Clinical Cancer Research

A Phase I Clinical Trial of Guadecitabine and Carboplatin in Platinum-Resistant, Recurrent Ovarian Cancer: Clinical, Pharmacokinetic, and Pharmacodynamic Analyses

Daniela Matei[1], Sharad Ghamande[2], Lynda Roman[3], Angeles Alvarez Secord[4], John Nemunaitis[5], Merry Jennifer Markham[6], Kenneth P. Nephew[7], Simone Jueliger[8], Aram Oganesian[9], Sue Naim[9], Xiang Yao Su[9], Harold Keer[9], Mohammad Azab[9], and Gini F. Fleming[10]

Abstract

Purpose: Epigenetic changes are implicated in acquired resistance to platinum. Guadecitabine is a next-generation hypomethylating agent (HMA). Here, we report the clinical results, along with pharmacokinetic (PK) and pharmacodynamic analyses of the phase I study of guadecitabine and carboplatin in patients with recurrent, platinum-resistant high-grade serous ovarian cancer, primary peritoneal carcinoma (PPC), or fallopian tube cancer (FTC).

Experimental Design: Guadecitabine was administered once daily on days 1 to 5 followed by carboplatin i.v. on day 8 of a 28-day cycle. Patients had either measurable or detectable disease. Safety assessments used CTCAE v4.

Results: Twenty patients were enrolled and treated. Median age was 56 years (38–72 years). The median number of prior regimens was 7 (1–14). In the first cohort ($N = 6$), the starting doses were guadecitabine 45 mg/m^2 and carboplatin AUC5. Four patients experienced dose-limiting toxicity (DLT; neutropenia and thrombocytopenia), leading to dose deescalation of guadecitabine to 30 mg/m^2 and of carboplatin to AUC4. No DLTs were observed in the subsequent 14 patients. Grade $\geq$3 adverse events $\geq$10% were neutropenia, leukopenia, anemia, nausea, vomiting, ascites, constipation, hypokalemia, pulmonary embolism, small-intestinal obstruction, and thrombocytopenia. Three patients had a partial response (PR), and 6 patients had stable disease (SD) >3 months, for an overall response rate (ORR) and clinical benefit rate of 15% and 45%, respectively. LINE-1 demethylation in PBMCs and promoter demethylation/gene reexpression in paired tumor biopsies/ascites were recorded.

Conclusions: Guadecitabine and carboplatin were tolerated and induced clinical responses in a heavily pretreated platinum-resistant ovarian cancer population, supporting a subsequent randomized phase II trial. *Clin Cancer Res; 24(10); 2285–93. ©2018 AACR.*

Introduction

High-grade serous ovarian cancer (HGSOC) is characterized by unique molecular features that include DNA repair deficiency, a *TP53*-mutated signature, and an initial high response rate to cytotoxic chemotherapy (1). Platinum is a key component of standard treatment for HGSOC, and development of platinum resistance is a well-defined clinical phenotype with negative survival implications (2). Most patients with advanced stage HGSOC develop platinum-resistant recurrence, which is essentially incurable, with life expectancy of less than a year (3). While genetic events associated with platinum resistance have been well characterized (4), emerging evidence also points to epigenomic alterations, such as DNA methylation and modifications of histone marks (5, 6) being linked to acquired chemoresistance. Such changes cause transcriptional repression of tumor suppressor genes (TSG) and of other genes associated with apoptotic responses to chemotherapy (7, 8). For example, promoter methylation causing silencing of TSGs (e.g., *BRCA1*, *MLH1*, *RASSF1A*, *DAPK*, *DOK2*, and *OPCML)* and of differentiation-associated transcription factors *HOXA10* and *HOXA11* (6, 9, 10) has been connected to tumor initiation and chemotherapy resistance in HGSOC (7, 8). These observations led to the hypothesis that inhibition of DNA methylation through pharmacological blockade could be used to reverse resistance to platinum.

Several previous single-arm trials used DNA methyl transferase (DNMT) inhibitors, also known as hypomethylating agents (HMA), as resensitizers to platinum (11–13). Prior phase I trials showed that combinations of platinum and HMAs (e.g., decitabine, 5-azacitidine) are tolerable and biologically active, as measured through global and gene-specific DNA methylation assays

[1]Northwestern University Feinberg School of Medicine, Chicago, Illinois. [2]Georgia Cancer Center at Augusta University, Augusta, Georgia. [3]USC Norris Comprehensive Cancer Center, Los Angeles, California. [4]Department of Obstetrics and Gynecology, Duke Cancer Institute, Division of Gynecologic Oncology, Durham, North Carolina. [5]University of Toledo College of Medicine and Life Sciences, Toledo, Ohio. [6]University of Florida College of Medicine, Gainesville, Florida. [7]Indiana University, Bloomington, Indiana. [8]Astex Pharmaceuticals, Cambridge, United Kingdom. [9]Astex Pharmaceuticals Inc., Pleasanton, California. [10]University of Chicago Medicine, Chicago, Illinois.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Daniela Matei, Northwestern University, 303 East Superior Street, Chicago, IL 60611. Phone: 312-503-4853; Fax: 312-503-4853; E-mail: daniela.matei@northwestern.edu

doi: 10.1158/1078-0432.CCR-17-3055

Translational Relevance

Preclinical models suggest that promoter methylation of tumor suppressor genes is associated with ovarian cancer progression and development of platinum resistance and that hypomethylating agents (HMA) able to reverse aberrant DNA methylation will resensitize ovarian tumors to platinum. Here, we report results of a proof-of-principle, dose-finding phase I clinical trial that tested the combination of a novel HMA, guadecitabine, and carboplatin in a heavily pretreated, platinum-resistant high-grade serous ovarian cancer patient population. The combination was found to be tolerable and clinically and biologically active, supporting further testing in a randomized phase II trial against FDA-approved therapy for recurrent ovarian cancer.

in peripheral blood mononuclear cells (PBMC) and tumors (11, 13). Subsequent phase II studies showed promising clinical efficacy of 5-azacitidine or decitabine and platinum combination regimens, including long progression-free survival (PFS) and high response rates (RR) in platinum-resistant ovarian cancer (13, 14), supporting further investigation of this strategy in recurrent HGSOC.

Guadecitabine (SGI-110) is a next-generation HMA that is a dinucleotide of decitabine (5-aza-2′-deoxycytidine) and deoxyguanosine. Guadecitabine is resistant to modification by cytidine deaminase, prolonging the exposure of tumor cells to its active metabolite decitabine, compared with parental decitabine (15). Guadecitabine is being studied as a single agent in acute myeloid leukemia (AML) and myelodysplasia (MDS; ref. 16). A phase I study in patients with AML or MDS identified the maximum tolerated dose of guadecitabine as 90 mg/m^2 subcutaneously (s.c.) daily for 5 days and the biologically active dose of guadecitabine of 60 mg/m^2 s.c. daily for 5 days based on evaluation of LINE-1 methylation in PBMC (16). A recent preclinical study demonstrated that the guadecitabine and carboplatin combination inhibited the growth of ovarian cancer xenografts more potently than either drug alone and induced demethylation along with reexpression of select TSGs and chemoresponsiveness-associated genes (17), supporting further investigation of this HMA in platinum-resistant disease.

Here, we report the results of a phase I trial testing a combination regimen of guadecitabine daily for 5 days followed by carboplatin in patients with recurrent platinum-resistant HGSOC, with planned escalating doses of guadecitabine in a 3+3 design. This regimen mirrored the combination strategy of decitabine and carboplatin previously tested (13), with platinum added at the point of maximal demethylation, as determined by time-dependent assessment of LINE-1 methylation in PBMCs. Pyrosequencing and quantitative PCR analyses determined guadecitabine-induced demethylation and gene expression changes, respectively, in paired PBMCs and tumor biopsies and PK analyses assessed the interaction between the two drugs. The combination of guadecitabine and carboplatin was found to be tolerable and biologically active, supporting its further testing in a randomized trial.

Materials and Methods

Patient population

Patients 18 years and older with platinum-resistant histologically or cytologically confirmed ovarian cancer, PPC, or FTC who had previously received carboplatin and paclitaxel treatment were eligible for treatment. Platinum resistance was defined as disease recurrence within 6 months of the last platinum-containing regimen. All grade 2 to 3 histological types were eligible. Eligible patients had acceptable organ function based on laboratory data, Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and were at least 3 weeks from the most recent dose of anticancer therapy. There were no limits on the number of prior regimens allowed, and platinum-based therapy was not required as the last previous treatment. Patients were required to have either measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 or detectable disease, defined as baseline values of CA-125 at least twice the upper limit of normal and (i) ascites and/or pleural effusion attributed to tumor or (ii) solid and/or cystic abnormalities on radiographic imaging that do not meet RECIST definitions for target lesions. Tumor biopsies, paracentesis or thoracentesis to recover tumor cells before and after treatment were required at baseline and on cycle 2 day 8, if clinically safe and feasible. Exclusion criteria included hypersensitivity to carboplatin, prior therapy with HMAs, progression on platinum treatment, left ventricular ejection fraction (LVEF) <50%, grade 2 or greater neuropathy, known brain metastases, other malignancies, active uncontrolled infections, or other life-threatening illness. The study was conducted in accordance with the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, applicable local regulatory requirements, and the principles enunciated in the Declaration of Helsinki. The protocol and informed consent form were reviewed and approved by an Institutional Review Board/Independent Ethics Committee at each study center prior to implementation. Patients provided written informed consent before enrollment. The study is registered on ClinicalTrials.gov, number NCT01696032.

Trial design and treatment

This was a multicenter, nonrandomized, open-label phase I study conducted at 7 centers in the United States. Guadecitabine was administered s.c. once daily on days 1 to 5 followed by carboplatin i.v. infusion on day 8 of 28-day cycles. Study treatment continued until disease progression, occurrence of unacceptable treatment-related toxicity, or patient withdrawal. In the dose escalation/deescalation design, cohorts of at least 6 patients were planned at each guadecitabine dose level to determine the maximum tolerated dose (MTD). In the absence of DLTs, the starting dose of guadecitabine of 45 mg/m^2 daily on days 1 to 5 was to be escalated only once to the full biologically effective dose (BED) of 60 mg/m^2 daily on days 1 to 5. An earlier study in patients with hematologic malignancies found that 90 mg/m^2 guadecitabine daily on days 1to 5 was well tolerated with no DLTs (16); the starting dose for this study was 50% of that dose (45 mg/m^2 daily on days 1–5) to accommodate the expected overlapping myelosuppression with carboplatin. In the presence of 1 DLT in 6 patients of the starting dose cohort, the cohort was to enroll 6 more patients at the same dose level. In the event that 2 or more DLTs occurred at the starting dose cohort, the next lower dose level of 30 mg/m^2 was to be evaluated. The MTD

was prespecified as the highest guadecitabine dose level (30–60 mg/m^2 daily on days 1–5) in combination with carboplatin at which no more than 1 of 6 patients or 2 in 12 experienced a DLT. Once the MTD was established, a total of 14 patients were to be treated at that dose and assessed for response.

Study endpoints and assessments

The primary endpoint was the incidence of DLTs and other adverse events (AE) to determine the MTD. Safety assessments consisted of procedures and laboratory assessments: recording of AEs, concomitant medications, complete or symptom-directed physical examination, weight, vital signs, ECOG performance status, 12-lead electrocardiograms (ECG), hematology, chemistry, and urinalysis. DLTs and AEs were graded by Common Terminology Criteria for Adverse Events (CTCAE) v4.0. DLT was defined as any of the following occurring during cycle 1 of therapy related to guadecitabine or the combination of guadecitabine and carboplatin: (i) any incidence of grade 4 thrombocytopenia or neutropenia lasting >7 days; (ii) grade 3 or 4 febrile neutropenia; (iii) any incidence of grade $\geq$3 nonhematologic toxicity, which could not be appropriately managed by supportive treatment; (iv) any incidence of failure to recover absolute neutrophil count (ANC) to 1,000 cells/mm^3 and/or platelets to >75,000 cells/mm^3 by cycle 1, day 42; (v) any other clinically significant AE placing patients at undue safety risk or resulting in discontinuation of treatment.

Efficacy endpoints, which were secondary endpoints in this study, included objective response rate [ORR: defined as complete response (CR) and partial response (PR) based on both measurable and detectable disease), progression-free survival (PFS) at 6 months, clinical benefit rate (CBR: defined as CR+ PR + stable disease for at least 3 months), percentage of patients with CA-125 reduction of at least 50%, duration of response (DOR), and overall survival (OS). Response was assessed using RECIST v1.1 (18) for patients with measurable disease, and modified Rustin criteria for patients with detectable disease (19, 20). At screening, disease measurements were obtained using computed tomography (CT) scans or magnetic resonance imaging (MRI), as appropriate. These were repeated at the end of every other cycle from cycle 1, day 1 for 6 cycles, then every 3 months until clinical and/or radiographic disease progression was evident.

Pharmacokinetics

Blood samples for determination of C_{max}, C_{min}, AUC and other secondary PK parameters were obtained during cycle 1 for assessment of guadecitabine, and its active metabolite decitabine, concentrations on day 1 at baseline (predose), 15 minutes, 30 minutes, 60 minutes, 90 minutes, and 2 hours, 4 hours, 6 hours, and 8 hours after dose, and for assessment of carboplatin concentrations on day 8 at baseline (predose), 30 minutes into the i.v.-infusion (end of infusion for patients given carboplatin over 30 minutes), 60 minutes (only for patients given carboplatin over 60 minutes to coincide with the end of infusion), and then 30 minutes, 1 hour, 2 hours, 4 hours, and 7 to 8 hours after the end of carboplatin i.v. infusion.

Pharmacodynamics

Exploratory pharmacodynamic (PD) endpoints included global LINE-1 DNA methylation analysis in PBMCs and ovarian tumor DNA, and methylation status of selected genes in tumor tissue before and after treatment. Changes in LINE-1 methylation levels in PBMCs were assessed at day 1, day 8, and day 15 of cycle 1, and day 1 and day 8 of cycles $\geq$2. Ascites or pleural fluid, or tumor biopsies (guided visually or by CT or ultrasound, according to institutional standards) were obtained at screening (baseline) and posttreatment (cycle 2, day 8 before carboplatin dose), if safe and feasible. DNA was extracted from PBMCs and tumor biopsies or ascites using the DNeasy Blood and Tissue Kit (Qiagen) at Covance. DNA concentrations were measured using a NanoDrop ND1000 Spectrophotometer. Sodium bisulfite conversion of genomic DNA was performed using the EpiTect Fast Bisulfite Conversion Kit (Qiagen) followed by pyrosequencing analysis for LINE-1 elements using a PyroMark Q24 platform at Astex. Pyrosequencing of specific gene promoters was performed by EpigenDx Inc. RNA was isolated from tumor biopsies or ascites using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen) following the manufacturer's protocol. Total RNA was reverse transcribed and analyzed by qRT-PCR with the LightCycler 480 SYBR Green I Master kit (Roche). Primers for selected genes were from Fisher Scientific. mRNA expression levels were determined using LightCycler software version 3.5 (Roche Applied Science), normalized to EEF1α1b, and using the $2^{-\Delta\Delta CT}$ method of relative quantification.

Statistical design and analyses

The sample size of at least 14 patients at the MTD provided a 51% probability of observing at least one adverse reaction to treatment if the true incidence of such reaction were 5% at the MTD. Also, 14 patients allowed the rejection of a response rate of 20% or more with a 95% confidence if no responses were observed in 14 patients treated at the MTD. The analysis data sets for efficacy and safety included all patients who received at least 1 dose of study treatment.

Descriptive statistics were used to summarize patient characteristics, and safety, efficacy, PD and PK endpoints. DOR, PFS and OS were estimated using the Kaplan–Meier method. Median durations of response, PFS, and OS as well as their corresponding 95% confidence intervals (CI) were also provided.

Results

Patients

Twenty-one patients enrolled and 20 patients were treated; 1 patient declined rapidly prior to initiation of treatment. Six patients were treated at the first dose level of guadecitabine 45 mg/m^2 and carboplatin AUC5 and 14 patients were treated at guadecitabine 30 mg/m^2 and carboplatin AUC4. All patients had measurable disease. Median age was 56 (range, 38–72; Table 1). All patients had platinum-resistant high-grade serous adenocarcinoma: 18 ovarian cancer, 1 FTC, and 1 PPC. The group was heavily pretreated with 14 patients having received $\geq$5 prior regimens. The median number of prior regimens was 7.0 (range, 1–14), and the median number of prior platinum-based regimens was 2.5 (range, 1–11).

Safety

The primary objective of stage 1 of the study was to assess safety and tolerability and determine the MTD for stage 2. Six patients were enrolled in the first cohort at 45 mg/m^2 guadecitabine and carboplatin AUC5. In this cohort, 4 of 6 patients had DLTs (grade 4 neutropenia in all 4 patients, and grade 4 thrombocytopenia in 2 patients). One patient was withdrawn from treatment due to the

Table 1. Patient characteristics

Demographics and baseline characteristics	G + C 30 mg/m^2 ($N = 14$)	G + C 45 mg/m^2 ($N = 6$)	Total ($N = 20$)
Age (y)			
Median	55.81	55.66	55.81
Range (min–max)	38.6–71.9	38.2–72.6	38.2–72.6
Race, *n* (%)			
Asian	0	1 (17)	1 (5)
Black or African American	1 (7)	0	1 (5)
White	12 (86)	5 (83)	17 (85)
Other	1 (7)	0	1 (5)
Ethnicity, *n* (%)			
Hispanic or Latino	1 (7)	0	1 (5)
Not Hispanic or Latino	13 (93)	6 (100)	19 (95)
Diagnosis, *n* (%)			
Serous ovarian cancer	13 (93)	5 (83)	18 (90)
Primary peritoneal carcinomatosis	0	1 (17)	1 (5)
Fallopian tube cancer	1 (7)	0	1 (5)
ECOG performance status, *n* (%)			
0	7 (50)	4 (67)	11 (55)
1	7 (50)	2 (33)	9 (45)
2	0	0	0
Number of prior regimens, *n* (%)			
1–2	1 (7)	2 (33)	3 (15)
3–4	3 (21)	0	3 (15)
≥5	10 (71)	4 (67)	14 (70)

Abbreviation: G + C, guadecitabine and carboplatin.

DLTs, guadecitabine was reduced to 30 mg/m^2 for subsequent cycles in this cohort for 4 of the 5 remaining patients, and 1 patient who did not experience any DLTs continued at 45 mg/m^2. Two patients also had the carboplatin dose reduced to AUC4 starting at cycle 2. For cohort 2, guadecitabine was reduced to 30 mg/m^2 and carboplatin was given at AUC4. Initially 6 patients were enrolled in cohort 2, and then another 8 patients were enrolled for a total of 14. No DLTs were encountered among the 14 patients treated at this dose level; therefore, guadecitabine 30 mg/m^2 on days 1 to 5 and carboplatin AUC4 on day 8 was subsequently declared the MTD. The median number of treatment cycles administered was 4 (range, 1–12). Causes for treatment discontinuation were progressive disease (12 patients), AEs (4 patients), patient decision (1 patient), and investigator decision (3 patients). Dose delays were required for 30% of cycles delivered to patients in cohort 1, and 26% of cycles delivered to patients in cohort 2. Five of 6 patients enrolled in cohort 1 and all patients in cohort 2 received >95% of the intended dose across all cycles. Eighteen of 20 subjects (90%) received at least 1 treatment with growth factor support.

Common treatment-related AEs included neutropenia (65%), nausea (55%), fatigue (50%), anemia (50%), injection site reaction (45%), thrombocytopenia (40%), leukopenia (35%), and vomiting (25%; Table 2). Six of 6 and 11 of 14 patients enrolled in cohorts 1 and 2, respectively, had a ≥3 grade AE (Supplementary Table S1). Grade ≥3 AEs occurring in more than 1 patient included neutropenia (50% in the 30 mg/m^2 group, and 100% in the 45 mg/m^2 group), leukopenia (29% and 33%, respectively), anemia (14% and 67%), nausea (14% and 33%), vomiting (14% and 17%), ascites (7% each), constipation (14% and 0%), hypokalemia (14% and 0%), pulmonary embolism (7% and 17%), small intestinal obstruction (14% and 0%), and thrombocytopenia (0% and 33%). The 2 events of ≥grade 3 thrombocytopenia occurred in patients in cohort 1 after treatment with 45 mg/m^2 guadecitabine and carboplatin AUC5, but did not occur in cohort 1 after dose reduction. Eight patients experienced carboplatin hypersensitivity reactions, but only 1 discontinued treatment permanently and 2 suspended treatment temporarily. One patient had a grade 3 carboplatin hypersensitivity reaction that was considered serious due to need for hospitalization; all other carboplatin hypersensitivity reactions were grade 1 or 2. Twelve patients (60%) had at least 1 serious AE. Serious AEs included nausea and vomiting (each in 4 patients), neutropenia (in 3 patients), constipation, small intestinal obstruction, and pulmonary embolism (each in 2 patients), and anemia, febrile neutropenia, thrombocytopenia, diarrhea, adverse drug reaction, pain, cholelithiasis, cellulitis, pneumonia, sepsis, postoperative fever,

Table 2. Treatment-related AEs occurring in 3 or more patients

Adverse event	G + C 30 mg/m^2 ($N = 14$)	G + C 45 mg/m^2 ($N = 6$)
Any related event	13 (93)	6 (100)
Neutropenia	7 (50)	6 (100)
Nausea	9 (64)	2 (33)
Fatigue	8 (57)	2 (33)
Anemia	6 (43)	4 (67)
Injection site reaction	7 (50)	2 (33)
Thrombocytopenia	3 (21)	5 (83)
Carboplatin reaction[a]	5 (36)	3 (50)
Leukopenia	5 (36)	2 (33)
Vomiting	3 (21)	2 (33)
Injection site pain	4 (29)	0
Constipation	3 (21)	1 (17)
Hyponatremia	3 (21)	1 (17)
Adverse drug reaction	2 (14)	1 (17)
Infusion-related reaction	1 (7)	2 (33)
Pyrexia	1 (7)	2 (33)

Abbreviation: G + C, guadecitabine and carboplatin.
[a]Carboplatin reaction includes events coded as drug hypersensitivity, adverse drug reaction, and infusion-related reaction.

transaminases increased, decreased appetite, failure to thrive, pain in jaw, cerebrovascular accident, seizure, mental status changes, and pleural effusion (each in 1 patient). Seven patients (35%) had a serious AE that was considered related to treatment (4 of the 7 patients with related serious AEs were in the 45 mg/m^2 group). Treatment-related serious AEs included neutropenia (in 3 patients), and anemia, febrile neutropenia, thrombocytopenia, nausea, adverse drug reaction, sepsis, and pain in jaw (in 1 patient each). One treatment-related death was recorded at the first dose level (sepsis). An additional patient died due to disease progression within 30 days of study treatment.

Efficacy

All patients had measurable disease and were assessed by RECIST. Efficacy outcomes are summarized in Supplementary Table S1. Because all but one patient in the 45 mg/m^2 guadecitabine group was dose reduced to 30 mg/m^2 for the second and subsequent cycles, efficacy was analyzed for all patients combined. Three patients had a PR and 6 patients had SD >3 months, resulting in an ORR (CR + PR) of 15% (3 of 20 patients) and a CBR (CR+PR+SD) of 45% (9 of 20 patients). The median DOR was 225 days (7.5 months) for the 9 patients with CBR. For the 3 patients who experienced a PR, the durations of response were 1.8, 4.2, and 7.5 months. Median PFS was 111 days (3.7 months), and the rate of PFS at 6 months was 35%. Median OS was 327 days (10.9 months) and the OS rate at 6 months was 70%. The median best reduction in CA-125 was 24%. Five of 15 evaluable patients (33%) had a CA-125 reduction of at least 50%.

Pharmacokinetics

PK analyses of guadecitabine and decitabine were conducted on samples collected at cycle 1 day 1. After a single s.c. dose of 45 or 30 mg/m^2 of guadecitabine, the plasma exposure profile for parent guadecitabine lasted up to 8 hours or longer if extrapolated. The average peak guadecitabine concentration (C_{max}) was 96.2 ng/mL at the 30 mg/m^2 dose and 109 ng/mL at the 45 mg/m^2 dose and occurred within 2 hours after dose (median T_{max}). The mean extent of exposure (measured as $AUC_{0\text{-}t}$) was 232 ng × h/mL at the 30 mg/m^2 dose and 363 ng × h/mL at the 45 mg/m^2 dose. The elimination of guadecitabine from plasma was fast with a mean $T_{1/2}$ el of 0.93 to 2.2 hours (Fig. 1A; Supplementary Table S2). As a result of the PK profile of parent guadecitabine in plasma, its active metabolite decitabine was rapidly and continuously formed via conversion, achieving an exposure window of >8 hours (Fig. 1B; Supplementary Table S3). Decitabine was detected at the first sampling time of 15 minutes after dose and peaked at a C_{max} of 22.6 ng/mL and 26.3 ng/mL for the 30 and 45 mg/m^2 guadecitabine doses, respectively. The mean decitabine $AUC_{0\text{-}t}$ was 68.3 ng × h/mL and 105 ng × h/mL for the 30 mg/m^2 and 45 mg/m^2 guadecitabine dose, respectively. The elimination of decitabine from plasma was fast with a mean $T_{1/2}$ el of 1.25 to 1.30 hours. The ratio of mean decitabine to mean guadecitabine AUC values was 0.72, calculated based on conversion to nmol/L, suggesting that more than 70% of guadecitabine was converted to the active metabolite, as measured in systemic circulation.

Pharmacodynamics

To determine the biological activity of the regimen, methylation of LINE-1 was measured by quantitative bisulfite sequencing in DNA extracted from PBMCs collected on days 1 and 8 of each cycle of treatment. The average LINE-1 demethylation at day 8 across multiple cycles compared with baseline was 19%, with no difference being observed between the 6 patients treated at 45 mg/m^2 (cohort 1) and the 14 patients who received 30 mg/m^2 (cohort 2). The average baseline LINE-1 methylation was 75% (range, 67%–82%) and was reduced to 60% after guadecitabine (range, 47%–72%; Fig. 2A). Importantly, the decreased levels of methylation observed during cycle 1 were maintained or decreased further during subsequent cycles (Fig. 2B), demonstrating preserved biological activity of the regimen throughout treatment. Of 9 patients experiencing significant LINE-1 hypomethylation in PBMCs, 7 had stable disease or a PR; while 2 of 3 patients

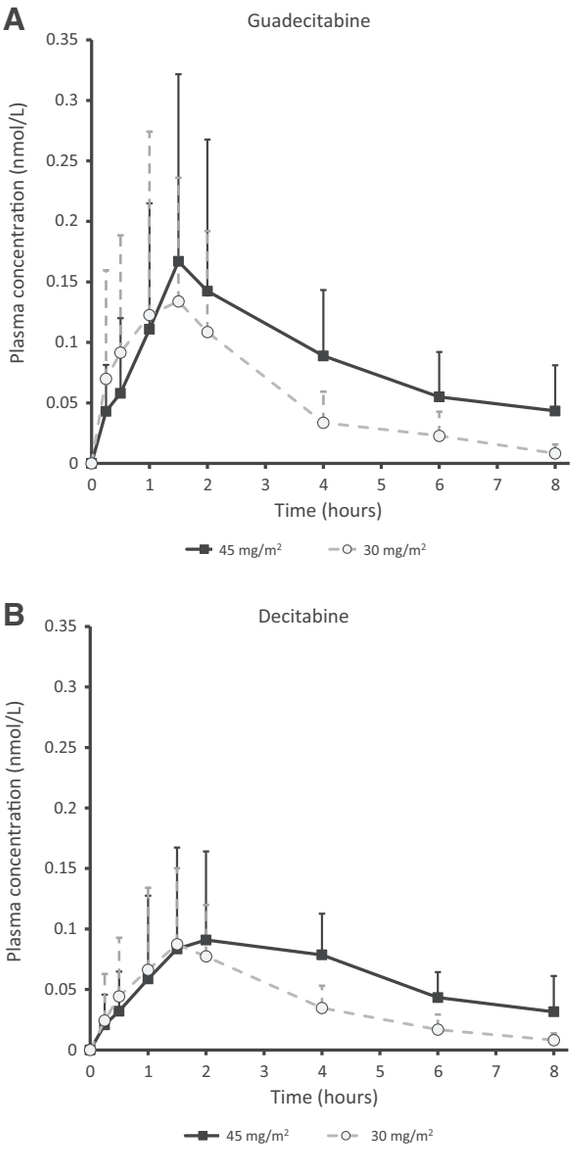

Figure 1.

PK analyses measured plasma concentrations of guadecitabine (**A**) and of the metabolite, decitabine (**B**), in plasma specimens during cycle 1 day 1. Average concentrations (±SD) from patients enrolled in the two cohorts (45 mg/m^2, n = 5 patients, and 30 mg/m^2, n = 14 patients) are presented for each time point.

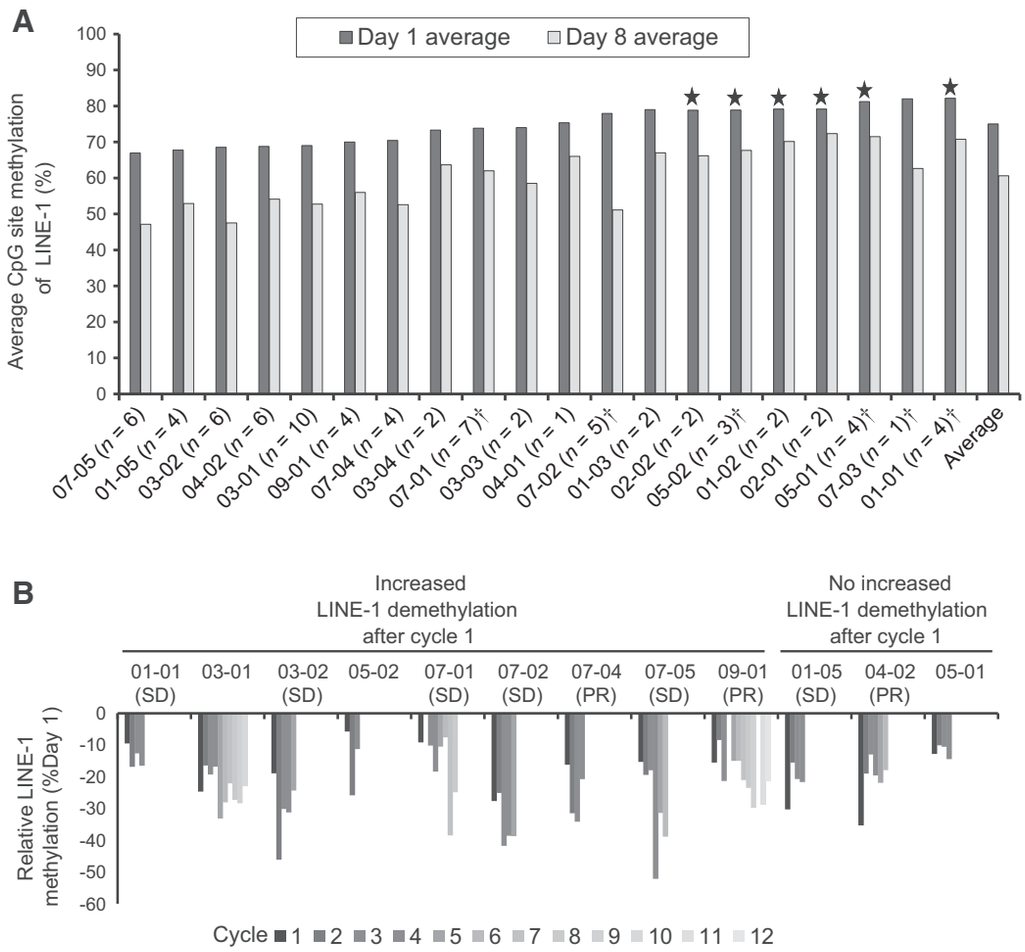

Figure 2.

PD analyses. **A,** Average LINE-1 methylation levels in PBMCs across multiple treatment cycles (number of cycles for each subject identified by *n* in the *x*-axis). Pretreatment methylation is shown as the mean LINE-1 methylation on day 1 of each analyzed cycle; posttreatment methylation is shown as the mean LINE-1 methylation on day 8 of each analyzed cycle. Patients treated with 45 mg/m^2 are noted with †. Patients who showed lower relative demethylation than average and higher basal methylation are marked with a star. **B,** Relative LINE-1 demethylation (day 8 vs. day 1) in PBMCs in each cycle of treatment for 12 patients who received more than 2 cycles of treatment. Patients showing increased LINE-1 demethylation after cycle 1 are on the left side of the figure, patients without increased LINE-1 demethylation are on the right side. SD, stable disease; PR, partial response.

without significant PBMC hypomethylation experienced stable disease or a response (Fig. 3A); thus, a correlation between clinical and biological response cannot be made in this patient set.

Tumor biopsies were obtained from 14 patients at baseline and paired tumor biopsies (one at baseline and one during treatment, generally at C2D8) were available for 10 patients. Nine pairs of tumor snap-frozen biopsies yielded sufficient amount of high-quality DNA and RNA for methylation and gene expression analyses. Methylation of the promoter of several genes previously identified as being epigenetically silenced or having a role in therapy resistance (11, 13, 21–23) was assessed. Overall, treatment-induced hypomethylation of tumor suppressor genes *RASSF1A* (Ras associated domain protein member 1A) and *DOK2* (docking protein 2), the cancer testis antigen genes *MAGEA2* and *MAGEA11*, and the receptors *FZD1* (frizzled 1) and *ESRRA* (estrogen-related receptor alpha) genes (Fig. 3A) was observed in tumor tissue; however, this did not reach statistical significance, due to large variability among the few samples analyzed. Gene-specific demethylation was generally associated with increased expression of the related transcripts, as measured by qRT-PCR (Fig. 3B).

Discussion

The results of the first stage of this trial demonstrate feasibility and tolerability of repetitive administration of guadecitabine and carboplatin combination therapy in women with heavily pretreated platinum-resistant ovarian cancer, supporting further testing of the regimen in the preplanned randomized portion of the study. As predicted by the known toxicity profiles of HMAs in other settings (24, 25) and by the results of the phase II trial of guadecitabine as single agent in recurrent AML (26), treatment-naïve AML (27) and MDS (data on file), the DLT in this trial was grade 4 neutropenia. These results are consistent with

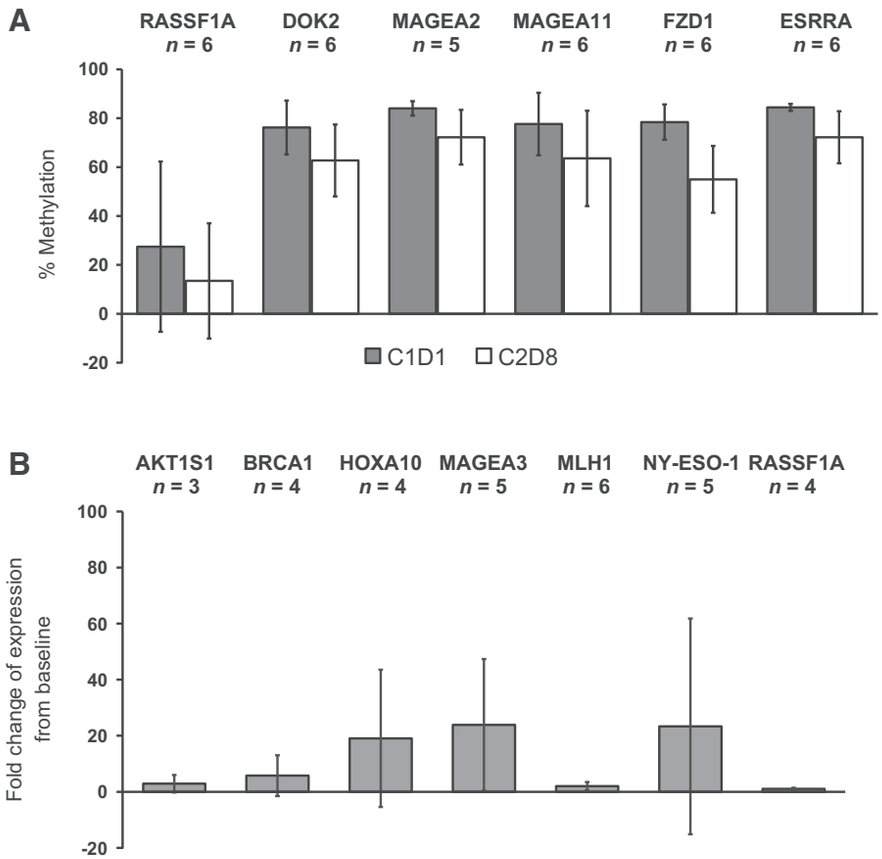

Figure 3.

Gene-specific methylation and expression levels in tumor biopsies. **A,** Promoter methylation was measured for selected genes in tumor DNA obtained before and after treatment. Bars, average values ±SD; *n*, number of patients included in each analysis. **B,** Gene expression levels were quantified by qRT-PCR in tumor RNA obtained before and after treatment. Bars, average values ±SD; *n*, number of patients included in each analysis.

observations from previous trials testing decitabine and carboplatin (11, 28, 29) or 5-azacitadine and carboplatin (14), where severe and prolonged neutropenia was also recorded. Administration of repetitive lower doses of the HMA has been found to be better tolerated compared with bolus administration (29). While most patients received the intended dose intensity, dose delays were encountered in approximately one third of patients. Guadecitabine and carboplatin dose reductions to 30 mg/m^2 daily for 5 days and to AUC4, respectively, and use of growth factor support in 90% of patients enabled safe delivery of the regimen in cohort 2 of this study, without additional severe toxicity. Mild gastrointestinal toxicity, fatigue, and injection site reactions were other common AEs in this trial. Carboplatin-associated hypersensitivity reactions, which had been previously reported with the decitabine and carboplatin combination (11, 13, 29), were also recorded in 8 patients enrolled on this trial, higher than the anticipated rate of allergic reactions upon reexposure to platinum (30). It had been speculated that HMAs might elicit immune reactivation by unmasking antigens silenced epigenetically (31), which may contribute to this phenomenon.

PD and PK analyses revealed that guadecitabine administered daily for 5 days induced potent global demethylation, as measured by pyrosequencing of LINE-1 repetitive elements in PBMCs. The level of guadecitabine-induced LINE-1 demethylation was similar to that seen in the AML trial, despite a lower dose (16) and was more pronounced (almost double) than previously observed with decitabine at 10 mg/m^2 daily for 5 days (11). Systemic plasma AUC exposures of the active metabolite decitabine in this population appeared to be higher based on guadecitabine dose-adjusted comparison with those seen in myeloid malignancies (16) and may explain the increased demethylation observed in this trial. Perhaps the longer exposure window to the active metabolite, decitabine, as demonstrated through the PK analyses performed here, permitting prolonged exposure of PBMCs to the active metabolite, explains the sustained demethylating effects. Importantly, both doses of guadecitabine tested in this trial induced similar levels of demethylation, providing reassurance that the lower dose found to be clinically tolerable in cohort 2 of this trial could be taken forward for efficacy assessment in the randomized portion of this study. Additionally, guadecitabine-induced demethylation was sustained or increased during subsequent cycles of treatment consistent with previous observations in hematologic malignancies where multiple cycles of administration of HMAs are required to reach biological and clinical effects (24).

The hypothesis addressed by the design of the trial is that guadecitabine will resensitize platinum-resistant tumors to platinum. This hypothesis is supported by preclinical studies, which have shown that treatment of platinum-resistant ovarian cancer cell lines and xenografts with an HMA reverses the resistant phenotype (17, 32, 33). One potential explanation is that the HMA would induce reexpression of TSGs or proapoptotic genes silenced epigenetically, permitting a response to chemotherapy. Examples of such TSGs repressed by DNA methylation include *MLH1*, *BRCA1*, *DOK2*, *RASSF1*, and others. Here, we observed demethylation of *DOK2* and *RASSF1* in response to treatment, but

basal levels of promoter methylation were low for both *BRCA1* (7%) and *MLH1* (4%). However, it has been challenging to point to a specific gene responsible for reversal of platinum resistance, particularly because a correlation between DNA methylation and gene expression has been difficult to prove *in vivo*. Additionally, variability and heterogeneity of tumor samples have limited the ability to point to a specific gene or mechanism reactivated in response to treatment-induced promoter hypomethylation in tumor tissue. We also acknowledge that tumor biopsies and malignant ascites samples analyzed here contain both tumor and stromal cells, which might impact results. However, bioinformatics analyses using deconvolution strategies on transcriptomic and methylomic data extracted from paired biopsies obtained from enrolled patients estimated that tumor content exceeded 60% and the observed global hypomethylation effects were independent of infiltrating immune cells (34).

A previous attempt to preselect patients based on levels of *MLH1* promoter methylation to enrich for a population likely to respond to decitabine in a prior trial was not successful due to a lower than predicted baseline *MLH1* promoter methylation (29), similar to that observed in our patient population. Thus, previous studies testing this hypothesis in xenografts or in humans have concluded that global effects of HMAs alter cancer-associated pathways, rather than specific transcripts (13, 17). Direct induction of multiple antitumorigenic mechanisms in tumor cells may include TGFβ and hedgehog signaling, immune reactivation pathways, and other metabolic networks (13, 17). Furthermore, guadecitabine has been shown to increase DNA damage induced by platinum by facilitating DNA adduct formation *in vitro* and *in vivo*, perhaps by modifying chromatin accessibility (17). Additional methylomic and transcriptomic analyses of tumor biopsies collected from this trial were recently reported (34).

The level of clinical activity observed in this trial (ORR of 15% and CBR of 45%) is remarkable for a heavily pretreated patient population, where 70% of the patients had more than 5 lines of prior therapy; however, we acknowledge that patients with aggressive platinum refractory disease were excluded from the study and that the size of the trial is small. Information on germline BRCA 1 or 2 mutational status was not collected, but given the small number of patients, it is not likely that presence of BRCA mutations impacted the observed effects. The data are consistent with our previous reports using low-dose decitabine and carboplatin in platinum-resistant ovarian cancer (11, 13) and justified proceeding with the stage 2 of the trial comparing this regimen in a randomized fashion to FDA-approved physician choice chemotherapy. Interestingly, lower response rates were observed in trials using bolus administration of HMAs with short half-life (e.g., decitabine; ref. 29), consistent with the concept that these agents are active only in cells in S-phase and that repeated daily administration captures more responsive cells, increasing the chances of inducing demethylation and a response. Full assessment of the clinical efficacy of the combination will be provided by the randomized phase of the trial and will be integrated with biological correlates derived from pre and posttreatment analyses.

Disclosure of Potential Conflicts of Interest

S. Ghamande is a consultant/advisory board member for Advaxis. A. Alvarez Secord is a consultant/advisory board member for Alexion, AstraZeneca, Clovis, Genentech, Janssen/Johnson & Johnson, Myriad, and Tesaro. M.J. Markham is a consultant/advisory board member for Astex. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: D. Matei, K.P. Nephew, M. Azab
Development of methodology: D. Matei, S. Jueliger, A. Oganesian
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Matei, S. Ghamande, L. Roman, A. Alvarez Secord, J. Nemunaitis, M.J. Markham, S. Naim, G.F. Fleming
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Matei, S. Ghamande, A. Alvarez Secord, K.P. Nephew, A. Oganesian, S. Naim, X.Y. Su, H. Keer, M. Azab
Writing, review, and/or revision of the manuscript: D. Matei, S. Ghamande, L. Roman, A. Alvarez Secord, J. Nemunaitis, M.J. Markham, K.P. Nephew, S. Jueliger, A. Oganesian, S. Naim, H. Keer, M. Azab, G.F. Fleming
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Matei, K.P. Nephew, S. Naim
Study supervision: D. Matei, A. Alvarez Secord, K.P. Nephew, S. Naim, H. Keer, M. Azab
Other (served as medical monitor): H. Keer

Acknowledgments

The authors thank Dr. F. Fang for technical assistance. This work was partly funded by National Cancer Institute Award CA133877-01, National Institutes of Health, and the V-Foundation (to D. Matei and K.P. Nephew).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 15, 2017; revised January 11, 2018; accepted February 27, 2018; published first March 2, 2018.

References

1. Bowtell DD, Böhm S, Ahmed AA, Aspuria PJ, Bast RC, Beral V, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer 2015;15:668–79.
2. Rose PG, Tian C, Bookman MA. Assessment of tumor response as a surrogate endpoint of survival in recurrent/platinum-resistant ovarian carcinoma: a gynecologic oncology group study. Gynecol Oncol 2010;117:324–29.
3. Mutch DG, Orlando M, Goss T, Teneriello MG, Gardon AN, McMeekin SD, et al. Randomized phase III trial of gemcitabine compared with pegylated liposomal doxorubicin in patients with platinum-resistant ovarian cancer. J Clin Oncol 2007;25:2811–18.
4. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers 2016;2:16061.
5. Watts GS, Futscher BW, Holtan N, Degeest K, Domann FE, Rose SL. DNA methylation changes in ovarian cancer are cumulative with disease progression and identify tumor stage. BMC Med Genomics 2008;1:47.
6. Barton CA, Hacker NF, Clark SJ, O'Brien PM. DNA methylation changes in ovarian cancer: implications for early diagnosis, prognosis and treatment. Gynecol Oncol 2008;109:129–39.
7. Balch C, Fang F, Matei DE, Huang TH, Nephew KP. Minireview: epigenetic changes in ovarian cancer. Endocrinology 2009;150:4003–11.
8. Barton CA, Clark SJ, Hacker NF, O'Brien PM. Epigenetic markers of ovarian cancer. Adv Exp Med Biol 2008;622:35–51.
9. Ibanez de Caceres I, Battagli C, Esteller M, Herman JG, Dulaimi E, Edelson MI, et al. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma, and peritoneal fluid from ovarian cancer patients. Cancer Res 2004;64:6476–81.
10. Fiegl H, Windbichler G, Mueller-Holzner E, Goebel G, Lechner M, Jacobs IJ, et al. HOXA11 DNA methylation–a novel prognostic biomarker in ovarian cancer. Int J Cancer 2008;123:725–29.
11. Fang F, Balch C, Schilder J, Breen T, Zhang S, Shen C, et al. A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in

patients with recurrent, platinum-resistant, epithelial ovarian cancer. Cancer 2010;116:4043–53.
12. Fang F, Zuo Q, Pilrose J, Wang Y, Shen C, Li M, et al. Decitabine reactivated pathways in platinum resistant ovarian cancer. Oncotarget 2014;5:3579–89.
13. Matei D, Fang F, Shen C, Schilder J, Arnold A, Zeng Y, et al. Epigenetic resensitization to platinum in ovarian cancer. Cancer Res 2012;72: 2197–205.
14. Fu S, Hu W, Iyer R, Kavanagh JJ, Coleman RL, Levenback CF, et al. 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. Cancer 2011;117:1661–69.
15. Yoo CB, Jeong S, Egger G, Liang G, Phiasivongsa P, Tang C, et al. Delivery of 5-aza-2′-deoxycytidine to cells using oligodeoxynucleotides. Cancer Res 2007;67:6400–08.
16. Issa JP, Roboz G, Rizzieri D, Jabbour E, Stock W, O'Connell C, et al. Safety and tolerability of guadecitabine (SGI-110) in patients with myelodysplastic syndrome and acute myeloid leukaemia: a multicentre, randomised, dose-escalation phase 1 study. Lancet Oncol 2015;16: 1099–110.
17. Fang F, Munck J, Tang J, Taverna P, Wang Y, Miller DF, et al. The novel, small-molecule DNA methylation inhibitor SGI-110 as an ovarian cancer chemosensitizer. Clin Cancer Res 2014;20:6504–16.
18. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
19. Rustin GJ. Use of CA-125 to assess response to new agents in ovarian cancer trials. J Clin Oncol 2003;21(10 Suppl):187s–93s.
20. Rustin GJ, Bast RC Jr, Kelloff GJ, Barrett JC, Carter SK, Nisen PD, et al. Use of CA-125 in clinical trial evaluation of new therapeutic drugs for ovarian cancer. Clin Cancer Res 2004;10:3919–26.
21. Zeller C, Dai W, Steele NL, Siddiq A, Walley AJ, Wilhelm-Benartzi CS, et al. Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. Oncogene 2012;31:4567–76.
22. Lum E, Vigliotti M, Banerjee N, Cutter N, Wrzeszczynski KO, Khan S, et al. Loss of DOK2 induces carboplatin resistance in ovarian cancer via suppression of apoptosis. Gynecol Oncol 2013;130:369–76.
23. Wang Y, Cardenas H, Fang F, Condello S, Taverna P, Segar M, et al. Epigenetic targeting of ovarian cancer stem cells. Cancer Res 2014;74: 4922–36.
24. Kantarjian HM, O'Brien S, Cortes J, Giles FJ, Faderl S, Issa JP, et al. Results of decitabine (5-aza-2′deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia. Cancer 2003;98:522–8.
25. Issa JP, Gharibyan V, Cortes J, Jelinek J, Morris G, Verstovsek S, et al. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. J Clin Oncol 2005;23:3948–56.
26. Roboz GJ, Kantarjian H, Yee KWL, Kropf PL, O'Connell CL, Griffiths EA, et al. Dose, schedule, safety, and efficacy of guadecitabine in relapsed or refractory acute myeloid leukemia. Cancer 2018;124:325–34.
27. Kantarjian HM, Roboz GJ, Kropf PL, Yee KWL, O'Connell CL, Tibes R, et al. Guadecitabine (SGI-110) in treatment-naive patients with acute myeloid leukaemia: phase 2 results from a multicentre, randomised, phase 1/2 trial. Lancet Oncol 2017;18:1317–26.
28. Appleton K, Mackay HJ, Judson I, Plumb JA, McCormick C, Strathdee G, et al. Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. J Clin Oncol 2007;25: 4603–09.
29. Glasspool RM, Brown R, Gore ME, Rustin GJ, McNeish IA, Wilson RH, et al. A randomised, phase II trial of the DNA-hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in combination with carboplatin vs. carboplatin alone in patients with recurrent, partially platinum-sensitive ovarian cancer. Br J Cancer 2014;110:1923–29.
30. Gadducci A, Tana R, Teti G, Zanca G, Fanucchi A, Genazzani AR. Analysis of the pattern of hypersensitivity reactions in patients receiving carboplatin retreatment for recurrent ovarian cancer. Int J Gynecol Cancer 2008; 18:615–20.
31. Srivastava P, Paluch BE, Matsuzaki J, James SR, Collamat-Lai G, Blagitko-Dorfs N, et al. Induction of cancer testis antigen expression in circulating acute myeloid leukemia blasts following hypomethylating agent monotherapy. Oncotarget 2016;7:12840–56.
32. Plumb JA, Strathdee G, Sludden J, Kaye SB, Brown R. Reversal of drug resistance in human tumor xenografts by 2′-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. Cancer Res 2000; 60:6039–44.
33. Li M, Balch C, Montgomery JS, Jeong M, Chung JH, Yan P, et al. Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. BMC Med Genomics 2009;2:34.
34. Fang F, Cardenas H, Huang H, Jiang G, Perkins SM, Zhang C, et al. Genomic and epigenomic signatures in ovarian cancer associated with re-sensitization to platinum drugs. Cancer Res 2018;78:631–44.