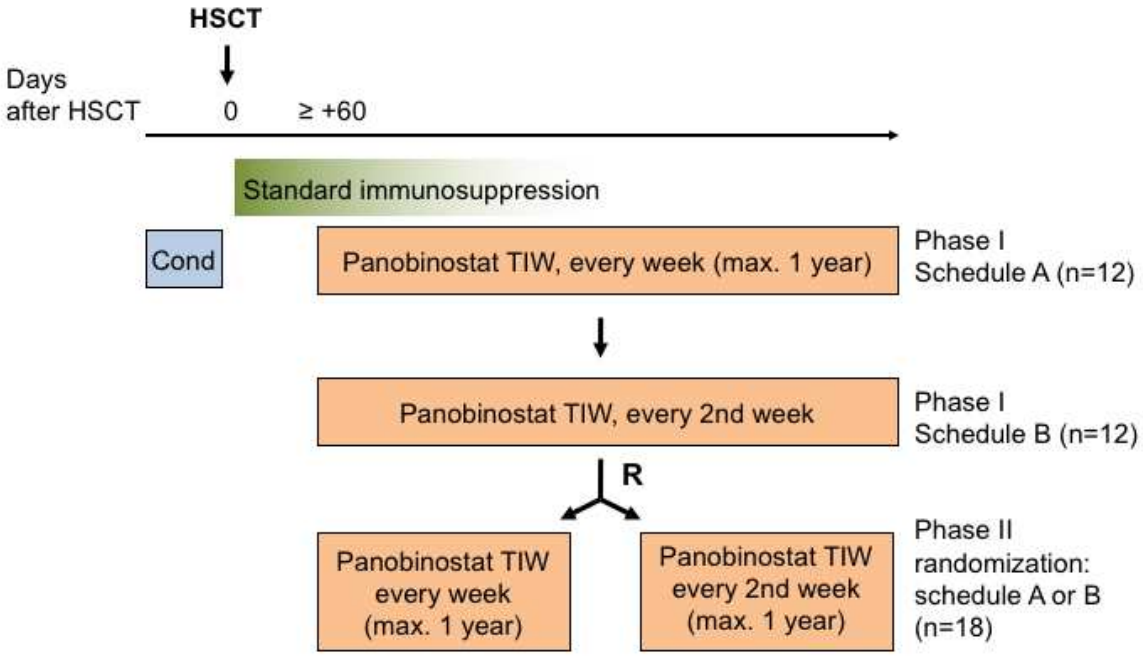


Figure 1B

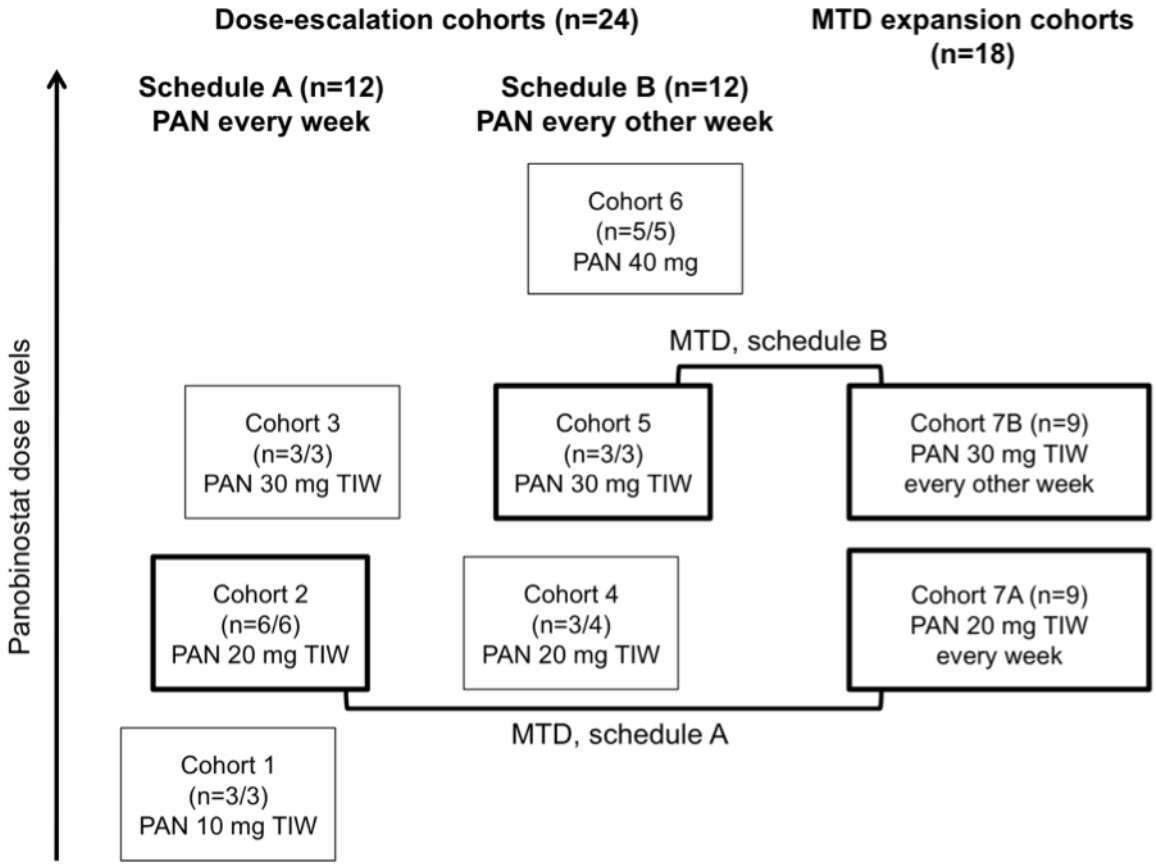


Figure 2

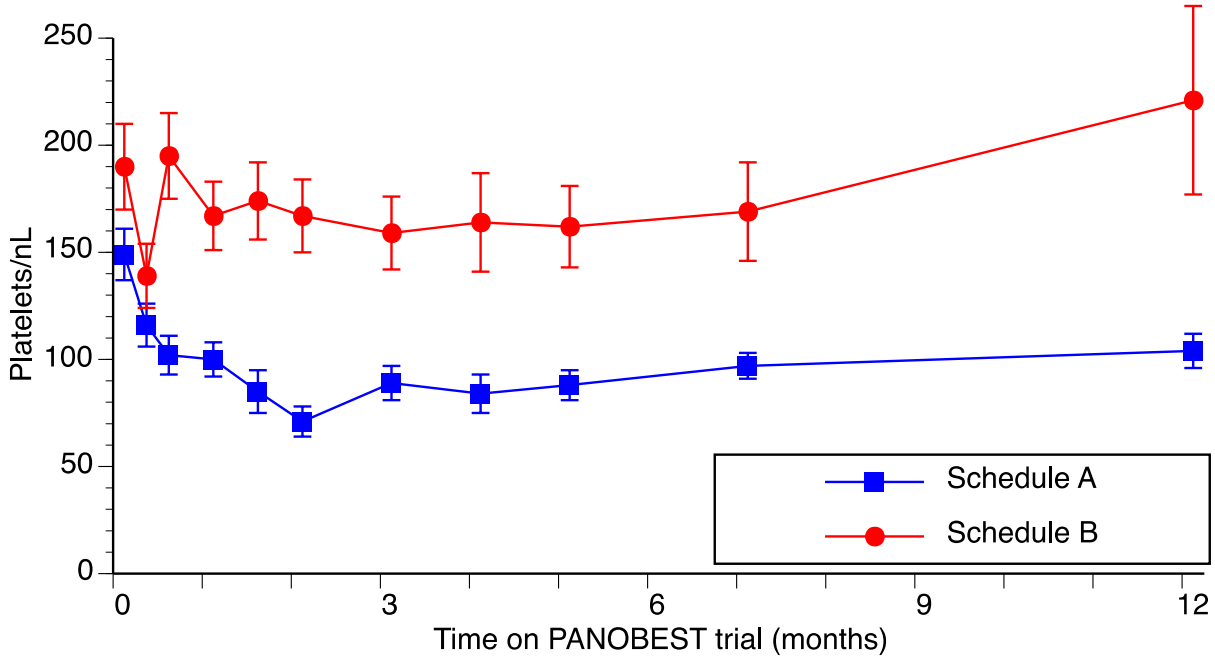

250
200
150
100
50
0
Platelets/nL
0
3
6
9
12
Time on PANOBEST trial (months)
Schedule A
Schedule B

Figure 3A: Overall Survival **B:** Relapse-free survival

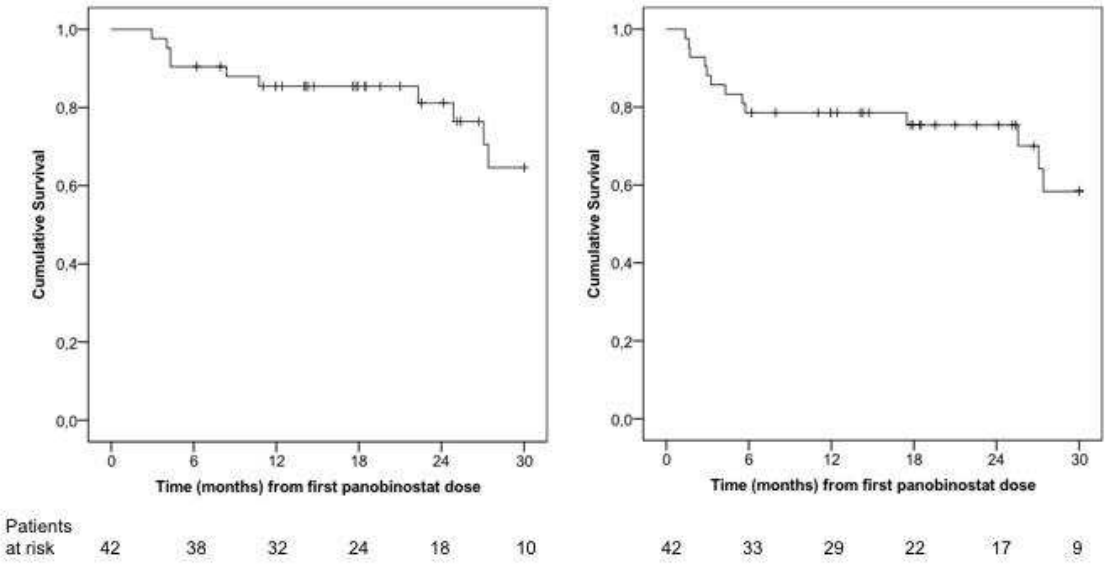


Figure 4A: Relapse and non-relapse mortality **B**: Moderate and severe chronic GvHD

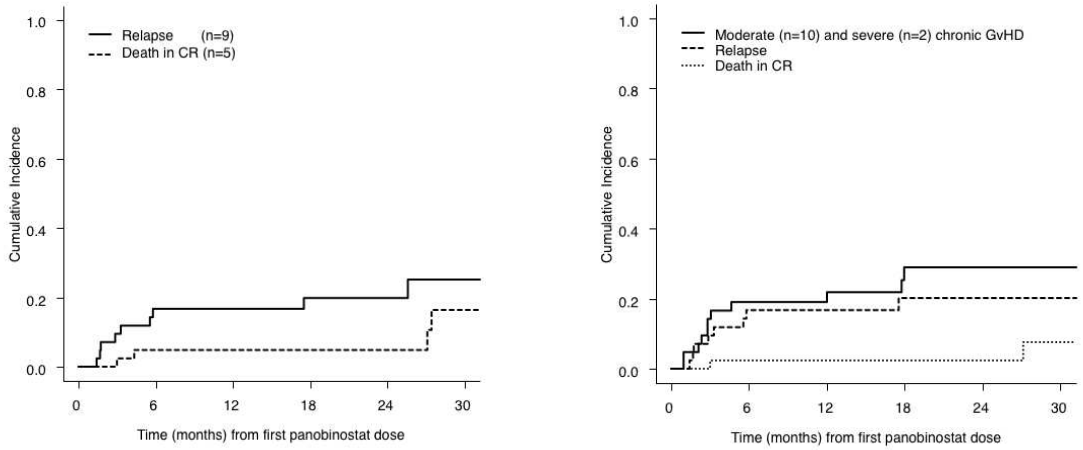
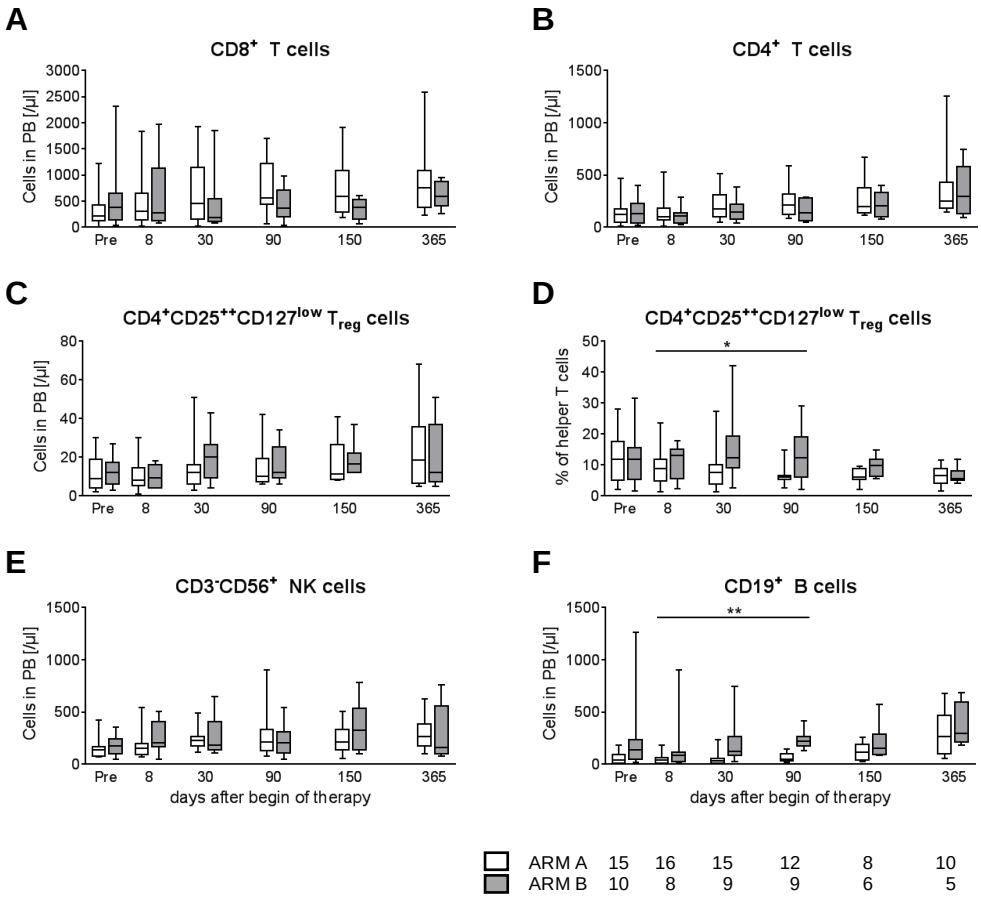


Figure 5


A
CD8+ T cells
Cells in PB [/µl]
B
CD4+ T cells
Cells in PB [/µl]
C
CD4+CD25++CD127low Treg cells
Cells in PB [/µl]
D
CD4+CD25++CD127low Treg cells
% of helper T cells
*
E
CD3-CD56+ NK cells
Cells in PB [/µl]
days after begin of therapy
F
CD19+ B cells
**
Cells in PB [/µl]
days after begin of therapy
Pre 8 30 90 150 365
ARM A 15 16 15 12 8 10
ARM B 10 8 9 9 6 5