Original Article

Phase 1b-2a Study to Reverse Platinum Resistance Through Use of a Hypomethylating Agent, Azacitidine, in Patients With Platinum-Resistant or Platinum-Refractory Epithelial Ovarian Cancer

Siqing Fu, MD, PhD[1]; Wei Hu, MD[2]; Revathy Iyer, MD[3]; John J. Kavanagh, MD[2]; Robert L. Coleman, MD[2]; Charles F. Levenback, MD[2]; Anil K. Sood, MD[2]; Judith K. Wolf, MD[2]; David M. Gershenson, MD[2]; Maurie Markman, MD[4]; Bryan T. Hennessy, MD[4,5]; Razelle Kurzrock, MD[1]; and Robert C. Bast, Jr, MD[4,6]

BACKGROUND: Sequential treatment with azacitidine can induce re-expression of epigenetically silenced genes through genomic DNA hypomethylation and reverse carboplatin resistance of epithelial ovarian cancer cells. A phase 1b-2a clinical trial of this sequential combination of azacitidine and carboplatin was initiated in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. **METHODS:** Patients with pathologically confirmed intermediate-grade or high-grade epithelial ovarian cancer who developed disease progression within 6 months (resistant disease, n = 18 patients) or during a platinum-based therapy (refractory disease, n = 12 patients) were eligible. All patients had measurable disease. **RESULTS:** Thirty patients received a total of 163 cycles of treatment. This regimen produced 1 complete response, 3 partial responses (overall response rate [ORR], 13.8%), and 10 cases of stable disease among 29 evaluable patients. For those patients who achieved clinical benefits, the median duration of the treatment was 7.5 months. The median progression-free survival (PFS) and overall survival (OS) for all patients were 3.7 months and 14 months, respectively. Patients with platinum-resistant disease achieved an ORR of 22%, with a median PFS of 5.6 months and a median OS of 23 months. The predominant toxicities were fatigue and myelosuppression. Correlative studies indicated that DR4 methylation in peripheral blood leukocytes was decreased during treatment in 3 of 4 objective responders (75%), but in only 5 of 13 nonresponders (38%). **CONCLUSIONS:** To the authors' knowledge, the results of the current study provide the first clinical evidence that a hypomethylating agent may partially reverse platinum resistance in patients with ovarian cancer. Further clinical evaluation of hypomethylating agents in combination with carboplatin is warranted. ***Cancer*** **2011;117:1661–9.** © *2010 American Cancer Society.*

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DNA methylation plays an essential role in regulating normal biologic processes as well as carcinogenesis.[1] Methylation of DNA is a heritable, DNA methyltransferase-induced modification of DNA structure that does not alter the specific sequence of base pairs responsible for encoding the genome, but can directly inhibit gene expression.[2] Two patterns of

Corresponding author: Siqing Fu, MD, PhD, Department of Investigational Cancer Therapeutics, Unit 0455, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030; Fax: (713) 794-4130; siqingfu@mdanderson.org

[1]Department of Investigational Cancer Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [2]Department of Gynecologic Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [3]Department of Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [4]Department of Gynecological Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [5]Department of Cancer Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [6]Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

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DNA methylation have been observed in cancer cells[3]: global hypomethylation across the genome and localized hypermethylation at specific CpG islands within the gene promoter regions of certain genes. Decreased methylation resulting from global hypomethylation may permit the expression of previously quiescent proto-oncogenes and pro-metastatic genes and promote tumor progression. Alternatively, an aberrant increase in methylation patterns at previously unmethylated sites, such as the promoter regions of tumor suppressor genes, may result in transcriptional silencing and an inability to control tumor development.[4-8]

Methylation microarray analyses of late-stage ovarian cancers identified 2 distinct groups based on tumor methylation levels that appeared to have prognostic significance.[9] Progression-free survival (PFS) after chemotherapy was found to be significantly shorter for patients with higher levels of methylation ($\leq$8 months) compared with those with lower levels ($\geq$12 months; $P < .001$), suggesting that a higher degree of CpG island methylation is associated with early disease recurrence and/or chemotherapy resistance.

Azacitidine is a hypomethylating agent that has been shown to induce the re-expression of hMLH1 in platinum-resistant ovarian cancer cell lines, leading to resensitization of treated cells to carboplatin.[10] Studies with clonogenic assays as well as human tumor xenografts have shown that the treatment of platinum-resistant ovarian cancer cells with hypomethylating agents such as decitabine or azacitidine increases sensitivity to platinum compounds, including carboplatin and cisplatin, in addition to other chemotherapeutic agents.[11,12] Our preclinical studies revealed that in platinum-resistant ovarian cancer cells, sequential treatment with azacitidine followed by carboplatin produced synergistic cytotoxicity. In addition, azacitidine enhanced the sensitivity of platinum-resistant ovarian cancer cells to carboplatin associated with a DR4-mediated caspase 8-dependent apoptosis.[13] In the current trial, we assessed the safety and efficacy of a sequential regimen in which azacitidine has been used to reverse resistance to carboplatin in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. The choice of this design is based on the finding that platinum is the most effective chemotherapeutic agent in the treatment of human epithelial ovarian cancer,[14-17] on previous clinical experience that reversal of epigenetic changes can overcome chemotherapy resistance in other tumor types,[18,19] and on our own preclinical data with ovarian cancer cell lines.

MATERIALS AND METHODS

Eligibility Criteria

Patients were eligible to participate in this trial if they had a pathologically confirmed diagnosis of intermediate-grade or high-grade epithelial cancers of the ovary, fallopian tube, or peritoneum that were considered platinum refractory (progressive disease [PD] while receiving or persistent disease after platinum-based therapy) or platinum resistant (PD within 6 months of treatment with a platinum-based regimen). Patients were at least aged 18 years and had measurable disease by imaging studies that had progressed within 3 months of study entry. All participants had an Eastern Cooperative Oncology Group performance status of $\geq$2. Additional eligibility criteria included adequate bone marrow function (absolute neutrophil count >1500/μL, hemoglobin >9.0 g/dL, and platelet count >75,000/μL), renal function (serum creatinine <1.5 mg/dL or a calculated creatinine clearance of at least 60 mL/minute), and hepatic function (serum total bilirubin <2.0 mg/dL and alanine aminotransferase <3-fold of the upper limit of normal). Patients were excluded if they had advanced hepatic metastases that occupied >75% of the hepatic parenchyma or if they had undergone high-dose chemotherapy for ovarian cancer.

Study Design

This study was a prospective, open-label, phase 1b-2a clinical trial of a sequential regimen using azacitidine to reverse resistance to carboplatin in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. The study was conducted at The University of Texas M. D. Anderson Cancer Center (MDACC) after the approval by the Institutional Review Board (IRB). All patients provided written informed consent before study entry using forms approved by the IRB. This study included a dose-escalation phase based on the classical "3 + 3" design and an expansion phase at the recommended phase 2 dosages. The primary goal of the current study was to define the safety and clinical responses of this regimen.

Treatment Plan

Treatment was administered on an outpatient basis at MDACC. Patients received azacitidine at a dose of 75 mg/m^2 subcutaneously daily for 5 days[20] and carboplatin at either an area under the curve (AUC) of 4 or an AUC of 5 intravenously over 1 hour on Day 2 every 28 days. Chemotherapeutic agents were reconstituted according to the manufacturers' manuals. All patients received

antiemetics according to best local practice. Patients with evidence of response or those without PD after the first 8-week treatment cycle were eligible to receive further treatment until prohibitive toxicity or PD developed.

Dose-limiting toxicity (DLT) was defined as a platelet count <20,000/μL, an absolute neutrophil count <500/μL for >7 days, neutropenic fever, or a delay of >7 days in the initiation of the next cycle at 100% dosage because of inadequate hematological parameters, as well as any grade 3 or greater nonhematological toxicity other than nausea, vomiting, or fatigue occurring during the first cycle of treatment. Carboplatin dosage was calculated using the Calvert formula = AUC × (GFR [glomerular filtration rate] + 25), in which the GFR was calculated by stable serum creatinine based on the Cockcroft-Gault equation.[21,22]

Safety and Efficacy Evaluation

All patients underwent evaluation including complete medical history, physical examination, and laboratory tests. Radiographic imaging studies (helical computed tomography [CT] or magnetic resonance imaging scan) were obtained to assess measurable disease whereas serum CA 125 was obtained to assess biochemical responses. Before each treatment, laboratory assessment, physical examination, ECOG functional status, toxicities, and concomitant medications were documented. The severity of adverse events was graded according to the Common Terminology Criteria for Adverse Events (version 3.0).

Efficacy endpoints included response rate, PFS, and overall survival (OS). All patients were observed until June 2008, when this protocol was closed, or until death. The World Health Organization (WHO) criteria of complete response (CR), partial response (PR), stable disease (SD), and PD were used to characterize tumor responses. Tumor size was determined by the product of 2 perpendicular diameters of marker lesions applied at the widest portion of the tumor. All the measurable lesions were evaluated every 2 cycles. Serum CA 125 levels were measured at baseline and before each cycle. The CA 125 response criteria of 50% (4 samples) and 75% (3 samples) reduction were used as a supplementary assessment of possible antitumor activity. To be evaluable for treatment response, a patient must have received at least 2 cycles of treatment.

DNA Extraction and Methylation Analysis

Blood samples from patients were collected into ethylenediamine tetraacetic acid (EDTA) vials at that time of enrollment and before each cycle of treatment. Plasma was separated by centrifugation and leukocytes were separated on Ficoll-Hypaque gradients and stored at −20°C until analyzed.

DNA was extracted from peripheral blood mononuclear cells (PBMC) obtained from patients at different time points before and after treatment using standard phenol-chloroform extraction. It should be noted that this will represent DNA from lysed normal blood cells as well as tumor cells.[23] Methylation analysis was performed using a methylation kit (EZ-96 gold; Zymo Research, Orange, Calif). MethPrimer software was used for the prediction of CpG island of DR4 (ACCESSION EF064713; GI: 117606477) and the design of methylation-specific primers. The sequence of primers for methylated DR4 promoter was forward, TTGGAGCGTAATGGTTTTATTTC; reverse, AATACCTATAATCCCAACCA CTCG, and that for unmethylated DR4 promoter was forward, GTTGGAGTGTAATGGTTTTATTTTG; reverse, AATACCTATAATCCCAACC ACTCAA. The methylation-specific (MSP) polymerase chain reaction (PCR) conditions were 94°C for 5 minutes with hot start, then 94°C for 30 seconds, 58°C or 60°C for 30 seconds, and 72°C for 1 minute repeated for 40 cycles. Universal methylated and unmethylated control DNAs were used for the positive control (Chemicon International, Temecula, Calif). All MSP PCRs were repeated twice separately. Methylated DR4 was normalized by both unmethylated DR4 and β-actin. Image analysis (Scion Image for Windows, Frederick, Md) was used for semiquantitative measurement of methylated and unmethylated DR4. We defined at least 10% of DR4 methylation changes as a cutoff value.

The hMLH1 methylation status in plasma DNA was determined by MSP PCR using a CPGWIZ hMLH1 amplification kit (Chemicon International) based on the manufacture's instructions.

Statistical Analysis

This study was divided into 2 parts: dose escalation was based on the standard 3 + 3 design and dose expansion was based on the Simon optimal 2-stage designs to enroll 27 patients assuming a p0 of 20%, a p1 of 35%, an α of .1, and a β of .1. Descriptive summary statistics were described as estimated proportions with 95% confidence intervals (95% CIs). Continuous variables not meeting the assumptions of normality (Shapiro-Wilk test) as well as nonparametric data were compared using the Fisher exact test. The intent-to-treat (ITT) population was

defined as all patients who were enrolled and received at least 1 partial dose of therapy, whereas the evaluable population was defined as patients who completed at least 2 cycles of therapy. WHO criteria for efficacy were used to estimate the overall response rate (ORR; CR + PR). All efficacy and safety analyses were conducted on an ITT basis. PFS and OS were estimated using the Kaplan-Meier method and log-rank test. PFS was defined as the interval from the first day of treatment with the study drug until documented PD or death due to any cause while the patient was on study or during the long-term follow-up period. OS was defined as the time period from the first day of treatment with the study drug until death. The association between the DR4 methylation in responders and nonresponders was analyzed using the chi-square test.

RESULTS

Patient Characteristics

A total of 30 patients (median age, 63 years; range, 37-73 years) who met the inclusion and exclusion criteria were recruited onto this study. Among these patients, 7 patients were enrolled during the dose escalation phase (with 1 early withdrawal) and 23 patients were enrolled during the expansion phase. Twenty-nine patients had received ≥2 cycles of therapy, whereas 1 patient voluntarily withdrew from the study during dose escalation and was replaced. She was evaluable for toxicity because she completed 1 cycle of therapy. The characteristics of these patients are listed in Table 1. It was noted that 18 patients had platinum-resistant and 12 had platinum-refractory ovarian cancer. Twenty patients had received ≥3 types of systemic chemotherapy.

Table 1. Patient Characteristics (N = 30)

Characteristic	No. of Patients (%)
Age (range), y	63 (37-73)
Pathology	
Serous	18 (60)
Clear cell	1 (3.3)
Endometrioid	1 (3.3)
Mucinous	1 (3.3)
Transitional	1 (3.3)
Undifferentiated	1 (3.3)
Mixed	7 (23.3)
Initial stage	
IB	1 (3.3)
IIC	1 (3.3)
IIIC	21 (70)
IV	7 (23.3)
Prior treatment	
1 regimen	2 (6.6)
2 regimens	8 (26.7)
3 regimens	6 (20)
4 regimens	7 (23.3)
5 regimen	4 (13.3)
6 regimen	1 (3.3)
8 regimen	2 (6.6)
Platinum sensitivity	
Resistant	18 (60)
Refractory	12 (40)

Antitumor Activity

Of 29 evaluable patients who received ≥2 cycles of treatment, 11 received ≥6 cycles of treatment and 6 patients received ≥10 cycles of treatment. One patient discontinued treatment because of intermittent rectal bleeding caused by tumor necrosis after having completed 21 cycles. The ORR was 13.8% (4 of 29 patients; 95% CI, 10.1%-17.5%): 1 patient achieved a clinical CR, 3 patients achieved a clinical PR, and 10 patients had SD as shown in Table 2. For those patients who achieved clinical benefits, the median duration of the treatment was 7.5 months. The median PFS was 3.7 months, whereas the median OS was 14 months (Fig. 1). Among 27 patients who were eligible for CA 125 response evaluation, 5 patients achieved a CR, 6 patients achieved a PR, and 10 patients had SD. Although not preplanned, subgroup analyses revealed that patients with platinum-resistant disease achieved an ORR of 22%, a median PFS of 5.6 months, and a median OS of 23 months, whereas patients with platinum-refractory disease achieved an ORR of 0%, a median PFS of 1.9 months, and a median OS of 10 months.

Toxicity

All 30 patients were evaluable for toxicity. No DLTs or treatment-related deaths were observed. The most common adverse events included fatigue and myelosuppression. Grade 2 or higher toxicities are summarized in Table 3. Side effects included neutropenia, anemia, fatigue, nausea, and pain/irritation at the injection sites. No greater toxicities were observed with sequentially administered azacitidine and carboplatin than would be expected from single-agent carboplatin based on historical experiences in a similar cohort of patients.

DR4 Methylation in PBMC and hMLH1 in Plasma DNA

To define changes in DNA methylation, DR4 and hMLH1 methylation levels were determined using MSP

Table 2. Major Characteristics of Individual Patients

Patient Access No.	Age, Years	Platinum Resistant or Refractory	Platinum-Free Interval, Months	Treatment-Free Interval, Months	Best Response by WHO	Best Response by CA 125	Progression-Free Survival, Months	Survival, Months[a]
1	51	Resistant	11	5	PR	CR	10	23
2	55	Refractory	3	1	NE	NE	1	3
3	45	Resistant	10	5	SD	NE	6	41
4	68	Resistant	13	3	PD	SD	2	7
5	62	Resistant	12	3	SD	PR	6	26
6	48	Refractory	1	1	PD	PD	2	5
7	44	Refractory	10	1	PD	PR	2	13
8	61	Resistant	14	5	SD	CR	10	21+
9	61	Resistant	9	4	SD	PR	5	24
10	69	Resistant	11	6	PD	SD	2	10
11	52	Refractory	10	1	PD	SD	2	18
12	69	Resistant	6	1	SD	SD	6	25+
13	63	Refractory	6	1	PD	SD	2	10
14	68	Resistant	32	4	SD	PR	4	37+
15	63	Refractory	10	1	PD	SD	4	5
16	66	Resistant	8	4	PR	CR	10	36
17	57	Refractory	6	1	PD	SD	2	14
18	62	Resistant	15	3	CR	CR	21	30
19	55	Resistant	31	3	PR	CR	16	36+
20	37	Resistant	7	1	PD	SD	2	4
21	64	Resistant	8	2	PD	PD	4	6
22	65	Refractory	7	1	PD	PD	2	3
23	51	Refractory	6	1	PD	PD	2	8
24	55	Resistant	6	2	PD	PD	2	11
25	58	Resistant	12	1	SD	PD	7	14
26	65	Resistant	14	1	SD	PR	14	32+
27	41	Refractory	6	3	PD	PR	2	2
28	73	Resistant	23	5	SD	SD	2	6
29	65	Refractory	1	1	PD	SD	2	6
30	48	Refractory	4	1	SD	NE	9	14

WHO indicates World Health Organization; PR, partial response; CR, complete response; NE, not evaluable; SD, stable disease; PD, progressive disease.
[a] A "+" sign indicates that patients were alive at the time of last follow-up.

PCR of DNA extracted from PBMC sampled from patients before and after carboplatin and azacitidine treatment or plasma DNA from patients before carboplatin and azacitidine treatment. When the dynamic changes of DR4 methylation in PBMC were analyzed in an objective responder (Fig. 2), we found that DR4 methylation decreased slightly during the first cycle, reached a nadir during the second cycle by approximately 50%, and then increased slightly after several cycles of treatment. In contrast, no difference in the hMLH1 methylation of plasma DNA was observed between objective responders and nonresponders (Fig. 3). In these heavily pretreated patients, hMLH methylation was found in 11 patients (Patients 3, 5, 7, 9, 10, 12, 13, 18, 20, 23, and 30) of 26 patients examined (42%), supporting the hypothesis that chemotherapy increased hMLH1 methylation.[23]

Among the 4 objective responders, 3 patients (75%) had a mean decrease of 40% (range, 23%-62%) in DR4 DNA methylation compared with baseline levels as detected by image analysis of methylated and unmethylated DR4 (Fig. 4). Only 5 of 13 nonresponders (38%) demonstrated similar decreases in the DR4 methylation levels, suggesting that DR4 hypomethylation is more frequently noted in patients who respond to azacitidine followed by carboplatin.

DISCUSSION

To the best of our knowledge, the current study provides the first clinical evidence that a hypomethylating agent may be able to partially reverse platinum resistance in patients with ovarian cancer. In addition, the current study included several interesting observations. First, several cycles of sequential therapy were required to reverse carboplatin resistance, which was supported by our observation that patients with platinum-refractory epithelial

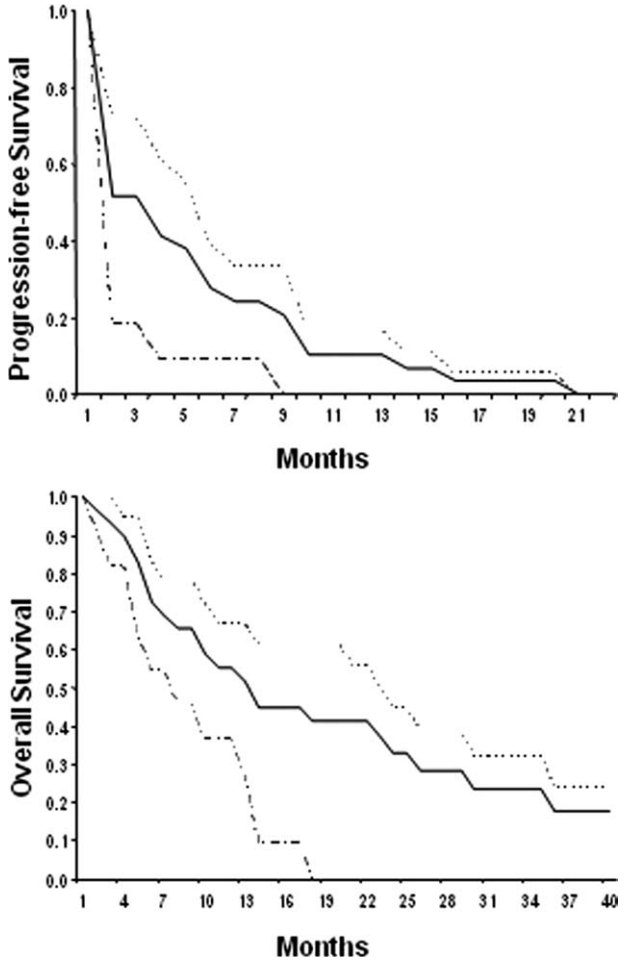

Figure 1. Progression-free survival and overall survival are shown for ovarian cancer patients treated with azacitidine and carboplatin as estimated by Kaplan-Meier curves and the log-rank test. Three groups of patients were analyzed. Straight line indicates all patients (n = 29); dotted line, patients with platinum-resistant disease (n = 18); dotted and dashed line, patients with platinum-refractory disease (n = 11).

ovarian cancer achieved no clinical response and were removed from the study after 2 cycles of treatment for PD. Second, patients who achieved a clinical benefit displayed mixed responses, suggesting tumor and response heterogeneity to the hypomethylating agent. The third observation is that hepatic metastases generally failed to respond to this sequential regimen, which might be caused by hepatic inactivation of azacitidine. Finally, no additional chemotherapy-related toxicities were observed in this small cohort of patients, indicating that the low dose of azacitidine that was sufficient to induce target hypomethylation did not augment the toxicity profile of chemotherapeutic agents when used in combination.

Table 3. Grade 2 or Greater Toxicity Profile Observed[a,b]

Toxicities	Grade 2	Grade 3	Grade 4
Leukopenia	16.7%	20.0%	0
Neutropenia	20.0%	13.3%	3.3%
Thrombocytopenia	10.0%	6.7%	3.3%
Anemia	33.3%	0	0
Nausea/vomiting	30.0%	6.7%	0
Constipation	20.0%	3.3%	0
Fatigue	33.3%	30.0%	0
Pain	43.3%	13.3%	0
Neuropathy	3.3%	0	0
Infection	0	3.3%	0
Metabolic	3.3%	0	0
Hepatic enzymes	0	3.3%	0
Alopecia	20.0%	0	0
Mucositis	3.3%	0	0
Rash	3.3%	0	0

[a] The severity of adverse events was graded according to the Common Terminology Criteria for Adverse Events (version 3.0).
[b] No dose-limiting toxicities were observed.

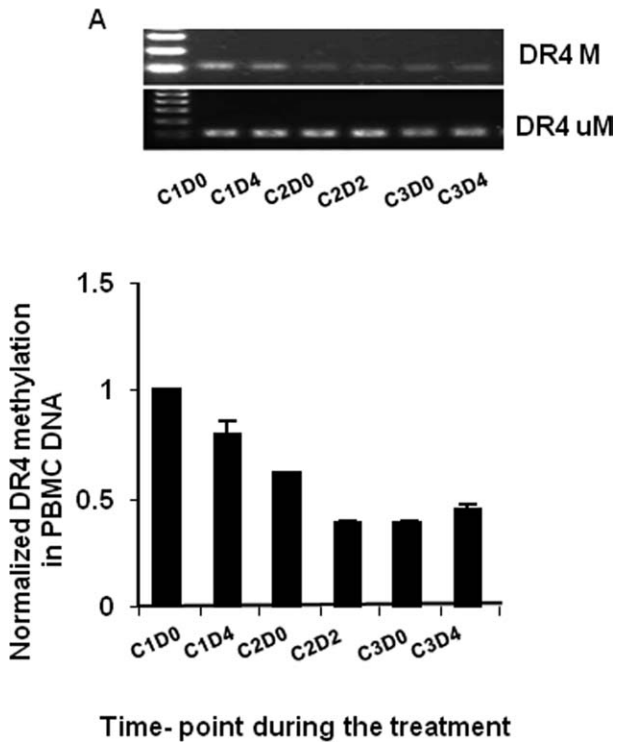

Figure 2. Dynamic changes in DR4 methylation in peripheral blood mononuclear cell (PBMC) DNA from 1 objective responder (Patient 18) are shown before and after treatment with azacitidine and carboplatin detected by (A) methylation-specific polymerase chain reaction and (B) semiquantitative analysis with a National Institutes of Health image tool. C indicates cycle; D, day; M, methylated DR4; U, unmethylated DR4.

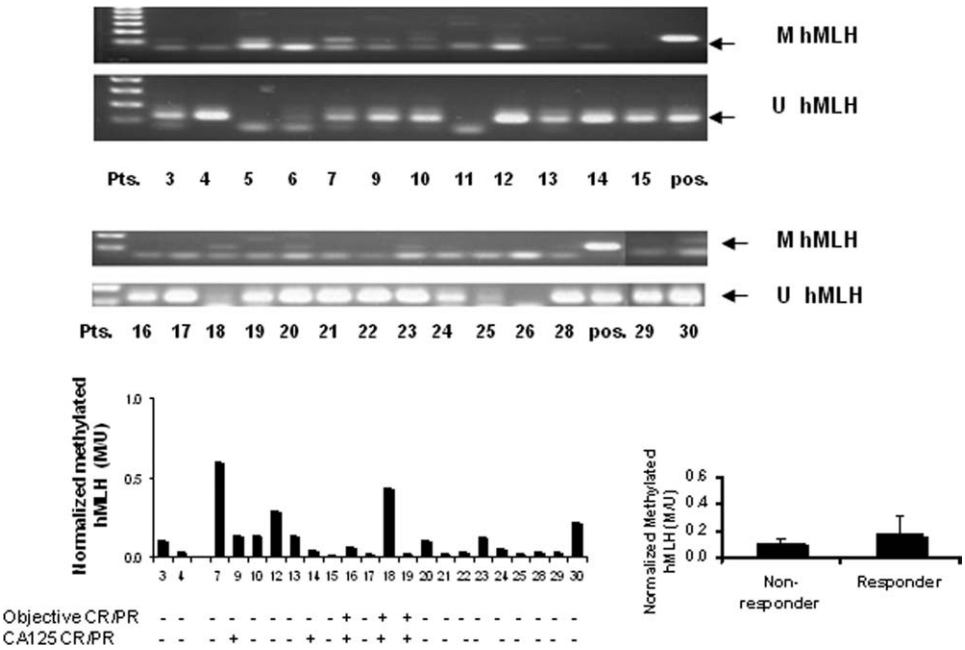

Figure 3. Basal level of hMLH methylation in plasma DNA from ovarian cancer patients is shown before treatment with azacitidine and carboplatin. (A) Polymerase chain reaction (PCR) is shown. (B and C) Semiquantitative measurement of normalized methylated hMLH is shown. pts indicates patients; pos, positive; M, methylated; U, unmethylated; CR/PR, complete response/partial response.

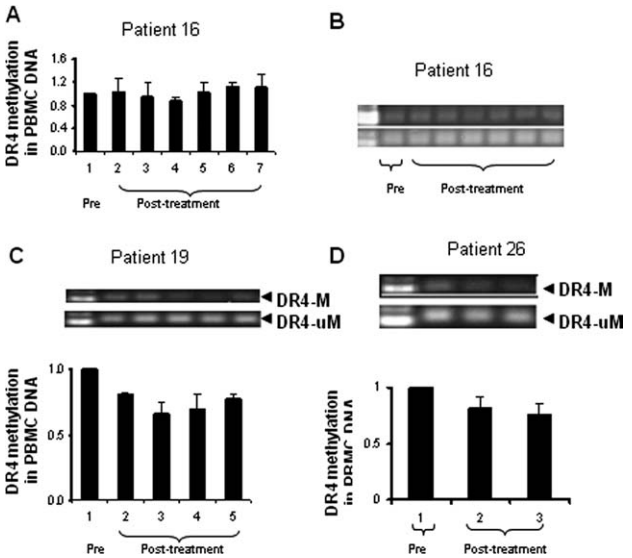

Figure 4. Dynamic changes in DR4 methylation are shown in peripheral blood mononuclear cell (PBMC) DNA from the other 3 objective responders (Patients 16, 19, and 26) before and after treatment with azacitidine and carboplatin as detected by methylation-specific polymerase chain reaction. (A and B) Changes in Patient 16 are shown. (C) Changes in Patient 19 are shown. (D) Changes in Patient 26 are shown.

Platinum-resistant or platinum-refractory epithelial ovarian cancer was chosen as a model for the following 4 reasons: advanced epithelial ovarian cancer displays multiple hypermethylation phenotypes involving known tumor suppressor genes; carboplatin has significant efficacy in treating ovarian cancer, although single-agent carboplatin induces minimal to no clinical response in patients with recurrent platinum-resistant and platinum-refractory ovarian cancer; and pretreatment with hypomethylating agents is able to reverse platinum resistance in platinum-resistant epithelial ovarian cancer cell models.[24-28]

The first retrospective study conducted at MDACC revealed that carboplatin retreatment resulted in a 21% PR rate in 33 patients with platinum-resistant epithelial ovarian cancer (79% had a platinum-free interval of at least 12 months and 2 patients were potentially sensitive to platinum). No patients with a platinum-free interval $<$12 months achieved an objective response.[29] It is interesting to note that a recent retrospective study at the same center identified 34 similar patients who received carboplatin retreatment. The median platinum-free interval from the time platinum was last received to retreatment

with carboplatin was 15.2 months. Only 2 patients achieved a PR (5.9%) whereas 21 patients achieved SD (61.7%).[30] Another retrospective study from the Memorial Sloan-Kettering Cancer Center identified 30 platinum-resistant ovarian cancer patients with a median platinum-free interval of 16.2 months from July 1997 through June 2001. At the time of platinum retreatment, 20 patients received single-agent platinum and 10 received platinum combinations with newer agents. Only 2 of 21 patients achieved a PR (9.5%) based on Response Evaluation Criteria In Solid Tumors (RECIST) by CT scans.[31] Moreover, in a phase 1 trial of bortezomib and carboplatin in patients with platinum-resistant ovarian cancer, 8 of 18 patients achieved SD, whereas 10 patients had PD.[32] Taken together, these data suggest that retreatment with carboplatin induces a very small percentage of objective responses (<10%). In the current study, treatment with azacitidine and carboplatin achieved an ORR of 22% based on the WHO criteria in patients with platinum-resistant ovarian cancer, whereas no objective responses were observed in patients with platinum-refractory ovarian cancer. It was noted that 2 of 4 responders had a platinum-free interval of <12 months.

The pathogenesis of ovarian carcinoma is poorly defined partly because of the lack of a tumor progression model as well as heterogeneous histological types.[33,34] Gene expression profiling of malignant ovarian cancer demonstrated that a significant number of hypermethylated genes were down-regulated.[35] A novel microarray system to assess gene expression, DNA methylation and histone acetylation in parallel, and to dissect the complex hierarchy of epigenetic changes has been developed using human ovarian cancer cell lines.[36] Aberrant CpG island methylation was found to be a major pathway leading to the inactivation of tumor suppressor genes and the development of cancer.[37-39] Further studies revealed that changes in DNA methylation were cumulative with PD.[8,40]

It is important to note that agents inducing hypomethylation do not benefit all patients with ovarian cancer. Therefore, the additional identification of predictive and surrogate biomarkers is needed for better management of this disease. Given our previous in vitro data that azacitidine enhanced the sensitivity of platinum-resistant ovarian cancer cells to carboplatin through reactivation of DR4 and induction of caspase 8-mediated apoptosis,[13] we tested whether a similar mechanism might exist in a patient population. Although the number of patients studied was small and differences did not achieve statistical significance, combining azacitidine with carboplatin was found to decrease DR4 methylation more frequently in patients who responded to this treatment than in those who did not, which is consistent with the possibility that reactivating silenced genes may be required for response. The basal methylation levels of the DR4 and hMLH1 gene promoters do not appear to be critical for predicting the response to this treatment strategy.[27,41]

The results of the current study suggest that a hypomethylating agent may enhance the response to platinum in patients with platinum-resistant ovarian cancer. The impact of epigenetic therapy might be further enhanced by the concomitant or sequential use of a hypomethylating agent and a histone deacetylase inhibitor before platinum treatment. Alternatively, it may be possible to maximize the therapeutic potential of epigenetic treatments through prolonged exposure to epigenetic agents, while concurrently administering a series of chemotherapeutic or biological agents.[24] Additional larger studies of the combination of azacitidine and carboplatin in patients with platinum-resistant ovarian cancer are warranted.

CONFLICT OF INTEREST DISCLOSURES

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