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Brief communication

Thrice weekly azacitidine does not improve hematological responses in lower-risk myelodysplastic syndromes: A study of the Hoosier Oncology Group

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ABSTRACT

Prolonged administration of methyl transferase inhibitors may increase response rates in myelodysplastic syndromes (MDS). Fourteen MDS patients with anemia and less than 10% marrow blasts received azacitidine 50 mg/m^2 thrice weekly for 2 weeks every 4 weeks; 7 also received weekly erythropoietin. The response rate of 43% did not improve the rates reported with other azacitidine administration schedules, so the study was closed. A decreased apoptosis of primitive erythroid progenitors and increased expression of $BclX_L$ was observed with treatment in responding patients compared to non-responders. Azacitidine may modulate $BclX_L$ and improve erythropoiesis through reduction of apoptosis in primitive erythroid progenitor population in MDS.

1. Introduction

Azacitidine, a cytidine nucleoside analog that acts via incorporation into RNA and DNA and inhibits DNA methyltransferase (DNMT), reduces or eliminates the need for red cell or platelet transfusions and improves quality of life in patients with MDS.

The optimal dose and schedule of azacitidine is unknown other than that repetitive cycles are necessary to assure adequate incorporation into DNA and clinical response. There is evidence that hypomethylating agents at low doses can more effectively induce differentiation, reduce proliferation and increase apoptosis [1]. This trial was conducted to evaluate prolonged exposure-per-cycle to azacitidine in lower risk MDS where such alterations may optimize therapeutic efficacy. Erythropoietin was also added to half the patients to assess possible additive or synergistic effect with azacitidine. Correlative studies were performed to explore erythroid differentiation in this setting.

2. Methods

2.1. Patient population

The study was conducted by the Hoosier Oncology Group (HOG); a community-based cooperative group with appropriate human subjects review. All patients gave informed consent prior to registration. Patients with transfusion-dependent MDS and less than 10% marrow blasts, no response to prior erythroid growth factor therapy, or a serum erythropoietin level of greater than 200 IU/L were eligible. Patients with poor liver or renal function (bilirubin >2 mg/dL, ALT and AST >2 times upper normal limit, and creatinine ≥1.5 times upper normal limit), active coronary artery disease, history of recent cerebrovascular accident or thromboembolic events, or poor performance status (Eastern Cooperative Oncology Group 3 or 4) were excluded.

2.2. Study design

Patients were randomized into two arms: one arm received subcutaneous azacitidine 50 mg/m^2 on Monday, Wednesday and Friday for two consecutive weeks in 28-day cycles; the other arm received the same treatment with additional 60,000 IU of subcutaneous erythropoietin (Procrit®; Centocor Ortho Biotech Services LLC, Horsham, PA) once a week. A bone marrow examination was performed at baseline and following cycles 3 and 6. Response was assessed based on the WHO IWG response criteria.

2.3. Correlative studies

Mononuclear low density bone marrow cells (LDBM) collected at baseline and following cycles 3 and 6 were evaluated for expression of CD34, CD36, CD71, and

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Table 1
Patient characteristics.

Patient	Sex/age	MDS type[a]	% Bone marrow blasts	Type of cytopenia[b]	Prior treatments	Cytogenetics
1	M/59	MDS/MPD	1	Anemia	Erythropoietin, Investigational	Normal
2	M/69	CMML-1	6	Anemia	Erythropoietin	Normal
3	M/83	RCMD	4	Anemia, Neutropenia	Investigational	Normal
4	M/75	RAEB-1	6	Anemia, Neutropenia	Lenalidomide, Thalidomide, Investigational	Normal
5	M/68	RAEB-1	6	Anemia	Erythropoietin	Normal
6	M/64	RCMD-RS	4	Anemia	Investigational	Normal
7	M/72	RAEB-1	8	Anemia, Thrombocytopenia	Cyclosporine, Investigational	Normal
8	M/66	RAEB-1	9	Anemia, Neutropenia	None	Normal
9	M/66	RAEB-1	9	Anemia, Neutropenia	None	Normal
10	M/78	RAEB-1	5	Anemia, Thrombocytopenia	None	del13(q12q14)
11	M/85	RCMD-RS	1	Anemia	Lenalidomide, Erythropoietin	−Y and t(1;2)
12	M/69	RA-RS	2	Anemia	None	Normal
13	F/65	RCMD-RS	4	Anemia	None	Del 7q
14	M/71	RCMD-RS	4	Anemia, Neutropenia	None	Complex

[a] MDS type by World Health Organization (WHO) classification.
[b] All patients had transfusion-dependent anemia.

Annexin V by flow cytometry. Quantitative RT-PCR reactions were performed for *GATA1*, erythropoietin receptor (*EpoR*), and $BclX_L$, as previously described [2].

2.4. Statistical analysis

Total BM cellularity, patient age, and flow cytometry data of CD34+, CD36+ and CD71+ subpopulations, as well as apoptosis, in pre- and post-treatment samples were compared using a two-sample *t*-test between responders and non-responders. For $BclX_L$ expression, comparisons were conducted using a repeated-measurement analysis of variance (ANOVA) model accommodating the within patient correlation among the observations at baseline and following cycles 3 and 6. All the analyses were performed using SAS 9.2.

3. Results and discussion

Fourteen patients completed a minimum of 3 cycles, with 8 completed 6 treatment cycles. Six patients did not continue beyond 3–5 cycles due to lack of response. Patient baseline characteristics are summarized in Table 1. In the azacitidine-only cohort, 3 patients experienced major erythroid response; while in the erythropoietin group, 2 major and 1 minor erythroid responses were observed. Findings suggested that the addition of erythropoietin did not contribute to the clinical efficacy of azacitidine; the analyses were therefore performed without separating the two groups. The overall response rate was 43% (6/14 patients) with major erythroid response of 36% (5/14 patients). Response was observed in 3 patients following three, and in 3 patients following six cycles. One of the major erythroid responders also obtained a major platelet response. One patient demonstrated a complete morphologic bone marrow remission, and 3 achieved a partial remission. The response rates were inferior to other previously reported azacitidine schedules, therefore the study did not continue accrual [3,4].

A median of 3 treatment cycles is required to achieve first response with azacitidine, nevertheless a number of patients experience response with further number of cycles [5]. Four patients in this study received only 3 cycles of treatment. The final response rate may have been greater if all patients had received more than 3 cycles. Furthermore, the total cumulative dose of azacitidine per cycle in this trial was 300 mg/m^2, while it was 375–525 mg/m^2 in the two reference studies [3,4]. It is not clear whether the unsatis-

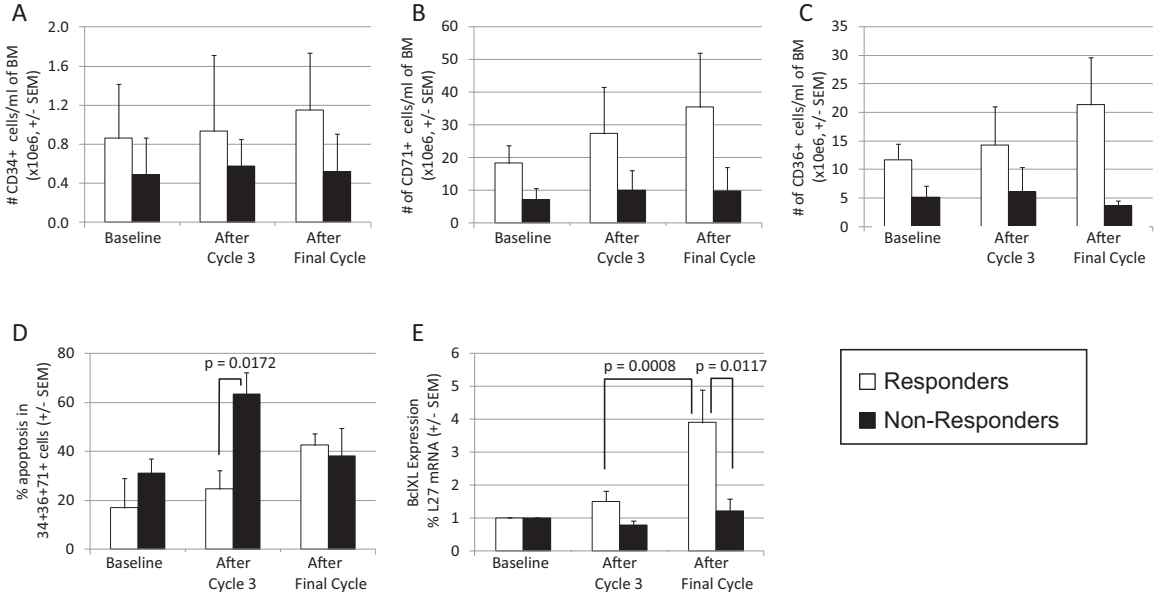

Fig. 1. Low density BM cells from responding and non-responding patients at baseline and following cycles 3 and 6: (A–C) number/mL of CD34+, CD71+ and CD36+ cells. (D) Percent apoptosis among CD34+, CD71+ and CD36+ cells. (E) Quantitative RT-PCR analysis for Bcl_{XL}.

factory response rate in our trial was due to the modified schedule, lower cumulative dose, or both.

Pre- and post-treatment samples from patients who completed at least 3 cycles of therapy were analyzed. Interestingly, the number of LDBM was significantly higher in responding patients compared to non-responders when all analysis time points, including pre-treatment, were pooled ($59.5 \pm 14.9 \times 10e6$/mL vs. $17.8 \pm 5.4 \times 10e6$/mL, respectively; $p = 0.01$). Based on the pathology readings, we observed a higher cellularity in the baseline bone marrow biopsy of responders compared to non-responders (mean ± SEM: $86.0 \pm 6.6\%$ vs. $55.8 \pm 5.9\%$, respectively; $p = 0.02$). A previous study found that patients presenting with a hypocellular marrow had better survival, independent of other risk factors and regardless of therapeutic approach, compared to patients with normo/hypercellular marrow [6]. However, in our study those with higher marrow cellularity demonstrated greater sensitivity to azacitidine. In addition, responders were significantly younger compared to non-responders (mean age in years ±SEM: 65.8 ± 1.9 vs. 74.9 ± 2.8, respectively, $p = 0.04$). Moreover, responders demonstrated a trend towards higher numbers of marrow CD34+, CD36+, CD71+ cells, which represent primitive erythroid progenitors (Fig. 1, Panels A, B, and C), and their double and triple positive erythroid subpopulations at all time points (data not shown). Apoptosis was more frequent among LDBM after cycle 3 in non-responders compared to responders (mean ± SEM: $26.6 \pm 7.8\%$ vs. $10.7 \pm 2.1\%$, respectively, $p = 0.06$); especially among CD34+, CD36+, CD71+ cells (mean ± SEM: $63.4 \pm 7.7\%$ vs. $24.7 \pm 9\%$, respectively, $p = 0.02$, Fig. 1, Panel D).

We had initially hypothesized that azacitidine-responsive patients, given promoter hypomethylation, would demonstrate increased *GATA1* and *EpoR* expression, reflecting improved erythroid differentiation. However, responding patients demonstrated no change in *GATA1* or *EpoR* expression (data not shown). The higher cellularity and less frequent apoptosis in responding patients led us to examine the pro-survival gene, $BclX_L$, shown previously to be underexpressed in low risk MDS [7]. Indeed, all responding patients demonstrated increased $BclX_L$ expression following 6 cycles of therapy (Fig. 1, Panel E). $BclX_L$ is a *GATA1*-responsive gene [8]; however, the lack of increased *GATA1* expression in responding patients suggests that in the context of MDS response to azacitidine, mechanisms other than *GATA1*-mediated expression, or perhaps a post-transcriptional modulation of *GATA1* activity regulate $BclX_L$.

Collectively, while results of this clinical trial do not support the use of thrice weekly scheduling of azacitidine in MDS patients, the correlative studies suggest that increased $BclX_L$ expression, induced by a *GATA1* expression-independent manner, as well as a reduction in apoptosis within the primitive erythroid progenitor population, play role in the azacitidine-induced erythroid response in these patients.

Conflict of interest

All authors have no conflict of interest to report.

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Contributions. H.S. supplied the acquisition of data, analysis and interpretation of data, drafting the article, revised it critically for important intellectual content, and gave final approval of the version to be submitted; R.J.C. provided the conception and design of the study, supplied the acquisition of data, analysis and interpretation of data, revised the article critically for important intellectual content, and gave final approval of the version to be submitted; C.M.O. provided the conception and design of the study, supplied the acquisition of data, analysis and interpretation of data, revised the article critically for important intellectual content, and gave final approval of the version to be submitted; E.M.C. provided the conception and design of the study; D.H., Z.Y., A.P, H.L.C., K.J.K., E.S.W., A.W., C.S. and S.C.N. supplied the acquisition of data, analysis and interpretation of data; Z.Y. and J.W. performed analysis and interpretation of data. L.C. provided the conception and design of the study, revised the article critically for important intellectual content, and gave final approval of the version to be submitted.

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