Randomized Open-Label Phase II Study of Decitabine in Patients With Low- or Intermediate-Risk Myelodysplastic Syndromes

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SUPPLEMENTARY MATERIAL

METHODS: SAMPLE SIZE AND STATISTICAL POWER CONSIDERATIONS

A maximum of 80 patients could be randomized and treated on this study. Approximately 5000 simulations per scenario were used to evaluate the performance of the adaptive randomization procedure of the Bayesian design under several different scenarios, as shown in Table S1. There was 80% power to select a superior schedule if the overall improvement rates were 10% and 30%, respectively. There was approximately 41% power to select a superior schedule if the overall improvement rates were 20% and 30%, respectively. When both treatment arms had a 10% overall improvement rate, the probability (Pr) for each one to be selected was about 19%, which is equivalent to the Type I error of a frequentist design.

Table S1: Operating Characteristics of Adaptive Randomization

	Schedule A	Schedule B
OIR	0.10	0.10
Expected no. of patients	34	35
Pr (select)	0.094	0.095
Pr (select early)	0.094	0.095
Pr (stop early)	0.21	0.21
OIR	0.05	0.20
Expected no. of patients	26	51
Pr (select)	0.002	0.69
Pr (select early)	0.002	0.68
Pr (stop early)	0.79	0.02
OIR	0.1	0.2
Expected no. of patients	30	47
Pr (select)	0.01	0.47

Pr (select early)	0.01	0.46
Pr (stop early)	0.50	0.03
OIR	0.20	0.20
Expected no. of patients	37	37
Pr (select)	0.12	0.14
Pr (select early)	0.11	0.14
Pr (stop early)	0.14	0.12
OIR	0.20	0.30
Expected no. of patients	32	47
Pr (select)	0.02	0.42
Pr (select early)	0.02	0.41
Pr (stop early)	0.42	0.03
OIR	0.10	0.30
Expected no. of patients	25	54
Pr (select)	0.0008	0.80
Pr (select early)	0.0008	0.80
Pr (stop early)	0.81	0.002

OIR, overall improvement rate; Pr, probability.

Based on previous studies, an overall improvement rate (OIR) of about 20% was expected in both arms. Therefore, it was assumed that OIR had a prior beta distribution (0.4, 1.6) with mean 0.20. OIRa and OIRb denoted the overall improvement rates for Schedule A and Schedule B, respectively. Beginning with the 21st patient in each arm and for each subsequent patient, OIRa was compared with OIRb, incorporating available data from all patients with evaluable responses. To avoid favoring one arm earlier in a large study, rather than assigning patients with posterior probability (Pa=Pr[OIRa>OIRb|data] and Pb=1-Pa), the following formula was used to assign patients:

$$Aa= \frac{\sqrt{Pa}}{(\sqrt{Pa}+\sqrt{Pb})}, \quad Ab=1-Aa,$$

wherein Aa denotes the probability of assigning patients to Schedule A, Ab denotes the probability of assigning patients to Schedule B, Pa is the posterior probability that

Schedule A is superior to Schedule B, and Pb is the posterior probability that Schedule B is superior to Schedule A.

If at any point during the study Pr(OIRa>OIRb|data)>0.95 (or <0.05), then Schedule A (or B) was selected as superior. If accruing information gave strong evidence that an OIR of 10% or greater was unlikely to be true for any treatment arm (Pr[OIRa >0.1|data]<0.05, or, Pr[OIRb >0.1|data]<0.05), assignment to that arm was stopped. If the maximum of 80 patients were enrolled and evaluated (the last patient had been on treatment for 3 cycles) and Pr(OIRa>OIRb|data)>0.95 (or <0.05), it was declared that Schedule A (or B) had a higher OR rate than schedule B (or A). Otherwise, the study would be considered inconclusive.

At each subsequent decision point for the calculation of posterior probability of Pa or Pb, patients with confirmed objective responses (complete response [CR], marrow CR [mCR], partial response [PR], or hematologic improvement [HI]) were considered successes and patients with progressive disease [PD] were considered failures. For patients who had only stable disease (SD), Markov Chain Monte Carlo draws using standard techniques (M=100,000) were adopted for the estimation.

METHODS: ***DNA Methylation and miR-29b Analysis***

DNA methylation was performed as previously described.[1] DNA was extracted using standard phenol-chloroform methods and was modified with sodium bisulfite. Both global and gene-specific assays of DNA methylation were performed. For global DNA methylation, the long interspersed nuclear element (LINE) assay using bisulfite pyrosequencing was used.[1] Gene-specific analyses were conducted on *p15, CSDA, ZNF582, ER,* and *PGRB* using pyrosequencing techniques (Table S2).[2-4]

For miR29b analysis, total cellular ribonucleic acid (RNA) was extracted with Trizol (Invitrogen; Carlsbad, CA). TaqMan MicroRNA reverse transcription kit (Applied Biosystems; Austin, TX) was used for reverse transcription reactions. For real-time PCR analysis, miR-29b TaqMan MicroRNA assays were purchased from Applied Biosystems and analyzed with TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA) with an Applied Biosystems Prism 7500 sequencing detection system. The U6 small nuclear RNA was used as internal control.[1]

TABLE S2. Primer Sequences Used for Pyrosequencing

Gene	Forward Primer	Reverse Primer	Biotin Primer	Sequencing Primer
LINE	5-TTTTGAGTT AGGTGTGG GATATA-3	5-AAAATCAA AAAATTCCC TTTC-3	5- AAAATCAA AAAATTCCC TTTC-3	5-AGTTAGGTGT GGGATATAGT-3
CSDA	5- GGAGTAGGG TTTTGGATTT TTATTAGT-3	5-GGGACACCGC TGATCGTTTA TTCACCCTAACTTT CCTTAATTCTACAA-3	5-GGGACACCGC TGATCGTTTA-3	5-GGATTTTTATTA GTATTTAAAGG-3
P15	5-GTTTTTTTTT AGAAGTAA TTTAGG-3	5-TCCTTCTA CRACTTAA AACC -3	5-TCCTTCTA CRACTTAA AACC-3	5- TTTTTAGAA GTAATTTAGG-3
ER	5-GGGAGGAA TAGGAGTAG GAGATTTTA-3	5-GGGACACCGC TGATCGTTTA CCCCCTCAAC AAAAAACAACTAAAA-3	5-GGGACACCGC TGATCGTTTA-3	5-TTGGGTT TAGGTTA-3
ZNF582	5-GGGTATTAGTT TTTGTTGGAT TTTAGAG-3	5- GGGACACCGC TGATCGTTTA TAAAAACCCCC CCTAAACTACC-3	5-GGGACACCGC TGATCGTTTA-3	5-TTTTTTAGGGTTT AAGTGTTAT-3
PGBR (PCR1)	5- TGTGGGTGGTAT TTTTAATGAGA-3	5-CCCCCTCACTAA AACCCTAAA-3		
PGBR (PCR2)	5-GAGAATTAG TTTTATTTG TTATTT-3	5- GGGACACCGC TGATCGTTTA CAACCCATTCCC AAAAAAAATC-3	5- GGGACACCGC TGATCGTTTA-3	5- GGGATTT GAGATTTT-3

NOTE: R indicates G or A.

RESULTS: *DNA Methylation*

As an exploratory analysis, global and gene-specific promoter DNA methylation were analyzed in 17 patients (Schedule A, n=9; Schedule B, n=8) using available samples. Methylation was analyzed on study days 0, 8, 15, and 22 of the first cycle of therapy. When LINE methylation data from patients on both schedules were pooled, no global hypomethylation effect was observed (Figure S1). Comparing LINE methylation by schedule, there was a trend toward induction of LINE hypomethylation on day 8 compared with baseline in patients on Schedule A but not in those on Schedule B (Figure S2). However, the small sample size does not allow any conclusions to be drawn. No correlation with clinical outcome was feasible.

Analyses were also conducted on levels of promoter methylation of 5 genes (*p15, CSDA, ZNF582, ER, PGRB*) reported to be methylated in MDS.[5,6] Only a decrease in promoter methylation of *PGRB* was observed (n=14; Figure S3).

Because miR29b has been associated with response to decitabine treatment,[7] miR29b levels were measured at baseline. Of 15 patients with available data, only 2 (13%) achieved a response. One patient had a high level of miR29b, and the other had an undetectable level. In general, baseline levels of miR29b were low in most patients (Figure S4).

FIGURE S1. Mean ± standard deviation of *LINE* methylation measured in 17 patients sequentially during the first cycle of decitabine therapy.

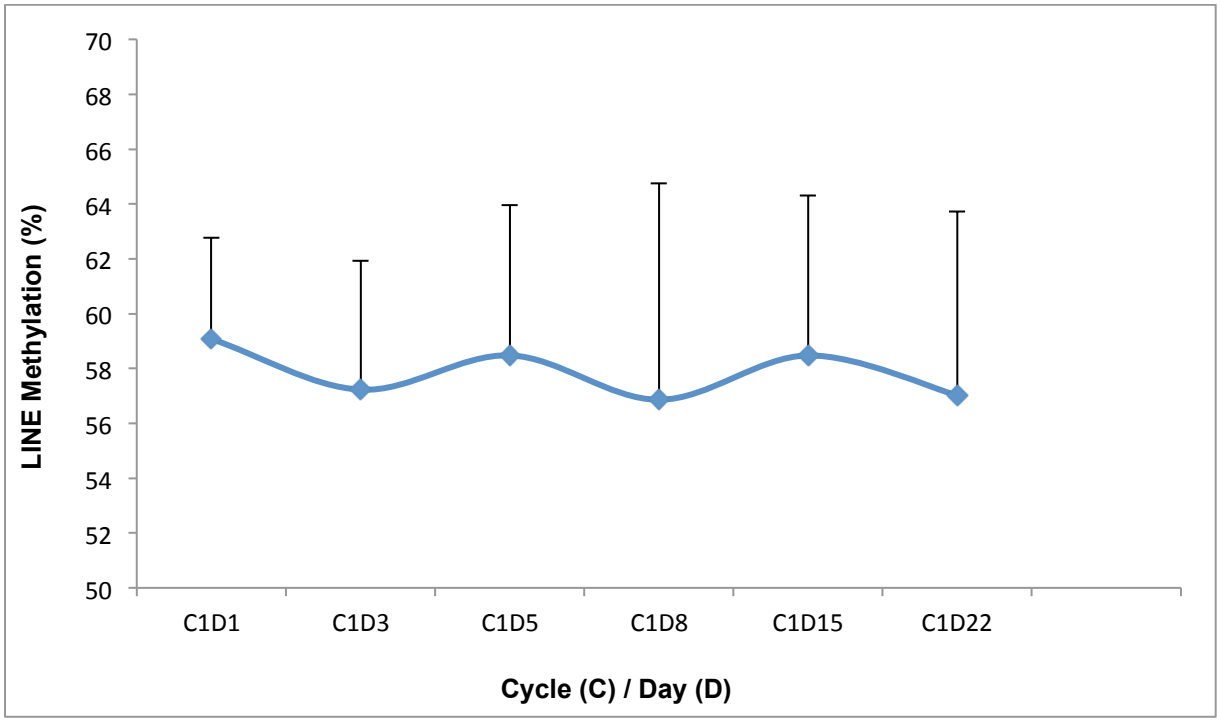


Figure S2. Sequential analysis DNA methylation during cycle 1 of therapy using LINE methylation (mean ± standard deviation). Schedule A: n=9; Schedule B: n=8.

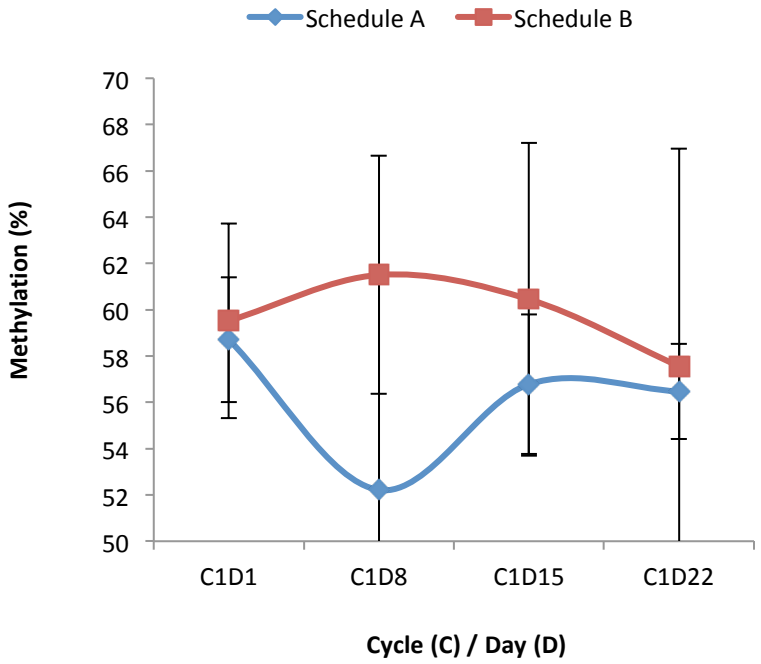


FIGURE S3. Mean ± standard deviation of promoter methylation of *PGRB* measured in 14 patients sequentially during the first cycle of decitabine therapy.

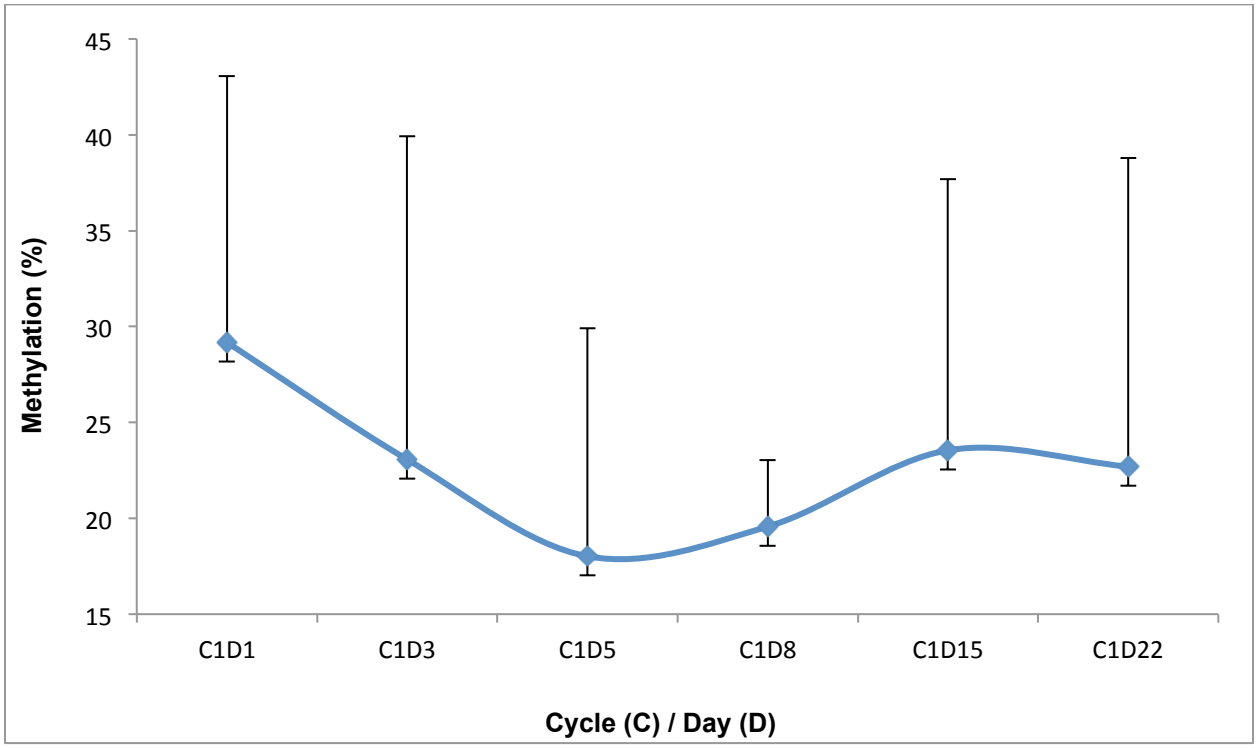
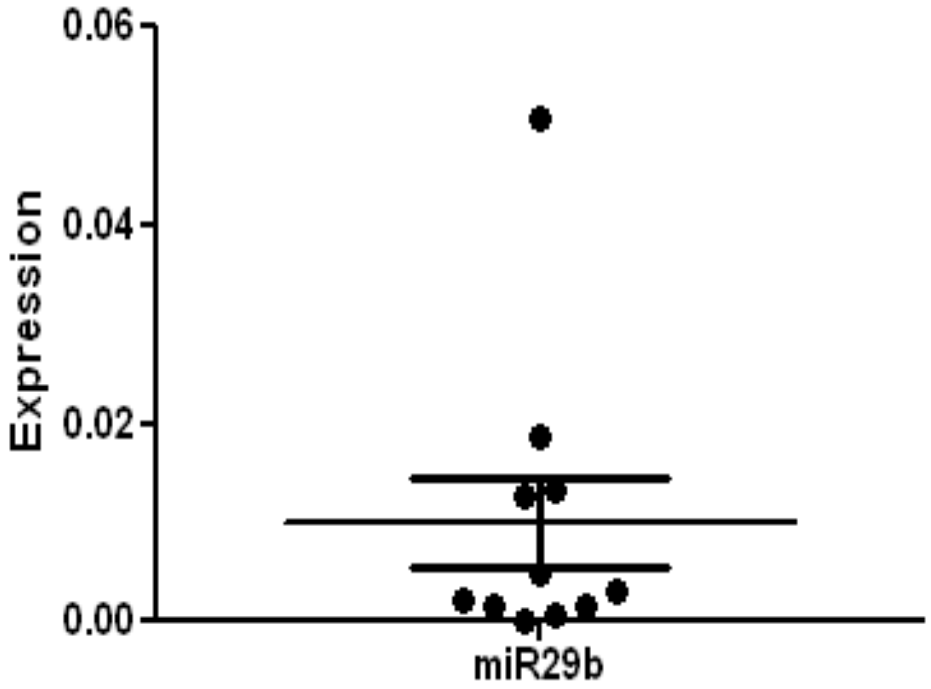


FIGURE S4. miR29b levels were analyzed in 15 patients at baseline by real-time PCR as described and are reported as expression levels. Bars represent mean ± standard error. Each symbol represents 1 patient. In 4 patients, expression levels were undetectable. There was no clear association between miR29b levels and response.



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