

## Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies

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**Decitabine (5-aza-2'-deoxycytidine) inhibits DNA methylation and has dual effects on neoplastic cells, including the reactivation of silenced genes and differentiation at low doses and cytotoxicity at high doses. We evaluated, in a phase 1 study, low-dose prolonged exposure schedules of decitabine in relapsed/refractory leukemias. Patient cohorts received decitabine at 5, 10, 15, or 20 mg/m<sup>2</sup> intravenously over one hour daily, 5 days a week for 2 consecutive weeks, doses 5- to approximately 30-fold lower than the maximum**

**tolerated dose (MTD). There were 2 groups that also received 15 mg/m<sup>2</sup> daily for 15 or 20 days. A total of 50 patients were treated (44 with acute myelogenous leukemia [AML]/myelodysplasia [MDS], 5 with chronic myelogenous leukemia [CML], and 1 with acute lymphocytic leukemia [ALL]), and the drug was well tolerated at all dose levels, with myelosuppression being the major side effect. Responses were seen at all dose levels. However, the dose of 15 mg/m<sup>2</sup> for 10 days appeared to induce the most responses (11 of 17 or**

**65%), with fewer responses seen when the dose was escalated or prolonged (2 of 19 or 11%). There was no correlation between P15 methylation at baseline or after therapy and response to decitabine. We conclude that decitabine is effective in myeloid malignancies, and low doses are as or more effective than higher doses. (Blood. 2004;103:1635-1640)**

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### Introduction

Epigenetic changes have acquired increased recognition as a driving force in human malignancies over the past few years.<sup>1,2</sup> DNA methylation in gene promoters has been demonstrated to accompany these epigenetic changes and to be essential for the maintenance of altered gene expression status in malignant cells.<sup>3</sup> Leukemias are characterized by high degrees of epigenetic changes marked by promoter methylation.<sup>4,5</sup> These observations led to the revival of interest in DNA methylation inhibitors as antineoplastic agents.<sup>6</sup> Decitabine (5-aza-2'-deoxycytidine; SuperGen, Dublin, CA) is a deoxycytidine analog that incorporates into DNA and forms irreversible covalent bonds with DNA-methyltransferases (Mts) at cytosine sites targeted for methylation.<sup>7</sup> This leads to DNA synthesis stalling and eventual degradation of DNA-Mts. Resumption of DNA replication in the absence of Mts results in gene hypomethylation and reactivation of gene expression, as has been demonstrated for multiple epigenetically inactivated loci.<sup>8-10</sup> Thus, decitabine has potentially dual effects on treated cells. At high doses, treated cells die via apoptosis triggered by the DNA adducts and DNA synthesis arrest. By contrast, at low doses, cells survive but change their gene expression profile to favor differentiation, reduced proliferation, and/or increased apoptosis. In the original description of decitabine-related differentiation, this dual effect was already apparent, with loss of the differentiation effect at high doses.<sup>11</sup>

Clinical development of decitabine was initiated more than 2 decades ago, with classical phase 1 studies that defined 1500 to 2250 mg/m<sup>2</sup> per course as the maximum tolerated dose (MTD), and

demonstrated a short half-life for the drug.<sup>6</sup> Dose-limiting toxicity was primarily hematologic, and phase 2 studies were largely disappointing in solid tumors, but encouraging in acute myelogenous leukemia (AML), myelodysplasia (MDS), and chronic myelogenous leukemia (CML). The decitabine regimens tested in these studies involved high doses and rarely gave the drug for more than 1 to 3 days per course. A lower dose schedule (15 mg/m<sup>2</sup> 3 times a day for 3 days) was reported to have encouraging activity in MDS.<sup>12</sup> An even lower dose (0.15 mg/kg daily for 10 days) was recently reported to have biologic efficacy in reactivating hemoglobin F in patients with sickle cell disease, with relatively little toxicity.<sup>13,14</sup> These observations, combined with the short half-life of the drug and its absolute requirement for DNA synthesis for activity, led us to conduct a phase 1 trial of decitabine, testing multiple low-dose longer exposure schedules, with the intent of finding an "optimal dose" for responses rather than a maximally tolerated dose. We found that, as predicted by *in vitro* studies, a low dose of decitabine was clinically optimal in producing responses in hematologic malignancies.

### Patients and methods

#### Study group

Patients entered in the study were required to have a diagnosis of AML, acute lymphocytic leukemia (ALL), MDS or myeloproliferative disease, or

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CML. All patients with CML or ALL had to have failed at least one prior regimen. Patients with AML and MDS were eligible if they had relapsed after induction chemotherapy, or if such therapy was felt to offer little potential benefit. Other eligibility criteria were adequate performance status (Eastern Cooperative Oncology Group 2 or better), and adequate cardiac (New York Heart Association Classes II-IV excluded) and hepatorenal functions (creatinine level, < 176.8  $\mu$ M; bilirubin level, < 34.2  $\mu$ M; and hepatic enzymes less than twice the upper limit of normal). All patients gave written informed consent indicating that they were aware of the investigational nature of the study, in keeping with the policies of the M. D. Anderson Cancer Center. Consent was also obtained for the (optional) correlative studies, which included the collection of additional blood samples.

## Treatment

The initial plan was to treat patient cohorts (6-8 patients) with decitabine at 5, 10, or 20 mg/m<sup>2</sup> daily intravenously over one hour for 10 days (5 days on, 2 days off, 5 days on), approximately every 6 weeks as indicated by follow-up counts and marrow studies. After the first 2 cohorts were accrued and clinical activity was confirmed, an intermediate dose of 15 mg/m<sup>2</sup> was added. After this first part of the study was concluded, we treated 2 cohorts of patients (3 each) at 15 mg/m<sup>2</sup> for 15 days and 20 days. Finally, a cohort of 11 patients was treated at 15 mg/m<sup>2</sup> daily for 10 days to confirm the drug activity at what was considered a reasonable dose schedule to pursue in phase 2 studies. No patient was dose-adjusted. If grade 3 or more nonhematologic toxicity was felt attributable to the drug, the patients were taken off the study. Patients experiencing grade 3 or worse hematologic toxicity were evaluated for persistent disease. If myelosuppression was attributed to disease, the treatment was continued at the same dose level unless clear evidence of progression was seen (such as rising blast count). If myelosuppression was attributed to the drug, treatment was discontinued. In general, patients were treated every 6 weeks, and additional courses were administered unless clear evidence of disease progression was observed.

## Response criteria and statistical considerations

Response criteria were as previously described.<sup>15,16</sup> A complete response (CR) required disappearance of all signs and symptoms related to disease, normalization of peripheral counts (absolute neutrophil count  $10^9$ /L or more, platelet count  $100 \times 10^9$ /L or more), and a normal bone marrow morphology with no dysplasia greater than grade I and 5% or less marrow blasts. A hematologic improvement (HI) was defined as for CR except for a platelet count increase by 50% to more than  $30 \times 10^9$ /L (normalized) but less than  $100 \times 10^9$ /L. In CML, a complete hematologic response (CHR) was similar to the definition of CR for acute leukemia, and was also categorized by the degree of suppression of Philadelphia chromosome (Ph)-positive cells as a complete cytogenetic response (Ph, 0%), partial cytogenetic response (Ph, 1%-34%), or minor cytogenetic response (Ph, 35%-90%). A partial hematologic response (PHR) was defined as a CHR but with persistence of peripheral immature cells (< 5% myelocytes + metamyelocytes), or persistent splenomegaly or thrombocytosis that, however, was reduced by 50% or more from pretreatment levels. A return to a second chronic phase referred to the disappearance of accelerated blastic-phase criteria. Complete responses had to have minimum response duration of 4 weeks. No such minimal duration was required for HI or PR. Remission was calculated from date of first response until relapse. Survival was calculated from start of therapy until death from any cause.

## Analysis of P15 DNA methylation

DNA was extracted from blood or bone marrow aspiration specimens using standard phenol-chloroform methods. After extraction, DNA was modified with sodium bisulfite. This induces deamination of unmethylated cytosines, converting unmethylated cytosine-phosphate-guanosine (CpG) sites to uridine-phosphate-guanosine (UpG) without modifying methylated sites. Bisulfite treatment of genomic DNA was performed as described,<sup>17</sup> and 2  $\mu$ g DNA was used. DNA was denatured in 0.2 N NaOH at 37°C for 10 minutes and incubated with 3 M Na-bisulfite at 50°C for 16 hours. DNA was then purified using the Wizard cleanup system (Promega, Madison,

WI) and desulfonated with 0.3 N NaOH at 25°C for 5 minutes. DNA was then precipitated with ammonium acetate and ethanol, washed with 70% ethanol, dried, and resuspended in H<sub>2</sub>O.

P15 methylation was analyzed using the combined bisulfite restriction analysis (COBRA) as previously reported.<sup>18,19</sup> This method allows the quantification of the ratio of methylated (restricted) versus unmethylated (unrestricted) polymerase chain reaction (PCR) product, or methylation density. PCR reactions were carried in 50- $\mu$ L reactions. In each reaction, 2  $\mu$ L bisulfite-treated DNA was used, as well as 1.25 mM deoxynucleoside triphosphate, 6.7 mM MgCl<sub>2</sub>, 5  $\mu$ L PCR buffer, 1 nmol primers, and 1 unit of Taq polymerase. P15 primer sequences were as follows: 5'GGAGTTA-AGGGGGTGGG; 3'CCTAAATTACTTCTAAAAA AAC. PCR fragments were digested with *Bst*UI and were separated in nondenaturing polyacrylamide gels that were stained with ethidium bromide. The proportion of methylated versus unmethylated product (digested vs undigested) was quantitated by densitometric analysis, determining the methylation density. Densitometric analysis was performed using a BioRad Geldoc 2000 digital analyzer equipped with the Quantity One version 4.0.3 software (BioRad, Hercules, CA).

## Results

### Study group

A total of 48 patients were entered in the study. There were 2 patients treated twice, at 2 different dose levels, having had a stem cell transplantation (and relapsed) in between the 2 registrations. The characteristics of these 48 patients are summarized in Table 1. Because responses were observed at the lowest dose tested, we used this parameter as an end point of the study and describe these first.

**Table 1. Characteristics of the study group**

Characteristic	No.
Age, y, median (range)	60 (2-84)
Male sex (%)	27 (56)
<b>Diagnosis (%)</b>	
AML	35 (73)
Untreated	5
Refractory or relapsed	30
First CR	
Less than 12 mo	28
More than 12 mo	2
Salvage number, 30 patients	
First	13
Second or more	17
MDS (%)	7 (14)
IPSS intermediate 1	2
IPSS intermediate 2	1
IPSS high	4
Untreated	2
Refractory or relapsed*	5
ALL (%)	1 (2)
CML (%)	5 (10)
Chronic	1
Accelerated	1
Blastic	3
<b>Karyotype (%)†</b>	
Good, Inv16; t(8;21)	2 (5)
Intermediate, normal	12 (29)
Poor, chromosome 11q23, 5 or 7 anomalies, others	28 (67)
No. of prior regimens, AML or MDS, median (range)	2 (0-11)

\*All first CR more than 12 months.

†Among 42 patients with AML or MDS.

**Table 2. Response to decitabine by dose level**

Decitabine, mg/m <sup>2</sup> × days	Dose, mg/m <sup>2</sup> per course	No. patients	CR	PR	HI	PHR or second CP*	Any response (%)
5 × 10	50	7	1	0	0	1	2/7 (29)
10 × 10	100	7	1	0	0	0	1/7 (14)
15 × 10	150	6	4	1	0	0	5/6 (83)
20 × 10	200	8	0	0	1	0	1/8 (13)
15 × 15	225	8	1	0	0	0	1/8 (13)
15 × 20	300	3	0	0	0	0	0/3 (0)
15 × 10	150	11	2	0	3	1	6/11 (55)
Overall (%)		50	9 (18)	1 (2)	4 (8)	2 (4)	16/50 (32)

CR indicates complete response; PR, partial response; HI, hematologic improvement; PHR, partial hematologic response; and CP, chronic phase.

\*CML patients only.

### Response to treatment

Of the patients, 5 did not complete the first cycle of therapy because of early death ( $n = 3$ ) or rapidly rising white blood cell counts ( $n = 2$ ). Responses by dose level and overall are shown in Table 2. Responses were observed at each dose level. The highest number of responses was observed at 15 mg/m<sup>2</sup> daily for 10 days (11 of 17 or 65%). Compared with the first 3 doses tested in which responses were seen in 14 (45%) of 31 patients, there were significantly fewer responses (2 of 19; 11%;  $P = .01$  by Fisher exact test) in patients treated at a higher dose (20 mg/m<sup>2</sup> for 10 days) or for longer periods of time (15 mg/m<sup>2</sup> for 15 or 20 days). This difference between the lower and higher doses was preserved when we considered only patients who achieved a complete remission (8/31 at the lower dose vs 1/19 at the higher dose,  $P = .06$  by Fisher exact test). The difference between the lower and higher doses was also preserved when limiting the analysis to the 44 patients with AML or MDS, where responses were seen in 10 of 25 patients at the lower doses versus 2 of 19 patients at the higher doses ( $P < .04$ ). Overall, objective responses were noted in 16 patients (32%). In 37 patients with AML, 5 (14%) achieved CR and 3 (8%) achieved PR. In 7 patients with MDS, 2 (29%) achieved CR and 2 (29%) achieved PR. In 5 patients with CML, 2 (40%) achieved CR and 2 (40%) achieved PR. As noted, however, these response rates are across different doses, and may thus be an underestimate.

Some responses observed were gradual. Among 14 patients who had more than 5% blasts in their bone marrow prior to therapy and subsequently achieved a clinical response, the median bone marrow blasts was 19% before treatment, 7% at day 14, and 2% at

days 21 to 28. In 3 responders with peripheral blasts more than 30% (54%, 51%, and 33%, respectively), these were reduced to 23%, 2% and 4%, respectively, by day 14, and to 9%, 7%, and 0%, respectively, by days 21 to 28. The median time to response was 45 days (range, 16-70 days). Bone marrow aspirates were done on 26 patients at day 14, 12 patients at day 21, and 16 patients at day 28. These revealed marked hypoplasia ("empty bone marrow") in 3 of 21, 1 of 12, and 2 of 16, respectively, suggesting that cytotoxicity is not a primary mechanism action of decitabine at these doses. However, patients did not have serial bone marrow biopsies done to more precisely evaluate cellularity on and after treatment. Of the 9 CRs, 8 occurred after one cycle of decitabine therapy; one patient required one additional cycle to achieve a CR. The median remission duration was 8 weeks (range, 4 to 59+ weeks). At last follow-up, 4 patients were alive, 3 of whom were disease-free. These 3 patients included 1 with AML who relapsed after allogeneic stem cell transplantation, achieved a CR following a course of decitabine, subsequently developed graft-versus-host disease, and remains in an unmaintained remission. A second patient with AML underwent transplantation after achieving a response to decitabine and remains disease-free. The third patient had CML accelerated phase, responded to decitabine, and was switched to imatinib mesylate when that drug became available.

Table 3 details the clinical characteristics of the responding patients. There were 10 patients (4 responders, 6 resistant) able to undergo allogeneic stem cell transplantation following decitabine therapy: 3 of the 4 responders relapsed 1.5, 3, and 5 months after transplantation; 1 remains in CR for 22+ months.

**Table 3. Characteristics of the responders following decitabine therapy**

Patient no.	Age, y/sex	Diagnosis	Salvage no.	BM blast %		Karyotype	Dose, mg/m <sup>2</sup> × days	Response	Response duration, wk	Survival, wk
				Before	After					
6	65/M	AML	1	41	0	-5/-7	5 × 10	CR	7	18
11	31/M	AML	2	16	3	t(3;3)	10 × 10	CR	21	62
20	55/F	AML	1	11	0	Diploid	15 × 10	HI	4	29
41	77/M	AML	1	35	2	Diploid	15 × 10	HI	4	43
44	78/M	AML	2	8	2	Diploid	15 × 10	HI	2	44
16	65/M	AML	1	60	3	-7	15 × 10	CR	17	35
19	48/F	AML	3	72	4	Diploid	15 × 10	CR	7	47
50	27/F	AML	4	5	0	Diploid	15 × 10	CR	59+	60+
48	21/F	MDS	0	13	1	Diploid	15 × 10	HI	13	16
17	72/M	MDS	1	9	1	-5/-7	15 × 10	CR	29	50
33	83/M	MDS	0	20	2	5q-, +8	15 × 15	CR	48	61+
22	64/M	MDS	1	10	1	+8 (100)	20 × 10	HI	1	44
7	43/M	CML	1	18	2	Ph (100)	5 × 10	PHR	8	111+
18	69/M	CML	1	1	0	Ph	15 × 10	CHR	9	19
43	45/F	CML	2	30	6	Ph+ other	15 × 10	2nd CP	1	20
46	55/M	CML	3	27	3	Ph+ other	15 × 10	CHR	8	28

### Side effects

The treatment was well tolerated overall. Nonhematopoietic side effects are detailed in Table 4. Asymptomatic but severe elevations in liver function tests, possibly related to therapy, were observed in 6 patients. In 5 cases, values returned to baseline within 2 weeks; the sixth patient died on day 21 with a bilirubin level of 290.7  $\mu$ M and evidence of sepsis. Febrile episodes were noted in 26 patients (52%). These included fever of unknown origin in 8 patients (16%) and documented infections in 18 patients (36%): bacterial in 6, fungal in 1, others in 3, minor infections in 1, pneumonia in 12.

We attempted to evaluate myelosuppression by studying time to CR and myelosuppression in patients receiving additional courses in remission. In patients achieving CR, the median time to platelet recovery more than  $100 \times 10^9/L$  was 39 days (range, 31-70 days), and the median time to granulocyte recovery more than  $10^9/L$  was 45 days (range, 33-70 days). A total of 10 courses of therapy administered to 6 patients in remission were analyzable for myelosuppression. In these, the median platelet nadir was  $45 \times 10^9/L$  (range, 20-200  $\times 10^9/L$ ), and, in the 6 courses with thrombocytopenia less than  $100 \times 10^9/L$ , median time to platelet recovery more than  $100 \times 10^9/L$  was 32 days (range, 24-58 days). The median nadir granulocyte count was  $0.32 \times 10^9/L$  (range, 0-3.0  $\times 10^9/L$ ), and, in the 7 courses with granulocytopenia less than  $10^9/L$ , median time to granulocyte recovery more than  $10^9/L$  was 36 days (range, 32-45 days). Of these 10 courses, 8 were administered at the dose of 15 mg/m<sup>2</sup> for 10 days, precluding meaningful analysis of myelosuppression at lower versus higher doses.

In this study, definition of a maximally tolerated dose was not a primary end point; dose escalation was stopped when it appeared to result in fewer responses. Thus, no formal maximally tolerated dose was reached on this regimen (although myelosuppression could not be properly evaluated because of the population studied).

### P15 methylation and response to decitabine

P15 methylation was analyzed by the quantitative COBRA technique in 29 patients who consented to sample collection for these studies. Samples consisted of cell suspensions obtained from peripheral blood immediately prior to therapy and at days 5 and 12 of decitabine administration. It has previously been shown that aberrant methylation can be detected in the peripheral blood of patients with AML and MDS. As shown in Table 5, P15 methylation averaged 11.2% in this group of patients, and 15 patients (52%) had levels of P15 methylation consistent with silencing (ie, > 10%). This group of 29 patients was representative of the entire population tested, and responses were seen in 7 patients (24%; 4 CR, 3 PR). At baseline, P15 methylation was similar in the group of patients who eventually responded to the drug, compared with nonresponders (mean of 5.2% vs 13.9%, 2-sided  $P = .12$  using a  $t$  test analysis). Demethylation on therapy was evaluated by also analyzing cell suspensions obtained from peripheral blood. Most patients had little change in peripheral blood counts at days 5 and

**Table 4. Nonhematologic side effects with decitabine (50 cases)**

Side effect	Grade II	Grades III/IV
Nausea, vomiting	2	1/–
Diarrhea	1	–/–
Skin rashes	1	–/–
<b>Liver dysfunction</b>		
Elevated enzymes	3	4/–
Increased bilirubin level	7	3/1
Creatinine elevation	5	0

**Table 5. P15 methylation before and after decitabine therapy**

	All patients, N = 29	CR + PR, n = 7	NR, n = 18	Inevaluable, n = 4
<b>Before Rx</b>				
Mean, %	11.2	5.2	13.9	9
Median, %	10.6	0	13	5.5
More than 10%, n (%)	15 (52)	2 (29)	11 (56)	2 (50)
<b>After Rx*</b>				
Mean, %	11.5	6.7	14.3	6.2
Median, %	3	1.5	10.3	0
More than 10%, n (%)	11/25 (44)	2/6 (33)	8/16 (50)	1/3 (33)

CR + PR indicates complete and partial remissions; NR, no response; inevaluable, inevaluable for a response; and Rx, decitabine therapy.

\*Excludes 4 patients for whom post-Rx samples were not available.

12, corresponding to the time of the methylation studies, suggesting that comparisons of methylation were not influenced by large changes in cell populations. In the 25 patients for whom on-treatment samples were available, on average, there was no significant decline in methylation at the day-5 and day-12 time points after treatment with decitabine, regardless of response (Table 5 and data not shown). There were 13 patients who started with P15 methylation less than 10%, and no case gained methylation to more than 10%. There were 12 patients who started with P15 methylation more than 10%. Of these, 1 case showed a significant increase (doubling or more) in P15 methylation after decitabine, while 3 cases showed significant decreases (halving or more) in their baseline methylation status. None of these cases responded to therapy. Of the 6 patients who achieved a clinical response and had baseline and on-treatment samples available, only 2 were methylated (1 had a CR, 1 had a PR), and these had not changed methylation at days 5 or 12 after therapy.

### Discussion

In this study, a low-dose prolonged exposure schedule of decitabine had significant activity in patients with refractory hematologic malignancies. The dose schedule suggested for future studies was 15 mg/m<sup>2</sup> daily for 10 days, as higher doses did not appear to be associated with an increased response rate. Although we treated a heterogeneous group of patients, 42 of the 48 patients had AML or advanced MDS, and all diagnoses were represented at the various doses. Moreover, the overall response rate of 25% in AML salvage was encouraging compared with an earlier study using higher doses of decitabine (75 mg/m<sup>2</sup> intravenously over 6 hours every 12 hours  $\times$  5 days = 500 mg/m<sup>2</sup> per course) and that showed only one CR among 17 patients treated (6%) (Table 6, based on H.M.K. et al, unpublished data, 1995). Mortality using the current regimen was also lower. Results with low-dose decitabine were better despite the worse characteristics of the study group (eg, number of prior salvage regimens and duration of first CR; Table 6). It is remarkable that increasing the dose of the drug appeared to actually reduce the number of clinical responses seen. Recognizing that this was not a controlled study, this observation is nevertheless consistent with the data on a dual mechanism of action for decitabine at low versus high doses,<sup>6,7,11</sup> and suggests that favoring the former mechanism may have optimized the use of this agent. One can speculate that, at higher doses, the drug may have effects that could suppress clinical responses, such as abrogation of an immune-mediated effect.<sup>20,21</sup>

The responses seen in this study tended to occur gradually and slowly, as observed in previous studies of decitabine.<sup>12,22</sup> For

**Table 6. Comparison of 2 study groups of patients with relapsed or refractory AML treated on low-dose versus higher-dose decitabine schedules**

Parameter	Current study	Higher-dose decitabine
Decitabine, mg/m <sup>2</sup> /course	50-300	750
No. patients treated (%)	32*	17
First salvage		
CRD1, less than 12 mo	11 (34)	9 (53)
CRD1, more than 12 mo	2 (6)	3 (18)
Second or more salvage	19 (59)	5 (29)
No. responses, CR + other (%)	5 + 3 (25)	1 (6)
No. induction deaths (%)	2 (6)	3 (18)
Median age, y	56	66
Median WBC count	2.8	4.9
Favorable or intermediate cytogenetics (%)	13/32 (41)	7/15 (47)

WBC indicates white blood cell.

\*There were 2 patients registered and treated twice on the study at 2 different dose levels, having had a stem cell transplantation (and relapsed) in between the 2 registrations.

several patients, blast counts continued to decrease weeks after cessation of therapy. While the mechanism of this phenomenon is difficult to ascertain, it is clearly different from responses observed following cytarabine treatment and favors a differentiation component of the responses observed following decitabine therapy. This is consistent with the proposed mechanism of action of low-dose decitabine, which affects silencing by aberrant methylation and ultimately “normalizes” the gene expression profile of malignant cells.<sup>3,11,23</sup> Multiple mechanisms may be involved in achievement of complete remissions, including apoptosis or senescence following normalization of gene expression, immune-mediated effects related to differentiation of leukemic cells into dendritic cells or changes in the cell-surface markers of malignant cells, or direct cytotoxic effects on leukemic cells.

Whether responses to decitabine are dependent on DNA demethylation is difficult to answer with the present data. While decitabine treatment in vitro results in gene-specific demethylation, many genes are induced that have no promoter methylation, including *P21*,<sup>24</sup> and this may be related to DNA methylation-independent changes in histone modifications.<sup>25</sup> The mechanism of this induction is likely indirect, but may be as important for response to decitabine as is DNA demethylation. Here, we studied *P15*, a gene specifically hypermethylated and silenced in hematologic malignancies,<sup>6</sup> and reported to be demethylated by decitabine in MDS.<sup>26</sup> We found no correlation between the presence of this anomaly and response to therapy. Moreover, while we demon-

strated substantial demethylation (by 50% or more) in 3 of 12 patients with high levels of *P15* methylation at baseline, demethylation of this gene after therapy was not associated with clinical response to decitabine. There are technical limitations to our approach. First, COBRA is not very sensitive, and measures only methylation of 1 or 2 CpG sites. The use of more sensitive methods,<sup>27</sup> particularly if coupled with quantitation,<sup>28</sup> may yield a different answer. Second, we used DNA extracted from peripheral blood here, and we did not attempt to fractionate cells and measure demethylation specifically in blasts. Aberrant methylation has been documented in the peripheral blood of patients with AML<sup>29</sup> and MDS,<sup>30</sup> but the choice of cells could clearly influence the results. Finally, it is of course possible that methylation/demethylation of genes other than *P15* is critical to response; thus large methylation profiling studies in patients treated with decitabine would be informative.

The low-dose prolonged exposure decitabine schedule was well tolerated and delivered on an outpatient basis. Of interest, in the current study, 4 of 5 patients with CML responded to low-dose decitabine, including a partial cytogenetic response in 1 patient, and 4 of 7 patients with MDS showed objective responses. This highlights several possible attractive investigational directions of decitabine, alone or in combination with imatinib mesylate in CML, and with cytarabine, topoisomerase I inhibitors, or thalidomide in MDS. This regimen can also be tested in phase 2 studies of other hematologic cancers (lymphoma, myeloma) or of solid tumors, where early decitabine studies were marred by toxicity related to the high doses used.<sup>6</sup> Many solid tumors, such as colon and lung cancer, have high degrees of aberrant methylation,<sup>31,32</sup> and, theoretically, should be attractive candidates for testing such therapy, particularly in stages of minimal residual disease (eg, resected tumor with high propensity for relapse). The favorable safety profile of this regimen also allows the design of rational decitabine combinations such as with histone deacetylase inhibitors, or with biologic response modifiers such as interferon alpha and all-*trans* retinoic acid.<sup>8</sup> The availability of subcutaneous and possibly oral formulations of decitabine will also produce interesting therapeutic strategies for sickle cell disease, where decitabine has shown activity, and in some of the indolent hematologic disorders such as MDS and CML.

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## References

- Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JPJ. Alterations in DNA methylation—a fundamental aspect of neoplasia. *Adv Cancer Res*. 1998;72:141-196.
- Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet*. 1999;21:163-167.
- Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science*. 2001;293:1068-1070.
- Issa JPJ, Baylin SB, Herman JG. DNA methylation changes in hematologic malignancies: biologic and clinical implications. *Leukemia*. 1997;11 (suppl 1):S7-S11.
- Melki JR, Clark SJ. DNA methylation changes in leukaemia. *Semin Cancer Biol*. 2002;12:347-357.
- Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. *Ann Intern Med*. 2001;134:573-586.
- Christman JK. 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*. 2002;21:5483-5495.
- Karpf AR, Jones DA. Reactivating the expression of methylation silenced genes in human cancer. *Oncogene*. 2002;21:5496-5503.
- Li Q, Ahuja N, Burger PC, Issa JPJ. Methylation and silencing of the *Thrombospondin-1* promoter in human cancer. *Oncogene*. 1999;18:3284-3289.
- Toyota M, Issa JP. Methylated CpG island amplification for methylation analysis and cloning differentially methylated sequences. *Methods Mol Biol*. 2002;200:101-110.
- Jones PA, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell*. 1980;20:85-93.
- Wijermans P, Lubbert M, Verhoef G et al. Low-dose 5-aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. *J Clin Oncol*. 2000;18:956-962.
- DeSimone J, Koshy M, Dorn L, et al. Maintenance of elevated fetal hemoglobin levels by decitabine during dose interval treatment of sickle cell anemia. *Blood*. 2002;99:3905-3908.
- Koshy M, Dorn L, Bressler L, et al. 2-Deoxy 5-azacytidine and fetal hemoglobin induction in sickle cell anemia. *Blood*. 2000;96:2379-2384.
- Kantarjian HM, Beran M, Ellis A, et al. Phase I study of Topotecan, a new topoisomerase I inhibitor,

- in patients with refractory or relapsed acute leukemia. *Blood*. 1993;81:1146-1151.
16. Kantarjian HM, Cortes J, O'Brien S, et al. Imatinib mesylate (STI571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase. *Blood*. 2002;99:3547-3553.
  17. Clark SJ, Harrison J, Paul CL, Frommer M. High sensitivity mapping of methylated cytosines. *Nucleic Acids Res*. 1994;22:2990-2997.
  18. Toyota M, Kopecky KJ, Toyota MO, et al. Methylation profiling in acute myeloid leukemia. *Blood*. 2001;97:2823-2829.
  19. Garcia-Manero G, Daniel J, Smith TL, et al. DNA methylation of multiple promoter-associated CpG islands in adult acute lymphocytic leukemia. *Clin Cancer Res*. 2002;8:2217-2224.
  20. Qudus J, Johnson KJ, Gavalchin J, et al. Treating activated CD4+ T cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. *J Clin Invest*. 1993;92:38-53.
  21. Zaharko DS, Covey JM, Muneses CC. Experimental chemotherapy (L1210) with 5-aza-2'-deoxycytidine in combination with pyran copolymer (MVE-4), an immune adjuvant. *J Natl Cancer Inst*. 1985;74:1319-1324.
  22. Kantarjian HM, O'Brien SM, Keating M, et al. Results of decitabine therapy in the accelerated and blastic phases of chronic myelogenous leukemia. *Leukemia*. 1997;11:1617-1620.
  23. Bender CM, Pao MM, Jones PA. Inhibition of DNA methylation by 5-aza-2'-deoxycytidine suppresses the growth of human tumor cell lines. *Cancer Res*. 1998;58:95-101.
  24. Milutinovic S, Knox JD, Szyf M. DNA methyltransferase inhibition induces the transcription of the tumor suppressor p21(WAF1/CIP1/sdi1). *J Biol Chem*. 2000;275:6353-6359.
  25. Kondo Y, Shen L, Issa JP. Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. *Mol Cell Biol*. 2003;23:206-215.
  26. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. *Blood*. 2002;100:2957-2964.
  27. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A*. 1996;93:9821-9826.
  28. Eads CA, Danenberg KD, Kawakami K, et al. MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res*. 2000;28:E32.
  29. Li Q, Kopecky KJ, Mohan A, et al. Estrogen receptor methylation is associated with improved survival in adult acute myeloid leukemia. *Clin Cancer Res*. 1999;5:1077-1084.
  30. Aoki E, Uchida T, Ohashi H, et al. Methylation status of the p15INK4B gene in hematopoietic progenitors and peripheral blood cells in myelodysplastic syndromes. *Leukemia*. 2000;14:586-593.
  31. Toyota M, Ahuja N, Ohe-Toyota M, et al. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A*. 1999;96:8681-8686.
  32. Zochbauer-Muller S, Gazdar AF, Minna JD. Molecular pathogenesis of lung cancer. *Annu Rev Physiol*. 2002;64:681-708.



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