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Phase I trial of low dose decitabine targeting DNA hypermethylation in patients with chronic lymphocytic leukemia and non-Hodgkin's lymphoma: Dose-limiting myelosuppression without evidence of DNA hypomethylation

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Abstract

Purpose—Targeting aberrant DNA hypermethylation in chronic lymphocytic leukemia (CLL) and non-Hodgkin's lymphoma (NHL) with decitabine may reverse epigenetic silencing in B-cell malignancies.

Methods—Twenty patients were enrolled in two phase I trials to determine the minimum effective pharmacologic dose (MEPD) of decitabine in patients with relapsed/refractory CLL (n=16) and NHL (n=4).

Results—Patients received 1–3 cycles of decitabine. Dose limiting toxicity (DLT) was observed in 2 of 4 CLL and 2 of 2 NHL patients receiving decitabine at 15 mg/m²/d days 1–10, consisting of grade 3–4 thrombocytopenia and hyperbilirubinemia. Six patients with CLL received decitabine at 10 mg/m²/d days 1–10 without DLT; however, re-expression of methylated genes or changes in global DNA methylation were not observed. Therefore, a 5-day decitabine schedule was

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Authorship

Contribution: K.A. Blum, D. M. Lucas, G. Marcucci, K.K. Chan, M.R. Grever, and J.C. Byrd designed and performed the studies. K.A. Blum and J.C. Byrd supervised, enrolled, and treated patients on the trial; analyzed and interpreted the data; and authored the manuscript. Z. Liu and D.M. Lucas designed and performed the global DNA hypomethylation analyses, and analyzed and interpreted the pharmacodynamic data. C. Plass helped design and interpret the correlative data. P. Chen, Z. Xie, and K.K. Chan developed and performed the pharmacokinetic analyses, and analyzed and interpreted the pharmacokinetic data. R. Baiocchi, T.S. Lin, D. Benson, S.M. Devine, J. Jones, L. Andritsos, and J. Flynn enrolled and treated patients on the trial. All authors reviewed the draft manuscript and approved its publication.

examined. With 15 mg/m²/d decitabine days 1–5, DLT occurred in 2 of 6 CLL and 2 of 2 NHL patients, consisting of grade 3–4 neutropenia, thrombocytopenia, and febrile neutropenia. Eight patients had stable disease. In 17 patients, there were no significant changes in genome-wide methylation or in target gene re-expression.

Conclusion—Dose-limiting myelosuppression and infectious complications prevents dose escalation of decitabine to levels associated with changes in global methylation or gene re-expression in CLL and NHL.

Keywords

decitabine; methylation; chronic lymphocytic leukemia; non-Hodgkin's lymphoma

INTRODUCTION

Epigenetic modifications including DNA methylation that favor transcriptionally repressed chromatin have been identified in B-cell malignancies, including chronic lymphocytic leukemia (CLL), mantle cell, and diffuse large B-cell non-Hodgkin's lymphoma (NHL). (Katzenellenbogen, *et al* 1999, Liu, *et al* 2006a, Nakatsuka, *et al* 2003, Raval, *et al* 2005, Raval, *et al* 2007, Rush, *et al* 2004, Takahasi, *et al* 2004) Specifically, aberrant hypermethylation has been described in *p15*, *p16*, *DAPK1*, *GRM7*, *ID4*, and *TWIST2*. (Katzenellenbogen, *et al* 1999, Nakatsuka, *et al* 2003, Raval, *et al* 2005, Raval, *et al* 2007, Rush, *et al* 2004, Takahasi, *et al* 2004, Yu, *et al* 2005) Furthermore, treatment of CLL and NHL cell lines and patient-derived cells with the DNA methyltransferase (DNMT) inhibitor decitabine (5-aza-2'-deoxycytidine) depletes DNMT and reverses transcriptional repression, leading to increased expression of these methylated target genes. (Katzenellenbogen, *et al* 1999, Liu, *et al* 2006a, Raval, *et al* 2005, Raval, *et al* 2007, Rush, *et al* 2004)

Targeting aberrant DNA hypermethylation with low dose decitabine has provided a new treatment strategy in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). (Blum, *et al* 2008, Blum, *et al* 2007, Issa, *et al* 2004, Issa, *et al* 2005, Kantarjian, *et al* 2007) and we postulated that a similar approach may be effective in patients with lymphoid malignancies. Therefore, we instituted two single institution phase I clinical trials to determine the minimum effective pharmacologic dose (MEPD) of decitabine: OSU 0336, a phase I trial of decitabine in patients with relapsed or refractory CLL; and OSU 0475, a phase I trial of decitabine in patients with relapsed or refractory NHL.

PATIENTS AND METHODS

Patient selection

Patients with relapsed or refractory CLL by 1996 National Cancer Institute (NCI) Working Group Criteria (Cheson, *et al* 1996) or diffuse large B-cell, transformed, or mantle cell NHL by 1999 World Health Organization Criteria (Harris, *et al* 1999) after at least one prior therapy were enrolled to OSU 0336 and OSU 0475, respectively. Patients with aggressive NHL eligible for autologous stem cell transplantation were not enrolled; however, prior stem cell transplantation was permitted. Eligibility requirements included age $\geq$ 18 years, Eastern Cooperative Oncology Group (ECOG) performance status 0–2, creatinine $\leq$ 2.0 mg/dL, total bilirubin $\leq$ 1.5 mg/dL, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$ 2.0 x the upper limit of normal. For NHL patients, an absolute neutrophil count (ANC) $\geq$ 1500/mm³ and a total platelet count $\geq$ 75,000/mm³ were also required. There were no restrictions on pre-treatment ANC or platelet counts for patients with CLL.

Patients with active infections including human immunodeficiency virus (HIV), central nervous system involvement, uncontrolled medical conditions, or autoimmune hemolytic anemia or idiopathic thrombocytopenia were not eligible. The Ohio State University Institutional Review Board approved the two trials and all patients provided written informed consent per institutional guidelines and in accordance with the Declaration of Helsinki.

Study Design

OSU 0336 and OSU 0475 were designed to independently determine the MEPD of decitabine in patients with relapsed and refractory CLL and NHL, respectively. Dose escalation in both trials began at a dose level of 15 mg/m²/day of decitabine administered intravenously over 1 hour on days 1–10. Twenty-eight days defined a cycle. Six patients were enrolled at each dose level. Dose escalation of decitabine by 5 mg/m²/day was planned until the MEPD where 1 or fewer patients experienced dose limiting toxicity (DLT) was reached.

MEPD was defined in both trials as the dose of decitabine that in at least 5 of 6 patients produced a 100% reduction in promoter methylation or a 100% increase in mRNA expression of selected genes (*DAPK1* and *ID4* for patients with CLL and *DAPK1*, *p15*, and *p16* for patients with NHL) compared to pre-treatment levels. DLT was defined during the first cycle as grade 3–4 non-hematologic toxicity in any patient, grade 4 neutropenia or thrombocytopenia lasting longer than 7 days in patients with NHL, grade 3–4 neutropenia or thrombocytopenia on day 35 in patients with NHL, or an ANC or platelet count $\leq$ 75% of pre-treatment levels by day 35 in patients with CLL.

Adverse events were graded according to NCI Common Toxicity Criteria for Adverse Events, version 3.0. Response was assessed according to 1996 NCI Working Group Criteria (Cheson, *et al* 1996) for patients with CLL and according to 1999 International Lymphoma Response Criteria (Cheson, *et al* 1999) for patients with NHL after every cycle.

Decitabine pharmacokinetics

Plasma concentrations of decitabine were measured on days 1 and 5 of cycle 1 using a previously validated liquid chromatography-tandem mass spectroscopy (LC-MS/MS) assay. (Liu, *et al* 2006b) Samples were collected immediately prior to and 5, 10, 30, 60, 65, 75, 90, 120, 150, 180, 240, and 360 minutes after decitabine for pharmacokinetic evaluations. Calculated parameters were obtained using standard compartmental or non-compartmental methods with WinNolin version 3.0 (Pharsight, Mountain View, CA).

Global DNA hypomethylation

Samples were first assessed for global changes in methylation to detect evidence of DNMT inhibition as previously described. (Liu, *et al* 2007, Liu, *et al* 2006b) Samples that showed evidence of methylation changes were then analyzed for specific promoter hypomethylation. (Raval, *et al* 2005, Raval, *et al* 2007) Peripheral blood samples were collected immediately prior to and post decitabine on days 3, 5, 15, and 22 of cycle 1 in patients with CLL. In patients with NHL, core needle biopsy samples for global DNA methylation assessment were obtained prior to decitabine treatment and on day 5 of cycle 1. Cells were purified by CD19 selection using magnetic activated cell sorting (MACSTM, Miltenyi Biotec, Germany), and genomic DNA was extracted from CD19-selected CLL and NHL cells using a DNeasy kit according to the manufacturer's protocol (Qiagen, Valencia CA). Samples were processed and analyzed immediately using a validated LC-MS/MS method. (Liu, *et al* 2007) The global DNA methylation level was expressed as the molar ratio of 5 mdC/(5mdC

+2dC). Expression of target genes *p15*, *p16*, *DAPK1* (Raval, *et al* 2007) and *ID4* (Yu, *et al* 2005) were evaluated using real-time RT-PCR.

Statistical Methods

Descriptive statistics including means, standard deviations, and frequencies were computed for pharmacokinetic and pharmacodynamic variables.

RESULTS

Patient Characteristics and dose escalation

Twenty patients with relapsed or refractory CLL (n=16) or diffuse large cell NHL (n=4) after a median of 4.5 prior therapies (range, 2–8) were enrolled to OSU 0336 and OSU 0475, respectively. Patient characteristics are described in Table 1. In CLL patients, the median age was 61 (range, 32–82) and 11 patients had Rai stage 3–4 disease. Two patients were previously transplanted and the majority of patients had adverse cytogenetics, including 3 patients with del(11q22.3), 4 patients with del(17p13.1), and 2 patients with a complex karyotype. In patients with diffuse large cell NHL, the median age was 72 (68–75), 50% of patients had stage IV disease, and all patients had a high risk International Prognostic Index score.

Decitabine dose escalation in both OSU 0336 and OSU 0475 began at 15 mg/m²/day administered 10 consecutive days in a 28 day cycle. DLT was observed in both trials at 15 mg/m²/day days 1–10, consisting of persistent thrombocytopenia < 75% of baseline on day 35 and grade 3 hyperbilirubinemia in 2 patients with CLL (Table 2A) and grade 4 neutropenia for more than 7 days in 2 patients with NHL (Table 2B). As a result, 6 patients with CLL were then enrolled at dose level –1.0, consisting of 10 mg/m²/day of decitabine on days 1–10. No DLTs were observed at this dose level; however, global DNA hypomethylation was not observed (Figure 2). Therefore, both trials were amended to explore a 5 day administration schedule starting at 15 mg/m²/day (dose level 1.1, Tables 2A and 2B), postulating that a shorter infusion schedule would permit dose escalation to the MEPD without DLT. With decitabine 15 mg/m²/day days 1–5, DLT was observed in 2 of 6 CLL patients consisting of grade 3 gram positive bacteremia and grade 4 febrile neutropenia. In NHL patients treated with decitabine 15 mg/m²/day days 1–5, 2 patients developed DLT consisting of grade 4 neutropenia and grade 4 thrombocytopenia for more than 7 days. As a result of these toxicities, further dose escalation of decitabine was halted.

Toxicity

Patients received a median of 1 cycle of decitabine (15 patients with CLL and NHL completed 1 cycle, 4 patients with CLL completed 2 cycles, and 1 patient with CLL completed 3 cycles). Patients were removed from the trial due to DLT or progressive disease. There was one treatment-related death due to cavitary pneumonia despite broad spectrum antibiotics and anti-fungal agents.

DLTs are described in Tables 2A and 2B and grade 3–5 toxicities are summarized in Table 3. Grade 3–4 hematologic toxicities were most prevalent, consisting of neutropenia in 45% of patients and thrombocytopenia in 55% of patients. Seven patients (6 CLL and 1 NHL) developed febrile neutropenia, with infections consisting of Streptococcal pneumonia (n=1), Aspergillus pneumonia (n=1), culture negative pneumonia (n=2), Staphylococcus epidermis bacteremia (n=1), and culture negative neutropenic fever (n=2). Other grade 3–4 non-hematologic toxicities included dyspnea (in 4 patients with pneumonia), fatigue, hyperbilirubinemia, and elevated ammonia (in the patient who died from cavitary pneumonia).

Patient response

In 20 patients, there were no complete or partial responses. Eight patients had stable disease (6 CLL and 2 NHL) after 1–2 cycles of decitabine. In these patients with stable disease, there were no clinically significant changes in lymph node size or in the absolute lymphocyte count, with a median pre-treatment value of 4,025 cells/uL (range, 760–61,380 cells/uL) compared to 7,300 cells/uL post-treatment (range 1,500–76,730 cells/uL). The remaining 12 patients progressed, including 3 patients with CLL who received 2–3 cycles of decitabine.

Decitabine Pharmacokinetics

Pharmacokinetic results on cycle 1 day 1 in 15 patients (11 with CLL and 4 with NHL) for patients receiving 15 mg/m²/day and 10mg/m²/day decitabine are shown in Table 4. In 15 patients, the mean C_{max} was 1.4 ± 1.0 μM, AUC was 61.2 ± 28.1 min*μM, total clearance was 1.5 + 1.2 ml/min/m², and beta half-life was 25.5 ± 6.9 min. There was a trend for an increase in C_{max} and AUC with increased decitabine dose (10 versus 15 mg/m²), but no differences were observed between the pharmacokinetic results in patients with CLL and NHL, nor with repeat dosing on days 5 and 10 of cycle 1 (data not shown). These results are similar to historical data in AML patients treated with single agent decitabine at OSU (Table 4 and (Blum, *et al* 2007). Representative plasma decitabine concentration versus time curves in patients with CLL and NHL treated with 10 mg/m² and 15 mg/m² of decitabine are shown in Figure 1.

Correlative studies

Expression of methylated target genes *DAPK1* (Raval, *et al* 2007) and *ID4* (Yu, *et al* 2005) were evaluated using real-time RT-PCR in 10 patients with CLL. No significant changes were noted in expression of either of these genes pre-treatment versus at multiple time points following administration of decitabine (data not shown). LC-MS/MS analysis of global DNA methylation demonstrated no significant changes in genome-wide methylation in CD19-selected CLL cells collected pre-treatment and on days 3, 5, 15, and 22 of each cycle (n=16 patients) or CD19-selected NHL cells from lymph node biopsies obtained pre-treatment and on day 5 (n=1 patient, Figure 2). Even with increasing doses of decitabine (10 mg/m² versus 15 mg/m²), DNA hypomethylation post-decitabine relative to pre-treatment methylation levels was not observed. Therefore, analysis of individual promoter methylation was not conducted.

DISCUSSION

The identification of hypermethylation in key regulatory genes in CLL and NHL that is reversible with DNMT inhibition (Katzenellenbogen, *et al* 1999, Liu, *et al* 2006a, Raval, *et al* 2005, Raval, *et al* 2007, Rush, *et al* 2004) justifies clinical evaluation of low dose decitabine in patients with relapsed and refractory CLL and NHL. However, in this trial of single agent decitabine in patients with previously treated CLL and NHL, dose-limiting myelosuppression and infectious complications precluded escalation to a dose associated with DNMT depletion and DNA hypomethylation. Twenty patients with either relapsed CLL or diffuse large cell NHL received decitabine at a starting dose of 15 mg/m²/day over 10 days. At this level, prolonged myelosuppression and transaminitis were dose-limiting. Subsequent dose reduction to 10 mg/m²/day was better tolerated in patients with CLL, with no DLTs; however, at this dose level no hypomethylation was observed in 5 of 6 patients. To permit further dose escalation, decitabine was then administered using a 5-day schedule. However, despite this decrease in duration of therapy, myelosuppression and infections precluded dose escalation of decitabine above 15 mg/m². Therefore, we conclude that the

maximum tolerated dose of decitabine in patients with relapsed or refractory CLL is 10 mg/m²/day days 1–10.

Similarly, in a phase I trial of decitabine in patients with relapsed or refractory solid tumors and T-cell lymphomas, after a median of 5 prior therapies, the recommended phase II dose of decitabine without growth factor support was 10 mg/m²/day days 1–5, with DLT consisting of febrile neutropenia.(Stewart, *et al* 2009) Although reductions of 2.5–6% in global DNA methylation were observed with 2.5–20 mg/m²/day decitabine, these reductions were not significant (p=0.40) and did not appear to be dose-dependent, with the greatest degree of hypomethylation occurring in the 10 mg/m² cohort. In addition, in this same trial, *p16* mRNA expression was not significantly altered by decitabine therapy and did not correlate with changes in global DNA methylation. One partial response was observed in a patient with thymoma and most patients discontinued therapy due to disease progression. (Stewart, *et al* 2009)

Therefore, this study and the study by Stewart et al.(Stewart, *et al* 2009) demonstrate that dose escalation of decitabine in patients with CLL, NHL, and solid tumors to an optimal clinical and biologic dose is limited by hematologic and infectious complications. These results contrast with previous trials in patients with AML and MDS, where decitabine doses up to 20 mg/m²/day days 1–5 or 1–10 are well tolerated and associated with meaningful clinical responses.(Blum, *et al* 2008, Blum, *et al* 2007, Issa, *et al* 2004, Issa, *et al* 2005, Kantarjian, *et al* 2007) Although a greater degree of infectious and hematologic complications are often tolerated in patients with AML and MDS, in the previous studies, the patients have sufficient hematologic recovery for re-treatment with decitabine within 28–42 days using 5–10 day schedules.(Blum, *et al* 2008, Blum, *et al* 2007, Kantarjian, *et al* 2007) In our trial, grade 3–4 neutropenia and thrombocytopenia persisting beyond day 35 as well as disease progression prevented patients from receiving additional cycles of decitabine therapy. Even 5-day schedules that are associated with less toxicity and higher response rates in patients with AML and MDS(Blum, *et al* 2008, Kantarjian, *et al* 2007) do not appear feasible in patients with B-cell malignancies, as 2 of 2 patients with NHL and 2 of 6 patients with CLL in our trials experienced prolonged myelosuppression and serious infections using a 5-day schedule.

Finally, and most importantly, our correlative study results in B-cell malignancies contrast strongly with those in AML. Our group previously reported that in relapsed or refractory AML patients treated with 15–20 mg/m²/day decitabine on a 10-day schedule, global DNA methylation declined to a median of 78% of baseline. This effect was associated with significant increases in *p15* and *ER* expression post-treatment.(Blum, *et al* 2007) No such reductions in global DNA methylation or increases in selected gene mRNA expression occurred with similar doses of decitabine in CLL and NHL patients. One possible reason for the lack of effectiveness of decitabine in the treatment of CLL is low expression of the hENT1 transporter responsible for the cellular uptake decitabine,(Fernandez Calotti, *et al* 2008, Marce, *et al* 2006) as compared to more proliferative diseases such as AML or mantle cell lymphoma. In other studies, hENT1 expression and/or function have been related to decitabine sensitivity.(Hubeek, *et al* 2005, Qin, *et al* 2009) Currently our group is exploring cellular uptake differences between CLL and AML patients. Additionally, the lower proliferation rate of CLL cells may limit decitabine incorporation into DNA and subsequent hypomethylation. If this were true, it would suggest that prolonged exposure to decitabine would be required to re-express genes. However, this study demonstrates that prolonged decitabine therapy is not feasible due to unacceptable myelosuppression in heavily pre-treated CLL and NHL patients. Lastly, as the pharmacokinetic data among CLL and NHL patients appears similar to those previously reported with decitabine in patients with AML,

(Blum, *et al* 2007) inadequate or elevated plasma levels cannot explain the decreased biologic activity or increased toxicity of decitabine in patients with lymphoid malignancies.

In conclusion, in patients with previously treated diffuse large B-cell lymphoma and CLL, dose-limiting myelosuppression, hyperbilirubinemia, and febrile neutropenia precludes dose escalation of decitabine to a minimum effective pharmacologic dose. However, since hypermethylation in the promoters of key regulatory genes involved in transformation and progression of CLL and NHL is well described, (Katzenellenbogen, *et al* 1999, Liu, *et al* 2006a, Nakatsuka, *et al* 2003, Raval, *et al* 2005, Raval, *et al* 2007, Rush, *et al* 2004, Takahasi, *et al* 2004) exploration of mechanisms of why decitabine does not lead to methylated target gene re-expression in CLL *in vivo* and evaluation of other novel agents that target epigenetic silencing is warranted.

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References

- Blum W, Klisovic R, Liu S, Garzon R, Kefauver C, Liu Z, Mickle J, Devine H, Devine S, Grever MR, Chan KK, Villalona-Calero M, Byrd JC, Marcucci G. Preliminary Results of a Phase II Study of Low Dose Decitabine as a Single Agent in Older Patients (age $\geq$ 60) with Previously Untreated Acute Myeloid Leukemia (AML). ASH Annual Meeting Abstracts. 2008; 112:2957.
- Blum W, Klisovic RB, Hackanson B, Liu Z, Liu S, Devine H, Vukosavljevic T, Huynh L, Lozanski G, Kefauver C, Plass C, Devine SM, Heerema NA, Murgo A, Chan KK, Grever MR, Byrd JC, Marcucci G. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. *J Clin Oncol*. 2007; 25:3884–3891. [PubMed: 17679729]
- Cheson B, Horning S, Coiffier B, Shipp M, Fisher R, Connors J, Lister T, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris N, Armitage J, Carter W, Hoppe R, Canellos G. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. *Journal of Clinical Oncology*. 1999; 17:1244–1253. [PubMed: 10561185]
- Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*. 1996; 87:4990–4997. [PubMed: 8652811]
- Fernandez Calotti P, Galmarini CM, Canones C, Gamberale R, Saenz D, Avalos JS, Chianelli M, Rosenstein R, Giordano M. Modulation of the human equilibrative nucleoside transporter1 (hENT1) activity by IL-4 and PMA in B cells from chronic lymphocytic leukemia. *Biochem Pharmacol*. 2008; 75:857–865. [PubMed: 18078872]
- Harris N, Jaffe E, Diebold J, Flandrin G, Muller-Hermelink H, Vardiman J, Lister T, Bloomfield C. World Health Organization classification of neoplastic disease of the hematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee Meeting -Airlie House, Virginia, November 1997. *Journal of Clinical Oncology*. 1999; 17:3835–3849. [PubMed: 10577857]
- Hubeek I, Stam RW, Peters GJ, Broekhuizen R, Meijerink JP, van Wering ER, Gibson BE, Creutzig U, Zwaan CM, Cloos J, Kuik DJ, Pieters R, Kaspers GJ. The human equilibrative nucleoside transporter 1 mediates in vitro cytarabine sensitivity in childhood acute myeloid leukaemia. *Br J Cancer*. 2005; 93:1388–1394. [PubMed: 16333246]

- Issa JP, Garcia-Manero G, Giles F, Mannari R, Thomas D, Faderl S, Bayar E, Lyons J, Rosenfeld C, Cortes J, Kantarjian H. Phase I study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood*. 2004; 103:1635–1640. [PubMed: 14604977]
- Issa JP, Gharibyan V, Cortes J, Jelinek J, Morris G, Verstovsek S, Talpaz M, Garcia-Manero G, Kantarjian HM. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J Clin Oncol*. 2005; 23:3948–3956. [PubMed: 15883410]
- Kantarjian H, Oki Y, Garcia-Manero G, Huang X, O'Brien S, Cortes J, Faderl S, Bueso-Ramos C, Ravandi F, Estrov Z, Ferrajoli A, Wierda W, Shan J, Davis J, Giles F, Saba HI, Issa JP. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood*. 2007; 109:52–57. [PubMed: 16882708]
- Katzenellenbogen RA, Baylin SB, Herman JG. Hypermethylation of the DAP-Kinase CpG Island Is a Common Alteration in B-Cell Malignancies. *Blood*. 1999; 93:4347–4353. [PubMed: 10361133]
- Liu TH, Raval A, Chen SS, Matkovic JJ, Byrd JC, Plass C. CpG Island Methylation and Expression of the Secreted Frizzled-Related Protein Gene Family in Chronic Lymphocytic Leukemia. *Cancer Res*. 2006a; 66:653–658.10.1158/0008-5472.CAN-05-3712 [PubMed: 16423993]
- Liu Z, Liu S, Xie Z, Blum W, Perrotti D, Paschka P, Klisovic R, Byrd J, Chan KK, Marcucci G. Characterization of in vitro and in vivo hypomethylating effects of decitabine in acute myeloid leukemia by a rapid, specific and sensitive LC-MS/MS method. *Nucleic Acids Res*. 2007; 35:e31. [PubMed: 17264127]
- Liu Z, Marcucci G, Byrd JC, Grever M, Xiao J, Chan KK. Characterization of decomposition products and preclinical and low dose clinical pharmacokinetics of decitabine (5-aza-2'-deoxycytidine) by a new liquid chromatography/tandem mass spectrometry quantification method. *Rapid Commun Mass Spectrom*. 2006b; 20:1117–1126. [PubMed: 16523529]
- Marce S, Molina-Arcas M, Villamor N, Casado FJ, Campo E, Pastor-Anglada M, Colomer D. Expression of human equilibrative nucleoside transporter 1 (hENT1) and its correlation with gemcitabine uptake and cytotoxicity in mantle cell lymphoma. *Haematologica*. 2006; 91:895–902. [PubMed: 16818276]
- Nakatsuka S, Takakuwa T, Tomita Y, Hoshida Y, Nishiu M, Yamaguchi M, Nishii K, Yang WI, Aozasa K. Hypermethylation of death-associated protein (DAP) kinase CpG island is frequent not only in B-cell but also in T-and natural killer (NK)/T-cell malignancies. *Cancer Sci*. 2003; 94:87–91. [PubMed: 12708480]
- Qin T, Jelinek J, Si J, Shu J, Issa JP. Mechanisms of resistance to 5-aza-2'-deoxycytidine in human cancer cell lines. *Blood*. 2009; 113:659–667. [PubMed: 18931345]
- Raval A, Lucas DM, Matkovic JJ, Bennett KL, Liyanarachchi S, Young DC, Rassenti L, Kipps TJ, Grever MR, Byrd JC, Plass C. TWIST2 Demonstrates Differential Methylation in Immunoglobulin Variable Heavy Chain Mutated and Unmutated Chronic Lymphocytic Leukemia. *J Clin Oncol*. 2005; 23:3877–3885. [PubMed: 15809452]
- Raval A, Tanner SM, Byrd JC, Angerman EB, Perko JD, Chen SS, Hackanson B, Grever MR, Lucas DM, Matkovic JJ, Lin TS, Kipps TJ, Murray F, Weisenburger D, Sanger W, Lynch J, Watson P, Jansen M, Yoshinaga Y, Rosenquist R, de Jong PJ, Coghill P, Beck S, Lynch H, de la Chapelle A, Plass C. Downregulation of Death-Associated Protein Kinase 1 (DAPK1) in Chronic Lymphocytic Leukemia. *Cell*. 2007; 129:879–890. [PubMed: 17540169]
- Rush LJ, Raval A, Funchain P, Johnson AJ, Smith L, Lucas DM, Bembea M, Liu TH, Heerema NA, Rassenti L, Liyanarachchi S, Davuluri R, Byrd JC, Plass C. Epigenetic Profiling in Chronic Lymphocytic Leukemia Reveals Novel Methylation Targets. *Cancer Res*. 2004; 64:2424–2433. [PubMed: 15059895]
- Stewart DJ, Issa JP, Kurzrock R, Nunez MI, Jelinek J, Hong D, Oki Y, Guo Z, Gupta S, Wistuba II. Decitabine effect on tumor global DNA methylation and other parameters in a phase I trial in refractory solid tumors and lymphomas. *Clin Cancer Res*. 2009; 15:3881–3888. [PubMed: 19470736]
- Takahasi T, Shivapurkar N, Reddy J, Shigematsu H, Miyajima K, Suzuki M, Toyooka S, Zochbauer-Muller S, Drach J, Parikh G, Zheng Y, Feng Z, Kroft S, Timmons C, McKenna R, Gazdar A. DNA methylation profiles of lymphoid and hematopoietic malignancies. *Clin Can Res*. 2004; 10:2928–2935.

Yu L, Liu C, Vandeusen J, Becknell B, Dai Z, Wu YZ, Raval A, Liu TH, Ding W, Mao C, Liu S, Smith LT, Lee S, Rassenti L, Marcucci G, Byrd J, Caligiuri MA, Plass C. Global assessment of promoter methylation in a mouse model of cancer identifies ID4 as a putative tumor-suppressor gene in human leukemia. *Nat Genet.* 2005; 37:265–274. [PubMed: 15723065]

Figure 1. Pharmacokinetics of decitabine. Plasma decitabine concentration versus time in a representative patient treated with 15 mg/m² decitabine (blue line), in a patient with CLL treated with 10 mg/m² decitabine (pink line), and in a patient with diffuse large cell lymphoma treated with 15 mg/m² decitabine.

Figure 2.

Global DNA hypomethylation changes (mean $\pm$ SD). Changes are assessed by LC-MS immediately prior to decitabine (pre-dose, clear) and days 3, 5, 15, and 22 of cycle 1 (post-dose, grey) in CD-19 selected CLL/NHL cells in patients treated with 10 mg/m² decitabine x 10 days (n=6 CLL patients), 15 mg/m² decitabine x 10 days (n=4 CLL patients), and 15 mg/m² x 5 days (n=6 CLL patients and 1 NHL patient).

Table 1

Patient characteristics

Characteristic	CLL (N=16)	NHL (N=4)
Age		
Median (range)	61 (32–82)	72 (68–75)
Gender		
Male : Female	12 : 4	3 : 1
Prior therapies		
Median (range)	5 (2–8)	3 (2–4)
Prior transplant	2 (13%)	1 (25%)
Autologous	1	1
Allogeneic	1	0
Refractory Disease		
Stage	Rai Stage	Ann Arbor
0–1	2 (13%)	2 (50%)
2	3 (19%)	0
3	1 (6%)	0
4	10 (63%)	2 (50%)
Bulky lymphadenopathy		
< 5 cm	4 (25%)	0
≥ 5 cm	12 (75%)	4 (100%)
Cytogenetics (FISH)		
+(12)	1 (6%)	
del(13q)	6 (38%)	
del(11q22.3)	3 (19%)	
del(17p13.1)	4 (25%)	
Complex karyotype	2 (13%)	
International Prognostic Index (IPI) Score		
0–1		0
2–3		0
4–5		4 (100%)

Table 2A

Dose escalation schema for CLL patients treated with decitabine on OSU 0336

Dose Level	Decitabine dose	No. patients	Dose limiting toxicities (DLT)
1.0	15 mg/m ² days 1–10	4	2 DLT: Thrombocytopenia < 75% baseline on day 35 and grade 3 hyperbilirubinemia
–1.0	10 mg/m ² days 1–10	6	0 DLT, no evidence of DNA hypomethylation
1.1	15 mg/m ² days 1–5	6	2 DLT: grade 3 and grade 4 febrile neutropenia with sepsis

Abbreviations: DLT, dose limiting toxicity

Table 2B

Dose escalation schema for NHL patients treated with decitabine on OSU 0475

Dose Level	Decitabine dose	No. patients	Dose limiting toxicities (DLT)
1.0	15 mg/m ² days 1–10	2	2 DLT: grade 4 neutropenia > 7 days in 2 patients
1.1	15 mg/m ² days 1–5	2	2 DLT: grade 4 neutropenia and grade 4 thrombocytopenia > 7 days

Abbreviations: DLT, dose limiting toxicity

Table 3

Grade 3–5 Adverse events

Grade 3–5 Adverse Events	Number of patients with at least one occurrence	
Hematologic	Number of patients (n = 20)	%
Neutropenia	9	45
Anemia	4	20
Thrombocytopenia	11	55
Infectious		
Febrile neutropenia	7	35
Gastrointestinal		
Anorexia	1	5
Diarrhea	1	5
Metabolic		
Elevated ammonia	1	5
Hyperbilirubinemia	1	5
Pulmonary		
Dyspnea	4	20
Cough	2	10
Neurologic		
Confusion	1	5
Somnolence	1	5
Other		
Fatigue	2	10
Tumor pain	2	10
Hypotension	2	10
Muscle weakness	2	10

Table 4

Decitabine pharmacokinetic results in patients with CLL, NHL, and AML

	C_{max} (μM)	T_{β1/2} (min)	AUC (μM·min)	CL (L/min/m²)
CLL (n=4) 10 mg/m ² d1	0.8 + 0.4	13.0 + 16.2	31.1 + 12.1	1.6 + 0.7
CLL (n=7) 15 mg/m ² d1	1.6 + 1.3	17.3 + 35.6	60.1 + 35.5	1.7 + 1.5
NHL (n=4) 15 mg/m ² d1	1.0 + 0.2	26.5 + 129.8	63.0 + 12.1	1.1 + 0.2
AML (n=8) 15 mg/m ² d1	1.3 + 0.9	35.7 + 30.7	51.1 + 12.1	2.4 + 2.2

Abbreviations: CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia.