A randomized phase 2 trial of epigenetic priming with guadecitabine and carboplatin in platinum-resistant, recurrent ovarian cancer

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ABSTRACT

PURPOSE:

Platinum resistance in ovarian cancer (OC) is associated with epigenetic modifications. Hypomethylating agents (HMAs) have been studied as carboplatin re-sensitizing agents in OC. This randomized phase 2 trial compared guadecitabine, a second generation HMA, and carboplatin (G+C) against second-line chemotherapy in women with measurable or detectable platinum-resistant OC.

PATIENTS AND METHODS:

Patients received either G+C (guadecitabine 30 mg/m^2 SC once-daily for 5 days and carboplatin) or treatment of choice (TC; topotecan, pegylated liposomal doxorubicin, paclitaxel, or gemcitabine) in 28-day cycles until progression or unacceptable toxicity. The primary endpoint was progression-free survival (PFS); secondary endpoints were RECIST v1.1 and CA-125 response rate, 6-month PFS, and overall survival (OS).

RESULTS:

Of 100 patients treated, 51 received G+C and 49 received TC, of which 27 crossed over to G+C. The study did not meet its primary endpoint as the median PFS was not statistically different between arms (16.3 weeks vs 9.1 weeks in the G+C and TC groups, respectively; $P = 0.07$). However, the 6-month PFS rate was significantly higher in the G+C group (37% vs. 11% in TC group; $P = 0.003$). The incidence of grade 3 or higher toxicity was similar in G+C and TC groups (51% and 49%, respectively), with neutropenia and leukopenia being more frequent in the G+C group.

CONCLUSIONS:

Although this trial did not show superiority for PFS of G+C versus TC, the 6-month PFS increased in G+C treated patients. Further refinement of this strategy should focus on identification of predictive markers for patient selection.

TRANSLATIONAL RELEVANCE

Although women with ovarian cancer (OC) initially respond to platinum-based chemotherapy, platinum-resistance commonly develops, leading to fatal outcomes. We set out to determine if epigenetic priming with a hypomethylating agent (HMA) prior to carboplatin improved progression-free survival (PFS) in platinum-resistant OC when compared with physician's choice chemotherapy in a randomized phase 2 trial. The median PFS and overall survival were not different, but the 6-month PFS rate was higher in the experimental group. Myelosuppression was the main toxicity observed with the experimental regimen and hypomethylating activity was measurable in PBMCs. Further development of the strategy will require identification of predictive biomarkers for patient selection.

INTRODUCTION

Advanced stage high-grade serous ovarian cancer (HGSOC), which is distinctively associated with a p53 mutated signature, has a poor estimated five-year survival of 50% (1). Although patients with HGSOC usually respond to initial platinum-based chemotherapy, relapses occur in most, leading to the development of platinum-resistance and subsequent death (2-3). Progression of HGSOC to a platinum-resistant state is caused by multiple mechanisms, including aberrant DNA repair responses, alterations in efflux pump proteins, and accumulated genomic and epigenomic modifications which impact the response of cancer cells to DNA damage. Adaptive responses include increased DNA methylation and modifications of histone marks (4-5), which cause transcriptional silencing of tumor suppressor genes (TSGs) and other genes required for chemotherapy-induced cell death (6-7).

Given preclinical data demonstrating that targeting DNA methylation to re-sensitize HGSOC to platinum is possible (8-11), we hypothesized this approach would restore platinum sensitivity in HGSOC patients (12,13). With early clinical studies demonstrating feasibility of this strategy (13-16), we set out to determine whether targeting DNA methylation induces clinically meaningful activity in platinum-resistant HGSOC by conducting a randomized phase 2 trial. The objectives were to measure and compare clinical outcomes of a combination regimen of the DNA methyltransferase inhibitor (DNMTI), guadecitabine, and carboplatin, versus FDA-approved physician's choice chemotherapy (liposomal doxorubicin, weekly paclitaxel, topotecan, or gemcitabine). Guadecitabine is a dinucleotide linking decitabine to guanosine via a phosphodiester bond. Guadecitabine is resistant to degradation by cytidine deaminase and has a longer half-life compared to other DNMTIs. In a dose-finding phase I trial (17), therapeutic

plasma levels of decitabine persisted beyond 8 hours. This pharmacokinetic profile provides a longer window of exposure to the hypomethylating agent (HMA), potentially exposing more cancer cells undergoing S-phase to the parent drug, decitabine, and promoting hypomethylation. Guadecitabine was shown to exert anti-tumor activity in OC xenografts as a single agent and in combination with carboplatin (11, 18, 19).

A recently reported phase 1 trial established the tolerable and biologically active dose of guadecitabine in combination with carboplatin (17). Guadecitabine was tolerable at 30 mg/m^2 SC daily for 5 days prior to carboplatin on Day 8 at an AUC of 4. Each cycle was 28 days and the regimen induced ~20% hypomethylation of long interspersed nuclear elements (LINE-1) in peripheral blood mononuclear cells (PBMCs), indicating biological activity. The phase 1 trial reported three patients with partial response (PR) and six patients with stable disease (SD) longer than 3 months (17), providing the rationale for conducting this randomized trial in women with platinum-resistant HGSOC. Here we report clinical outcomes with G+C as compared to physician's choice FDA-approved chemotherapy for OC in this high-need patient population.

METHODS

Trial Design and Patient Population:

This was a multicenter, randomized, open-label phase 2 trial conducted at 20 centers in the US, UK, and Canada. Eligible patients were ≥18 years old with platinum-resistant histologically- or cytologically-confirmed recurrent high-grade serous, or grade 2-3 endometrioid, mixed cell or clear cell epithelial OC; primary peritoneal carcinoma (PPC); or fallopian tube (FT) cancer. All

patients were required to have received carboplatin and taxanes. Platinum-resistance was defined as recurrence within 6 months of the last platinum-containing regimen. 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 one of the following: (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 were performed to recover tumor cells and were required at baseline and on Cycle 2 Day 8, if clinically safe and feasible. Eligible patients had acceptable organ function based on laboratory data, Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and were ≥3 weeks from their last therapy. Exclusion criteria included carboplatin hypersensitivity, prior HMA therapy, progression on platinum treatment, left ventricular ejection fraction <50%, grade 2 or greater peripheral neuropathy, known brain metastases, other malignancies, active infections, or life-threatening illnesses. The trial was conducted in accordance with the International Council for Harmonisation Good Clinical Practice guidelines and applicable local regulatory requirements according to the Declaration of Helsinki. Local Institutional Review Boards and Independent Ethics Committees reviewed and approved the protocol and the informed consent form. Patients provided written informed consent before enrollment. The trial is registered on ClinicalTrials.gov as NCT01696032. Trial protocol and amendments are available as Supplements 1 and 2, respectively.

Randomization, Trial Intervention and Clinical Outcomes:

Eligible subjects were randomly assigned (1:1) to receive a 28-day treatment cycle of either a G+C combination treatment (guadecitabine 30 mg/m^2 SC once-daily on Days 1–5 and

carboplatin IV AUC 4 on Day 8), or treatment choice (TC) of topotecan IV (3.5–4.0 mg/m^2/wk administered on Days 1, 8 and 15), pegylated liposomal doxorubicin IV (PLD; 40–50 mg/m^2 administered on Day 1), paclitaxel IV (60–80 mg/m^2/wk administered on Days 1, 8, 15 and 22), or gemcitabine IV (800–1000 mg/m^2 administered on Days 1, 8 and 15); treatment choice in the TC arm was at the investigator's discretion. Randomization was stratified by number of prior chemotherapies and by treatment center using an unblinded approach using a centralized web-based system. Concomitant medications and therapies were allowed, as deemed necessary for supportive care and safety of subjects; administration of other anti-cancer agents was not permitted. Treatment in both arms continued until disease progression or unacceptable toxicity. If the investigator decided to stop carboplatin treatment after 4 or more cycles, guadecitabine could be continued until progression or initiation of an alternative anti-cancer treatment. Crossover from the TC arm to the G+C arm was permitted after evidence of disease progression in the standard therapy arm.

The primary endpoint was PFS. Secondary efficacy endpoints included objective response rate (ORR: defined as complete response [CR] and partial response [PR] based on both measurable and evaluable disease), PFS at 6 months, clinical benefit rate (CBR: defined as CR+ PR + stable disease for at least 3 months), proportion of patients with CA-125 reduction of at least 50%, duration of response (DOR), and overall survival (OS); in subjects crossing over from the TC to the G+C arm, ORR was measured. Response was assessed using RECIST v1.1 for patients with measurable disease (20), and modified Rustin criteria for patients with detectable disease according to CA-125 criteria (21-22). Tumor measurements were obtained by CT or MRI at screening, after every 2 cycles for the first six cycles, and every three months until progression.

Safety was assessed by subject-reported and investigator-observed adverse event (AE) recording, along with physical examination, 12-lead electrocardiograms, hematology, chemistry, and urinalysis with each cycle. There was a 30-day (+5 day) safety visit after the last treatment. AEs were graded by Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Treatment-emergent AEs (TEAEs) were defined as events that first occurred or worsened after the first dose of trial drug given on the first day of the first treatment cycle until 30 days after the last dose of treatment. Related serious AEs (SAEs) that occurred more than 30 days after the last dose were also considered TEAEs; AEs occurring after the start of an alternative anti-cancer treatment were not considered TEAEs. Patients lost to follow-up were included in statistical analyses to the point of their last evaluation.

Exploratory pharmacodynamic endpoints included quantitative analysis of LINE-1 methylation in PBMCs and tumor DNA, and of selected gene promoters in tumor tissue. Blood samples for methylation assays were collected weekly during Cycle 1 and on Day 1 and Day 8 thereafter. Global DNA methylation was evaluated by sodium bisulfite pyrosequencing for LINE-1 CpGs using PyroMark Q24 as previously described (17). Ascites, pleural fluid, or fresh tumor biopsies were obtained at screening and on Day 8 of Cycle 2 for assessment of methylation of selected genes listed in the supplementary information (Supplementary Table S1). DNA was extracted from tumor biopsies or ascites using DNeasy Blood & Tissue Kit (Qiagen, Netherlands) and LINE-1 and specific gene pyrosequencing was performed at EpigenDx Inc (Hopkinton, MA).

Statistical Design and Analyses:

It was estimated a sample size of ≥96 patients randomized 1:1 into two treatment arms would provide approximately 80% power to detect a difference between the two PFS curves (median

PFS of 15 vs. 28 weeks for the TC and G+C arms) at 5% significance level using a two-sided log-rank test, assuming uniform accrual of subjects over 12 months, a 24-month trial duration and an exponential distribution of the PFS endpoint. PFS, OS, and 95% confidence intervals (CIs) were evaluated using the Kaplan-Meier method. PFS and OS were compared using the log-rank test, while ORR and CBR were compared using Fisher's exact test. Subjects still alive with no progression and those who withdrew were censored on the date of the last adequate tumor, CA-125, or clinical progression assessment. Subjects initiating subsequent anti-cancer therapy, including those who crossed over, were censored accordingly, but prior to the initiation. Survival time was censored on the last date the subject was known to be alive or lost to follow-up before reaching the event of death. Efficacy and safety data for subjects who crossed over were tabulated separately once guadecitabine was first administered. All analyses are descriptive and inferential statistical tests and CIs were two-sided with alpha equal to 0.05 unless otherwise specified. The database was locked for analysis on July 7, 2016 with mature PFS data; 97 of the 100 treated patients progressed or did not survive and all patients discontinued protocol therapy at this time (Figure 1). LINE-1 and gene-specific methylation level differences before and after G+C treatment were determined using paired *t*-tests. SAS version 9.3 was used for all statistical analyses.

RESULTS

One hundred and three patients with HGSOC, FT cancer, or PPC were enrolled and randomized (52 G+C, 51 TC) and 100 received treatment (51 G+C, 49 TC; Figure 1). Baseline characteristics are summarised in Table 1 and were well balanced between the two arms in terms of age, performance status, prior therapy, and ethnicity. More patients randomized to the G+C arm had PPC compared to those randomized to TC (10 vs. 0). Most subjects were white, with a median

age of 62 years, and all received prior platinum-based therapy (Table 1). Of the patients randomized to TC, 11 received weekly paclitaxel, 15 received liposomal doxorubicin, 20 received topotecan, and 3 received gemcitabine. Patients in the G+C arm received more treatment cycles than subjects in the TC arm (median of 4.0 vs. 2.0 cycles, respectively), with 59% of subjects in the G+C arm receiving at least 3 cycles of treatment and 37% receiving at least 6 cycles of treatment vs. 47% and 31% of subjects in the TC arm, respectively. Fifty-five percent of patients from the TC arm crossed over to G+C arm following progression (Figure 1). Disease progression was the most common reason for discontinuing treatment (~80% of patients in each group; Figure 1). The most common TEAEs occurring in more than 5% of the trial population are reported in Table 2. AE frequencies between the two arms were similar, but neutropenia, diarrhea, nausea and vomiting were more common in the G+C arm (Tables 2 and 3).

The median duration of PFS in the G+C arm was 16.2 weeks compared to 9.1 weeks in TC arm (*P*=0.07; Figure 2A and Table 4). The 6-month PFS rate was 37% in the G+C arm (95% CI, [0.24; 0.50]) compared to 11% in the TC arm (95% CI, [0.04; 0.22]; *p*=0.003) and did not meet the pre-specified criterion for superiority (HR 0.686, 95% CI, [0.456; 1.030]; Figure 2 and Table 4). There was no difference between the two arms in OS (43 and 40 weeks in the G+C and TC arms, respectively; Figure 2B and Table 4), OS survival rate at 6 months (0.72 and 0.67 in the G+C and TC arms, respectively; Table 4), overall response rate (ORR; 16% and 8% in the G+C and TC arms, respectively; Table 4), or clinical benefit response by RECIST v1.1 or CA-125 (Table 4, Supplementary Table S2). Twenty-seven patients from the TC arm crossed over post-progression into the G+C arm and received a median of 3 cycles (14 subjects received ≥3 cycles

and 5 subjects received ≥6 cycles) with a CA-125 response being confirmed in 6 of 21 evaluable subjects (29%). Patient disposition and outcomes are included in Supplementary Table S3.

To determine the biological activity of the G+C regimen, LINE1 methylation was assessed in PBMCs from 48 patients randomized to the G+C arm. Similar to the first stage of this trial (17), LINE1 hypomethylation approximated 20% (C1D8 vs. C1D1; range +15% to -55%; Supplementary Figure S1A) (17). In 15 patients who continued treatment beyond 2 cycles and for whom PBMCs were available, LINE1 hypomethylation observed during Cycle 1 was maintained or increased during subsequent cycles (Supplementary Figure S1B), indicating that G+C maintains its biological effects throughout treatment. Correlation between clinical response and pharmacodynamic effects as measured by LINE-1 hypomethylation in PBMCs was not observed. Promoter methylation of selected genes representing TSGs (23-24) or tumor antigens known to be methylated in OC (25-26) was measured in bisulfite-converted DNA obtained from paired tumor biopsies on C1D1 and C2D8 (n = 8 paired specimens). Treatment-induced hypomethylation of *MAGE-A2* and *MAGE-A3* promoters in tumor DNA was significant (Supplementary Figure S1C). A non-significant decrease in promoter CpG methylation was also observed for LINE-1 and for the tumor antigens *NY-ESO-1* and *MAGE-A11*, but not for the TSGs *RASSF1A, MLH1* and *BRCA1* (data not shown) or for the differentiation associated gene *HOXA11*. Taken together, these results provide evidence that G+C treatment exerts *in vivo* hypomethylating activity detectable in PBMCs and tumors.

DISCUSSION

This is the first randomized study comparing a regimen of G+C to standard of care chemotherapy for recurrent platinum-resistant OC. Although the 6-month PFS rate was higher in the G+C arm than the TC arm, the study did not meet its primary endpoint in this heavily pre-treated population. These results are comparable with previous single-arm phase 2 studies using an epigenetic priming with decitabine (13-14) or 5-azacitadine (15) prior to carboplatin. Those trials used repetitive low doses of DNMTIs, which is similar to the strategy employed with this class of HMAs in hematological malignancies (27-28). The repetitive administration of the HMA increases drug exposure of cells undergoing S-phase and incorporation of the nucleoside analogue into the replicating DNA, trapping DNMTs and inhibiting *de novo* methylation.

In contrast, a previous trial conducted by the Scottish Gynecological Trials Group that used bolus administration of decitabine on Day 1 prior to administration of carboplatin a week later was prematurely closed due to high hematological toxicity and indicated lower efficacy of the combination regimen compared to carboplatin alone (29). This trial reported reduction in efficacy with the addition of decitabine to patients with partially platinum sensitive recurrence when given in conjunction with carboplatin (29). Whether the difference in administration (bolus vs. low-dose repetitive administration) was solely responsible for the differences in levels of clinical activity remains unknown. The clinical efficacy differences with this trial may be attributable to the Scottish trial's inclusion of less heavily pre-treated subjects who retained partial platinum sensitivity. Since increased DNA methylation is observed in advanced bladder cancer, colon cancer, cholangiocarcinoma, and germ cell tumors (30), DNMTI-induced

sensitization to platinum or to chemotherapy is also explored in these settings with early promising results having been reported recently in colon cancer (31).

The G+C regimen had myelosuppression as the main toxicity. Prolonged neutropenia required growth factor support in >80% of the patient population to maintain the intended every-4-week administration of the combination. However, infections were rare and no episodes of neutropenic sepsis were recorded. Hypersensitivity and other adverse infusion reactions were observed in 9 (18%) and 8 (15%) patients in the G+C arm compared with 6% in the TC arm in this trial, which is concordant with similar observations from prior trials of DNMTIs and carboplatin (13, 29). This is most likely due to increased exposure to platinum therapy in the experimental arm, but it is also possible HMA treatment may augment type II allergic reactions.

The study has few limitations. While all patients in this trial had platinum-resistant disease, platinum-refractory disease was excluded. Given that carboplatin was not included among the potential control regimens, and could conceivably induce clinical benefit in selected patients, this trial cannot exclude the activity of single-agent carboplatin in this population. Additionally, topotecan administration in the TC arm followed a weekly administration schedule. While this schedule was favored among treating oncologists due to its favorable toxicity profile and early reports of activity (32), the regimen was subsequently shown to induce a decreased response rate compared to the schedule using daily administration for 5 days, although OS was not affected (33). Chemotherapy with bevacizumab became FDA-approved and an accepted standard for patients with platinum resistant OC after results of Aurelia trial were reported (34), which occurred after the inception of this protocol. Of note is that prior therapy with bevacizumab was

not excluded, and 33 patients enrolled in this trial had received bevacizumab. The shorter median PFS observed in the control group of this study (~2 months) compared to the Aurelia trial (3.4 months; 34) reflects a more heavily pre-treated group patients included here (mean of 3-4 prior regimens) for whom limited treatment options currently exist.

High-quality nucleic acids were extracted from tumor biopsies from 40 subjects at baseline and from 8 patients after two cycles of G+C. The precise mechanism by which G+C induces anti-tumor responses remains unknown. Our tissue- and cell-based analyses showed a number of genes and pathways involved in DNA repair and response to chemotherapy (e.g., *DOK2, miR193a, 14-3-3σ, RASSF1A)* are silenced through promoter methylation and re-expressed after guadecitabine treatment (35). Using overexpression or knock-down strategies, we have shown some of these pathways restore platinum sensitivity in OC cell lines and xenografts (10, 35). It is likely that not one gene, but a more global genomic program is reactivated in response to DNA hypomethylation, allowing tumor cells to undergo apoptosis in response to chemotherapy. Since preclinical models show that guadecitabine selectively eliminates chemotherapy-resistant OC stem cells (11) by inducing a cellular differentiation program, the G+C regimen may exert anti-tumor activity through multiple mechanisms. The low number of post-treatment biopsies collected in the trial limits the strength of the conclusions we can draw regarding the mechanisms elicited by this HMA *in vivo*.

This randomized trial demonstrated that epigenetic priming in combination with carboplatin did not increase PFS compared to standard chemotherapy, but improved 6-month PFS in platinum-resistant OC. Although these results do not support development of this strategy for an

283 unselected population, they suggest a subgroup of patients might have benefitted from G+C
284 treatment. Future studies should focus on developing predictive markers to enrich a patient
285 population more likely to benefit from the use of HMAs.
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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Angeles Alvarez Secord reported being paid for consulting or participating in an advisory role for Alexion, Aravive, AstraZeneca, Clovis, Janssen/Johnson & Johnson, Mesano, Myriad, Roche/Genentech, and Tesaro, and received research funding from Amgen, AbbVie, Amgen, Astellas Pharma Inc., Astex Pharmaceuticals Inc., AstraZeneca, Boerhinger Ingelheim, Bristol Myers Squibb, Eisai, Exelixis, Endocyte, Roche/Genentech, Incyte, Merck, PharmaMar, Prima Biomed, and Tesaro. Sarah Blagden reported serving in a consultant or advisory role for Novartis, Octimet and Roche, receiving travel, accommodation and expense reimbursement from NuCana, BioMed and Tesaro, receiving research funding from NuCana, BioMed, Sierra Oncology, Incyte, DesigneRx and Tesaro, and holds patents or receives royalties from RNA Guardian Ltd. Susana Banerjee reported receiving honoraria from AstraZeneca and Tesaro, serving in a consultant or advisory role for AstraZeneca, Tesaro, Clovis, Seattle Genetics, and receiving research funding from AstraZeneca. John Nemunaitis disclosed employment with Gradulis, leadership roles with Gradulis and Symvivo. He has stock or other ownership interest to disclose with Gradulis, received honoraria from AstraZeneca, has consulted for AstraZeneca and Symvivo, participated in a speaker's bureau for AstraZeneca, received research funding from Gradulis, holds patents or receives royalties from Gradulis, receives travel, accommodations, or expenses from AstrazZeneca, Symvivo and Gradulis, and been paid to provide expert testimony on behalf of Foundation Medicine. Hal Hirte reported receiving honoraria from AstraZeneca, Merck and Roche. Diane Provencher reported consulting and advising AstraZeneca. Benjamin Schwartz reported receiving honoraria from NOVADAQ. Patricia Braly reported participating in a speakers' bureau for Myriad, Invitae, Tesaro, AstraZeneca, Clovis and Roche, and receiving research funding from Tesaro, AstraZeneca, Merck, Janssen, Pharma Mar and Xenetic. Geoffrey

Hall reported receiving honoraria from and serving in a consultant or advisory role for AstraZeneca and IQVIA. Daniela Matei reported serving in a consulting or advisory role for Genentech, Tesaro, AstraZeneca, and Anydyn, and receiving travel, accommodation and expense reimbursement from Genentech. The following authors are employed by Astex Pharmaceuticals Inc: Aram Oganesian, Sue Naim, Yong Hao, Harold Keer, Mohammad Azab and Simone Jueliger.

AUTHORS' CONTRIBUTIONS:

Conception and design: Matei, Nephew, Azab, Oganesian, Naim, Hao, Keer.

Development of methodology: Matei, Nephew, Azab, Oganesian, Naim, Hao, Keer.

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): All authors.

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Matei, Nephew, Azab, Oganesian, Naim, Hao, Keer.

Writing, review, and/or revision of the manuscript: All authors.

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Matei, Oza, Azab, Naim, Hao, Keer.

Study supervision: Matei, Oza, Azab, Jueliger, Oganesian, Naim, Hao, Keer.

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FIGURE LEGENDS

Figure 1. Disposition of subjects in the trial. AUC indicates the target area under the concentration-versus-time curve.

Figure 2. Survival of subjects assigned to G+C arm versus TC arm. A: Kaplan-Meier estimates of progression-free survival with the G+C treatment and TC regimens. B: Kaplan-Meier estimates of overall survival with the G+C treatment and TC regimens. For subjects in the TC group who crossed over to receive G+C, OS time was censored at the crossover time point.