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Carboplatin And Cyclosporin A In The Treatment Of Recurrent Epithelial Ovarian Cancer

Eve Elizabeth Overton
Yale University, eve.overton@gmail.com

The Use Of Carboplatin and Cyclosporin A In The Treatment of Recurrent Epithelial Ovarian Cancer

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

By
Eve Overton
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Abstract:

The aim of this study is to report our institutional experience with the use of carboplatin (carbo) and concurrent cyclosporin A (CsA; combined treatment abbreviated as carbo/CsA) for recurrent epithelial ovarian cancer (EOC) and to assess the efficacy and tolerability of this regimen in treatment of the treatment of EOC. The medical records of patients diagnosed with ovarian cancer and treated with carbo/CsA from 2000 to 2015 at Yale New Haven Hospital were reviewed. Histological classification and staging were determined by the World Health Organization and Ann Arbor systems, respectively. Kaplan-Meier was used to calculate progression free survival (PFS) and overall survival (OS). Objective response was defined by RECIST criteria on computerized tomography (CT) or by a >50% reduction in CA-125. Statistics were run using STATA software. Fifty-four patients were identified carbo/CsA and had adequate documentation for analysis. Patients received a total of 265 cycles of carbo/CsA with a mean of 4.9 cycles per patient (range 1-10). Mean PFS was 7.7 months (SD 5.2), median 5.8 months, with a range of 1 to 25.4 months. OR by CT was observed in 7 patients, objective response by CA-125 was observed in 17 patients. There were a total of 49 patients with adequate data for measure of objective response by either CT or CA-125. By either method, a total of 19 patients exhibited objective response for an overall response rate of 38.8%. The rates of OR were significantly different in patients who were platinum resistant versus platinum sensitive (p= 0.015). Two of fifteen patients (13.3 %) defined as platinum resistant demonstrated objective response while 17 of 34 patients (50.0%) with platinum-sensitive disease experienced objective response. Five patients (9.3%) discontinued therapy due to toxicity. Most common grade III or IV toxicities included anemia (7.4%) and nausea and vomiting

(5.6%) with other grade III reactions including hypertension, headache, and pancytopenia, and allergic reaction. Most common low grade toxicities included headache (24%), nausea and vomiting (18.5%), and fatigue (16.7%). Both PFS and OS were significantly higher in patients that demonstrated objective response on carboplatin/CsA than those who did not. There was no significant association of, age at treatment, number of prior chemotherapeutic regimens, histology or disease with likelihood objective response or length of PFS. We find that carbo/CsA has activity in platinum sensitive patients. It also demonstrates limited but present activity in platinum resistant patients. The regimen was adequately tolerated by patients and revealed a similar side effect profile to prior studies of this regimen.

Keywords: ovarian neoplasm, cyclosporin A, carboplatin, platinum resistance, platinum sensitivity

List of Abbreviations/Acronyms included: AUC, area under the time versus concentration curve; Carbo, carboplatin; CsA, cyclosporin A; CT, computerized tomography; EOC, epithelial ovarian cancer; OS, overall survival; PFS, progression free survival.

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Background

Ovarian cancer is the most common cause of gynecological cancer death in the Western world and the second most commonly diagnosed gynecological cancer.[1] Approximately 27% of gynecologic cancers are of ovarian origin, but 53% of all gynecologic cancer deaths occur in women who have ovarian cancer. With a mean age of at diagnosis of 63 in the US, five year survival for the disease ranges from 30 to 39% overall. This rate has remained stable despite development of new treatments.[2] The high mortality of ovarian cancer can be attributed largely to late recognition; approximately seventy-five percent of women will be diagnosed at stage III disease or higher.[3]

Late diagnosis is attributable to the disease's late, nonspecific clinical presentation and the absence of effective screening measures in the general population.[4,5] Typical subacute clinical presentation includes abdominal pain, changes in bowel habits and gastrointestinal complaints, or adnexal mass. These vague symptoms can be associated with either early or late stage and are frequently evaluated in the outpatient setting, where significant delay in diagnosis, up to one year in 11% of patients, may occur.[6,7] Acute presentation may occur, with symptoms of bowel obstruction, pleural effusion or deep venous thrombosis (DVT). This presentation is frequently the result of bulky disease and thus patients will already be at advanced stage. [8]

Approximately 95% of all ovarian cancers are epithelial in origin (EOC) and thus the primary focus of therapeutic development.[9] The remaining 5% of cancers develop from the other cell-types found in the ovary, including sex-cord stromal, granulosa, germ cell or mixed cell–type tumors[5]. In its healthy state, the ovarian surface epithelium consists

of a single layer of flat to cuboidal cells and lacks prominent distinguishing features.[10] The pathophysiology of EOC is poorly understood, and etiology likely varies with histological subtype. There are several primary mechanistic theories of pathogenesis of EOC which are not mutually exclusive. The “incessant ovulation hypothesis” proposes that with each episode of ovulation, the ovarian epithelium undergoes minor trauma, and is additionally exposed to estrogen rich follicular fluid during repair, and that the chronically repeated formation of these stromal epithelial clefts and inclusion cysts may be the source of future neoplasm.[11,12] This theory is supported by evidence that the risk of ovarian cancer increases linearly with number of lifetime menstrual cycles.[11,12] Others have proposed the “Gonadotropin hypothesis.”[13] This theory posits that systemic gonadotropins, such as luteinizing hormones (LH) and follicular stimulating hormone (FSH) overstimulate the ovarian epithelium, leading to increased cellular proliferation and eventually to malignant transformation. *In vitro* studies have demonstrated the presence of gonadotropin receptors on ovarian epithelium and have shown higher neoplastic conversion in ovarian epithelial cells lines with overexpression FSH receptors, but the mechanism of the this effect is not understood.[14,15] The gonadotropin hypothesis has been challenged however, by histological evaluation has demonstrated that many EOC’s may arise from the fallopian tube, and that frequently the origin cell of neoplasm in EOC is not from the ovary.[16] Further, This finding, paired with the fact that an increase in endometrioid and clear cell ovarian cancer rates in women with a history of endometriosis has has led to a modification of the “incessant ovulation” theory: the “incessant menstruation” hypothesis. This theory posits that chronically refluxing menstrual products through patent fallopian tubes, and the presumed inflammatory response that follows eventually leads to neoplasm. In particular iron-containing tissue and blood may place oxidative stress on fallopian and ovarian tissue.[17,18]

Risk and protective factors for EOC reflect these mechanistic theories of oncogenesis. Established risk factors include age, and positive family history. Possible risk factors include infertility, increased BMI, and tobacco and alcohol use.[19] Protective factors are typically those that disrupt continuous ovulation. These included lactation, increasing parity and incomplete pregnancies, lactation, and oral contraceptive use.[20] Additionally, surgical procedures such as TAH, and tubal ligation confer weak protective benefit against ovarian neoplasm, while prophylactic bilateral salpingo-opherectomy reduces risk of ovarian cancer by 90%.[9] A subset of ovarian cancers are hereditary in nature, with 7% of patients reporting a family member with a history of the disease. Most heritable cases attributable to mutations in BRCA1 or BRCA2, genes well known for their breast cancer association.[21] EOC is diagnosed variably across geographical and socioeconomic boundaries which likely reflect both genetic and modifiable risk factors. It has highest incidence in developed nations, with rates as high as 10 per 10,000 women in North America and Europe, with lowest rates seen in Asia and Africa. Increased rates of ovarian cancer are seen in populations who migrate to areas of higher incidence, highlighting the effect of nongenetic risk factors. Within North America, risk of EOC disproportionately affects Caucasian women, with intermediate rates in Hispanic women, and lowest incidence in African American and Asian populations.[9]

Screening measures, including transvaginal ultrasound and monitoring of serum biomarkers markers, have shown no benefit when applied to women without major risk factors.[22,23] CA-125 is the most commonly used serum biomarker in ovarian cancer. It is a glycoprotein which is overexpressed by ovarian cancer tumor cells but also is present in healthy mullerian tissue. Elevation (levels >35) is typical in ovarian cancer but may

also be seen during menstruation in premenopausal women or concurrent with other intra abdominal inflammation This lack of specificity and low positive predictive value in low risk populations limits its utility in the general population.[24] However, CA-125 remains a valuable way to track response to therapy and to monitor for disease progression.[25] CA-125 can also accurately be used as post-treatment surveillance to identify recurrent disease.[26] While CA-125 elevation detects recurrence earlier than awaiting symptoms or imaging evidence of disease, there is some evidence that there is no survival benefit to defining and treating progression on CA-125 in the absence of clinical symptoms. [27,28]

EOC is often initially suspected as a result of clinical presentation, by abnormalities found with noninvasive imaging or by elevated CA-125. However, histological sampling is required to confirm diagnosis. This sample is typically obtained via post-oophorectomy tissue biopsy, but may also be acquired through paracentesis, thoracocentesis or image guided biopsy.[29,30] The histology of EOC is heterogeneous, with four primary histological types of epithelial ovarian cancer: serous, endometrioid, clear cell, and mucinous. Serous histology accounts for 70-80% of EOC, and its low and high grade variants are often considered to be distinct entities.[1,31] As mentioned above, these histological variants likely represent different pathophysiological means of development, and an increasing number of antineoplastic agents are developed with the goal of providing therapy to specific histological subtypes. [32]

Diagnosis is typically completed concurrently with staging. EOC must be staged surgically. Operative procedures typically include total hysterectomy, bilateral salpingo-oophorectomy and sample of pelvic and paraaortic lymph nodes. Additional procedures,

including pelvic exenteration and resection of segments of small bowel, splenectomy, and partial hepatectomy may be required in order to optimally resect gross residual disease. Staging is broadly determined on a tumor, node, metastasis (TNM) system. Several revisions to the international standards of EOC staging, the FIGO guidelines, have occurred over the last several decades, with the most current guidelines presented in 2014.[33] Those patients determined to have initially unresectable disease or who are poor surgical candidates will first undergo neoadjuvant platinum-based chemotherapy prior to staging surgery. Neoadjuvant chemotherapy has demonstrated noninferiority to upfront surgical staging in this subpopulation with some improvement in perisurgical morbidity and mortality.[34,35] In either upfront or delayed surgery, maximal cytoreduction with initial staging procedure has shown a strong correlation to overall survival.[36]

The standard of care for EOC is well-established for patients receiving upfront surgery, regardless of histological subtype of disease.[37] Maximally cytoreductive surgery and staging is followed by platinum-based combination chemotherapy, most commonly carboplatin (carbo) and paclitaxel.[38–40] Platinum based chemotherapies are active in a variety of solid tumors. They act primarily by intercalation of DNA, specifically by forming covalent bonds at nucleophilic centers on purine bases of DNA. Platinum allows for cross-linking between two adjacent guanines. This DNA damage activates the signal transduction pathways that eventually lead to apoptosis.[41] Paclitaxel (trade name Taxol) is a taxane derived from derived from the bark of the pacific yew tree which acts by stabilizing and promoting microtubule assembly, thus inhibiting mitosis.[42] While initial trials used cisplatin in these multi-drug chemotherapeutic regimens, carboplatin has largely replaced cisplatin in first-line adjuvant therapy. Studies comparing cisplatin/taxol to carbo/taxol found noninferiority in progression free survival (PFS) and overall survival

(OS) between arms, but an improved toxicity profile, including decreased nausea and vomiting, appetite loss, and fatigue was demonstrated in patients treated with carboplatin.[38,43,44]

Encouraging initial response rates of 60 to 75% are seen with first-line platinum-based chemotherapy. However high relapse rates, up to 80% in patients with stage III- IV disease, require a return to therapy.[45] Disease progression is assessed with multiple modalities in ovarian cancer and may be defined by increasing CA-125, increase in gross disease on CT imaging, or via clinical progression. Patients with initial sensitivity to platinum-based therapy will return to a similar platinum-based regimen after relapse.[46]

Patients who fail to respond to platinum therapy or who experience a disease relapse within 6 months of treatment are considered platinum-resistant. This may occur with first line therapy (sometimes described as “platinum refractory” disease). Platinum resistance tends to increase from the initial rates 25% to 40% nonresponse seen in first line therapy with subsequent platinum therapies. The development of platinum-resistant disease is thought to be multifactorial, in part because platinum acts so broadly within the cell. Studies have indicated that cancer cells may increase in proficiency of DNA repair, decreased cellular uptake or increased excretion of drug, increased damage tolerance have been proposed.[47] In some cases, resistance may development in a manner similar to drug-resistant microbes, with the clonal outgrowth of platinum-resistant tumor cells initially present at low levels that then dominate the tumor when placed in a platinum-rich environment.[48] Prognosis is poor in platinum resistant patients. Second line treatments are more heterogeneous and the benefit of any given regimen, with such chemotherapeutic agents as doxorubicin and topotecan, has a lower response rate than

initial therapy, with 10-30% overall response.[2] This subpopulation historically has an expected survival of less than 12 months.[49] The challenge of poor outcomes in platinum-resistant and refractory EOC has made the development and identification of alternative second-line and salvage regimens a research priority.[50,51]

One novel chemotherapeutic regimen that has been studied in patients with platinum-resistant disease is the combination of carboplatin and cyclosporin A (carbo/CsA). Cyclosporin A is a widely used immunosuppressant, perhaps best known for its use in the prevention of rejection in solid organ and bone marrow transplant recipients where it to limit cellular immunity via inhibition of calcineurin, a T-cell activator, and subsequent down regulation of T-cell activity.[52,53] However, CsA's potential application in oncology was pursued after *in vitro* studies on the late 1980s and and early 1990s of cisplatin-resistant ovarian cancer cells demonstrated reversed resistance to multiple antineoplastic agents after exposure to cyclosporine.[54,55] Kashani- Sabet and colleagues in particular theorized that cyclosporin impacts multiple mechanisms of resistance. The group demonstrated that thymidylate synthase (dTMP) and dihydrofolate reductase, enzymes critical in Folate metabolism and dTMP synthesis, were upregulated in platinum resistant ovarian cancer cell lines. They demonstrated a similar elevation in c-fos and DNA polymerase β gene expression, enzymes perceived to act in repair of cisplatin-induced DNA damage through DNA synthesis. They further observed that with exposure to cyclosporin, both resistance to cisplatin was reversed and concomitant downregulation all of these enzymes was observed after either a one-time administration of the drug or weekly exposures.[47] Further *in vitro* studies have attributed platinum resistance reversal with cyclosporin to an alteration of the pharmacokinetics of cisplatin.[56]

These promising *in vitro* studies that established the activity of CsA in decreasing drug-resistance led to clinical trials assessing its promise as both a chemosensitizer of platinum in platinum-sensitive tumors and as chemomodulator of platinum resistance.[57,58] Chambers and colleagues conducted several Phase I and II trials to assess efficacy and the feasibility of delivering intraperitoneal and intravenous CsA. These trials were conducted in conjunction with carboplatin *in vivo* in research based out of Yale New Haven Hospital.[59–61]

In an initial phase I trial, the group administered carbo/CsA to 29 patients with heavily-pretreated recurrent EOC, 24 of whom were defined as platinum resistant. Patients received a total of 114 cycles of the regimen with escalating dosing of CsA via IV. Cyclosporin was administered in an escalating loading dose, 6 to 10 mg/kg, then as a continuous 24h infusion from 2.5 to 14.5 mg/kg/day. Carboplatin was targeted to an area under the time versus concentration curve (AUC) of 6 mg/ml x mm and was not dose escalated. The dose limiting toxicity in this trial was thrombocytopenia, with grade 3 to 4 thrombocytopenia found in 35% of patients grade 3 neutropenia, grade 4 nausea and vomiting were seen in 10% and 14% of patients respectively. At the maximum tolerated cyclosporin dose of 10 mg/kg a target blood concentration of >1 µg/ml was determined (lower than the >3µg/ml concentration of CsA is used as a modulator of multidrug resistance). Five objective responses were achieved, all in platinum sensitive patients, and an additional 5 patients had a 75% or greater reduction in CA-125.[59]

The Chambers group also conducted the sole clinical trial examining the effect of carbo/CsA with intraperitoneal rather than peripheral infusion. In this pharmacokinetic

and phase I study, 35 patients were administered CsA, first alone then in conjunction with intraperitoneal carboplatin via IP catheter for dwell times of four hours. At 20 mg CsA/kg, there was no difference in mean blood CsA levels (0.9 microgram/ mL) or mean IP CsA concentrations (1,000 micrograms/mL) when administered alone or with carboplatin. The most common toxicity was anemia, seen in 66% of patients. Common toxicities at the maximum CsA dose delivered (34.6 mg/kg) were anemia, leukopenia, thrombocytopenia, and hypertension. There were three objective responses in this trial, which lasted for three to eleven months. The group considered the intraperitoneal to systemic exposure favorable (1/1000) and found that malignant ascites were particularly well-controlled with this regimen.[61]

These findings formed the dosing regimen for a follow up phase II study conducted by the same group. Fifty-one patients with recurrent EOC received a total of 235 cycles of carbo/CsA via peripheral infusion, as a second to sixth line therapy. Patients had previously received one to three courses of platinum-based chemotherapy. Dosing followed the max tolerated doses of the Phase I: CsA was infused as a loading dose of 10 mg/kg over 5 h, followed by carboplatin infused in a one-time dose over 30 mm at an AUC of 6 mg/ml x mm, then a 24-h continuous infusion of 11.6 mg/kg CsA. Eight patients received more than six cycles every 28 days, thirty-four patients received three to six cycles; and nine patients received only one or two cycles. Thirty-eight patients were evaluable for objective response, and in an additional nine patients, CA-125 was the only marker of response. Four patients had no marker of disease. Of those patients who could be evaluated, 74% were platinum resistant. There were nine objective responses. Platinum resistant patients demonstrated a 14% response rate, and in platinum-sensitive patients there was a 50% response rate. No responses were seen in

patients who had only received one to to two cycles. Patient maintained stable disease for three to nineteen months. The regimen was well tolerated, with the most common grade III to IV toxicities seen were thrombocytopenia (22%) and hypertension (18%). [60]

More recent Phase II trials conducted by Morgan and colleagues have have shown mixed results.[58,62,63] Their group assessed Carboplatin/CsA in a small phase II trial in platinum-resistant EOC patients.[62] Dosing was lower than the Chambers studies: CsA was begun as an IV loading dose of 6 mg/kg over two hours. A continuous infusion of 9 mg/kg of CsA over 24 hours was then delivered.[59,60] Carboplatin dosage was initiated at an AUC of 4 between cyclosporin infusions with an increase of 1 AUC available if the no grade 3 to 4 toxicities developed. Twenty-three patients were enrolled and received a total of 58 courses of carbo/CsA. There was modest partial reversal of resistance observed, with only one case of objective response and seven cases of disease stabilization for a median of 4.9 months. This result was in contrast to *in vitro* findings in the same manuscript, which replicated earlier findings that CsA reversed platinum resistance in clonogenic assays.[62]

A second phase II trial conducted by Morgan and colleagues evaluated the combination of carboplatin/CsA with the addition alpha-interferon with results indicating inadequate reversal of clinical resistance in patients with platinum refractory ovarian cancer.[63] Alpha-interferon was added in this study as it had demonstrated some chemomodulatory activity previously both in conjunction with cisplatin and as a single agent.[64,65] In this study, 31 patients, 19 of whom had platinum-resistant disease, received a total of 86 courses of carbo/CsA/alpha-interferon. Three patients (10%) experienced partial response, while an additional nine patients maintained stable disease. Grade III toxicities

included myelosuppression, hypertension, and headache. Dosing was the same at their 2003 study, and while lower than dosing in the Chambers trial, the group found that blood levels >1μm/mL CsA were attained, the level determined to reverse platinum resistance in *in vitro* studies. Morgan and colleagues suggested that perhaps longer CsA exposures may increase efficacy of the regimen but the prospect of significant toxicities made increasing dosage infeasible. The combined results of these two studies led the Morgan group to conclude that carbo/CsA did not demonstrate adequate clinical benefit to warrant further study.[58,62]

The combination of carboplatin and cyclosporin A has been used at Yale since the 1990s and continues to be a treatment option in selected patients as late-line therapy. This practice continues despite the findings described by Morgan and colleagues due to the promising result of the Chambers group at Yale New Haven Hospital and due to continued anecdotal tolerability and response the patient cohort assessed in this review. Our institutional experience with this regimen has not yet been shared in the literature, and to our knowledge there have been no retrospective reviews of use of this regimen at other institutions. We hypothesize that we will see response rates similar to those demonstrated by the Chambers group, given that these studies were conducted at the same institution and with similar protocols to the current sample.[60] We describe these outcomes to help to further understand the efficacy of carbo/CsA in the treatment of recurrent ovarian cancer, to evaluate its tolerability, and to assess what patient characteristics are common to patients selected for carboplatin/CsA therapy.

Materials and Methods

Chart Review Strategy

A retrospective chart review was performed of patients managed at Yale New Haven Hospital from January 1995 until February 2015 who were diagnosed with ovarian cancer and who were treated with carboplatin/CsA at any point in their therapy. This project has received approval from Yale's institutional review board. Charts were identified through billing data records. Medical records, including patient demographics, date of birth, date of diagnosis, date of death (if applicable), pathology reports, histology, operative reports, chemotherapy/radiation treatment records, medical history and other relevant data regarding treatment including patient medical history, all medications, past history of surgeries, demographic data including ethnicity, smoking and employment history, pregnancy history including number of pregnancies, outcomes, and all recurrences with dates and treatment. Staging for this used the International Federation of Gynecology and Obstetrics (FIGO) 2000 criteria. These data were accessed using the EPIC electronic medical record system which contained access to historic electronic medical records. Patient information was deidentified and coded numerically in a password protected electronic database on a secure Yale server.

We reviewed prior studies of carboplatin/cyclosporin A in order to define relevant factors for this study.[60,62] We divided chart review data into the following subsections: patient characteristics, initial presentation, treatment prior to carbo/CsA, carbo/CsA course, treatment and survival following carbo/CsA. Within patient characteristics we sought the following variables: patient date of birth, age at initial diagnosis, ethnicity, age of onset of menses, age of menopause, parity, duration of use of oral contraceptive pills and

hormone replacement therapy, BRCA status (if available). In the initial presentation section, the following variables were recorded: initial platelets prior to any intervention, initial CA-125 prior to any surgical or chemotherapeutic intervention, date of primary staging procedure, procedure completed, if neoadjuvant chemotherapy was utilized or upfront surgery was completed, in cases of neoadjuvant therapy the agents used and number of cycles completed were recorded. Histological subtype and clinical stage of disease were recorded. Of note, in patients who initially receive neoadjuvant chemotherapy (NACT), true surgical staging was not attainable due to preemptive chemotherapeutic intervention. Generally, patients who required neoadjuvant chemotherapy have bulky, unresectable disease consistent with late stage illness. At this institution, these patients are typically documented clinically as being “stage X.” However, to provide consistency within staging, we counted neoadjuvant chemotherapy recipients to be stage IIIC or above, rather than using “stage X” convention specific to our institution. Those patients who received no clinical staging or who did not have exploratory laparotomy prior to NACT we classified as a stage IIIC. For those patients who remained a clinical stage IV at the time of staging surgery or who had ex-lap prior to staging that indicated stage IV disease, stage IV was indicated. In the “treatment prior to carboplatin/CsA section” variables included: initial adjuvant chemotherapy, number of cycles, and date of completion, all following chemotherapies and number of cycles were recorded, date of last chemotherapy completion prior to initiation of the chemotherapeutic regimen of interest and date of relapse. Of note, we considered neoadjuvant therapy followed immediately by adjuvant therapy with the same regimen so be a single course of chemotherapy. Platinum sensitivity was recorded as a binary (yes or no) based on whether patients had every progressed within 6 months of completion of treatment on any prior platinum chemotherapeutic regimen. Additional

surgical procedures and history of radiation therapy were documented. During the carbo/CsA period, start and end date of carbo/CsA therapy were documented, and cycles on carbo/CsA. CA-125 at the beginning, following each cycle when available, at the end of therapy was recorded. Where available, we included CT imaging of disease prior to and following carbo/CsA therapy. As our set was obtained from patients-off trial we had variable documentation of CT evaluation of disease and CA-125. Described toxicities and severity of toxicities were recorded. Toxicity was defined by the the 2010 Common Toxicity Criteria of the National Cancer Institute (CTCAE) on a 1-4 grading scale.[66] These ratings were based upon narrative documentation of toxicities and laboratory data within clinical notes. Reason for discontinuation of carbo/CsA, date of progression following therapy, ensuing chemotherapeutic regimens with number of cycles, date of late contact, and date of death were recorded after the completion of carbo/CsA.

Statistical Analysis

Statistical analyses were completed using STATA 13 (Statacorp LP, College Station, Texas) software. Demographic statistics were calculated using mean, median, range and standard deviation. Comparative statistics were performed using student's t test for continuous variables, Wilcoxon-Mann-Whitney test was used when comparing nonparametric continuous variables and Fisher's exact test was used for categorical variables. Univariate and multivariate linear and logistic regression were also performed. Survival was determined by Kaplan-Meier survival curves.

All data collection, analysis and manuscript preparation were conducted by this author with feedback from faculty mentors.

Results

Charts of 88 patients were initially identified with a diagnosis of ovarian cancer and a record of therapy with carboplatin and cyclosporin. After initial review, 54 patient charts included adequate data available for analysis. Charts were excluded when key information was lacking. This included: date of death, cycles on carbo/CsA, relapse after carbo/CsA, Date of disease progression, and notes documenting patient visits during carbo/CsA regimen. Charts that were excluded for inadequate information differed from included charts by date of treatment. Twenty-three of thirty-four excluded patients were treated before 2006, when electronic medical records became available. The remaining eleven excluded patients were either lost to follow up or had relevant portions of their care completed at an outside facility without accessible medical records.

Demographics

Fifty-four patients were included with a diagnosis of epithelial ovarian cancer who received a total of 265 cycles of carbo/CsA. Patient mean age of diagnosis was 61.8 with a range of 33 to 87. There were 50 Caucasian patients, one Asian, one African American patient and two Hispanic patients in the sample. A subset of patients had all or some of their basic obstetric and gynecological history available. Forty-seven patients had documentation of parity, with mean number of gestations at 2.5 (median 2) range of 0 to 11, with mean number of live births 2.0 (median 2) with a range of 0 to 10. 14.7% of patients were nulliparous and 23.4% had never experienced a live birth. Age of onset of menses was available for 26 patients, with a mean of 13.1 (range 10 to 17) and age of menopause available in 37 patients, with a mean of 49.5 (range 29 to 68). For the 24 patients who had both menarche and menopause ages available, the mean years of menses was 35.9 (range 21 to 55). History of extrinsic hormone exposure was limited.

Thirty-two patients had documented history of oral contraceptive use, with eleven of thirty-two (34.3%) patients reporting OCP use. Eight of ten (80%) patients with documentation regarding hormone replacement therapy had a positive history.

Patient Disease characteristics

Patients were skewed towards late-stage disease. Fifty-three patients had adequate staging information, one patient was listed as “unknown” due to having received primary therapy at an outside facility. The remaining patients ranged from stage IIC through Stage IV disease using FIGO 2000 criteria. Frequency breakdown was as follows: 1 stage IIC, 2 stage IIIA, 34 stage IIIC, 16 stage IV, 1 unknown. With regard to histology, 37 patients (72.6%) had serous disease. Of patients with non-serous histology, 3 had endometrioid disease, 1 clear cell, 4 mucinous, 7 were poorly differentiated, and 3 patients had mixed histology disease.

Eleven Patients had documented BRCA testing available. Of these, 4 were negative, 4 were positive for BRCA1 and 3 were positive for BRCA2. Seven patients had a history of another primary malignancy, with 6 having a history of breast cancer, and one prior rectal cancer. Two of the patients with breast cancer were documented to be BRCA+.

Initial CA-125 prior to any chemotherapy or surgery was available in 32 patients. Mean initial CA-125 was 4604, median 1030 (range 71.9- 62756). Initial platelets prior to intervention were available in 34 patients, with a mean of 421.6, median of 39, with a range of 156 to 709. Thirteen patients (24.2%) received neoadjuvant chemotherapeutic courses. All patients with neoadjuvant chemotherapy courses received platinum-based therapies. One patient received 5 cycles of neodjuvant chemotherapy, while the eleven

remaining patients received a typical 6 cycle course. When operative notes were available, we assessed whether primary staging surgery had attained optimal cytoreduction. 38 patients had this data available, of these 26 (68.4 %) had optimal cytoreduction while 12 (31.6%) were suboptimally cytoreduced.

By the time patients were placed on carboplatin/CsA, they had undergone a median of 3 prior chemotherapies (mean 3.2), with a range of 1 to 9 prior courses. Patients had received from 1 to 4 previous platinum based chemotherapies, with a median of one prior regimen (mean 1.6). Thirty-seven patients (68.5%) were considered platinum sensitive at initiation of carboplatin/CsA, and 17 (31.5%) had demonstrated platinum resistance. Thirteen patients (24%) had undergone an interval debulking prior to carbo/CsA. Two patients had received radiation therapy prior to receiving carbo/CsA. Unfortunately, patient functional status was only explicitly documented in four patients and thus was not included for analysis.

When comparing platinum sensitive to platinum resistant patients, they did not differ significantly in terms of ethnicity, age at diagnosis, histology or stage of disease. Median interval between diagnosis and initiation of carbo/CsA was significant at $p=0.02$. Platinum resistant patients had significantly higher number of courses of prior chemotherapy and number of prior courses of platinum based chemotherapy (Fisher's exact $p < 0.0001$ and $p = 0.0001$respectively).

Carboplatin Regimen and Dosing

All patients on study received carboplatin/CsA via port or PICC line rather than intraperitoneally. Regimen was scheduled on a 28 day cycle in 53 of 54 patients, with a

single remaining patient on a 21 day cycle. Initial planned course was 6 cycles in all patients. Dosing information was available in a subset of patients but was unavailable in the earliest treated patients in our set due to the absence of pharmacy notes in the EMR prior to 2008. Forty-two patients received cyclosporin at the same dosing as described by Chambers and colleagues, with a CsA was infused as a loading dose of 10 mg/kg over 5 h, followed by carboplatin infused over 30 minutes or via desensitization challenge, followed by a 24-h continuous infusion of 11.6 mg/kg of cyclosporin [60]. Carboplatin dosing was available in 43 patients. Planned carboplatin dosing protocols varied between an AUC of 5 and AUC of 6. Thirty-two patients (74.4%) received AUC of 5, and eleven received AUC of 6 (25.6%). There were not blood levels of either agent available for analysis. As in other studies of carbo/CsA, each cycle of chemotherapy required an inpatient visit.

Of note, 17 patients of 54 (31.5%) required platinum desensitization dosing at some point during their carbo/CsA regimen due to history of carboplatin reaction, positive skin testing, or symptoms of platinum allergy during the carboplatin/CsA course.

Therapeutic Effects

A total of 265 cycles was received by 54 patients with patients receiving a range of 1 to 10 cycles, with a mean of 4.9 cycles per patient (median 5.5). Objective response was measured both by CT and CA-125 changes. As patients were not included on a clinical trial, they had less frequent imaging than protocoled patients in the Chambers and Morgan group studies described above. Typically, those patients with imaging data available had CT imaging prior to initiation of therapy, and underwent CT after

completion or with suspected progression on treatment. Initial CT was available in 47 patients prior to initiation of therapy. Forty patients in this group (85.2%) demonstrated gross disease on CT. Thirty-one patients had imaging following completion of therapy. Response was defined by RECIST criteria.[67] Twenty-eight patients had both initial and repeat CT imaging available, and 5 of these patients did not have gross disease initially, leaving 23 patients with potential to evaluate for objective CT response. Seven objective responses (39.4%) were seen in in this population. An additional 4 patients had no gross disease at either initiation or completion of therapy. Remaining patient CTs at completion included five patients with mixed response or stable disease, and fifteen patients demonstrating progression. Three exams documenting progression did not have comparison imaging before therapy.

Objective response by CA-125 was defined by the guidelines developed by Rustin and colleagues in 2004 and used in multiple clinical trials of ovarian cancer treatment since that time.[68] CA-125 reduction of 50% or greater from pretreatment CA-125 level and with a confirmatory CA125 one month following this level, given an initial pretreatment CA-125 at least two times the upper limit of normal and taken within two weeks of initiating therapy.[69,70] There were 41 patients with CA-125 at initiation who had CA-125 at twice the upper limit of normal (>70). Of these patients, 17 (41.4 %) demonstrated an objective response by these criteria. An additional three patients saw a decrease of 50% or greater from initial CA-125 which was not sustained on repeat measurement at one month. Duration of response during treatment varied from 2 to 8 cycles in length, with a mean of 4.6 cycles (median 5, SD 1.9). Serial CA-125 following completion of treatment was not available and thus not included in these response durations.

There were a total of 49 patients with adequate data for measure of objective response by either CT or Ca-125. By either method, a total of 19 patients exhibited objective response for an overall response rate of 38.8%. There was both CA-125 and CT response in 5 patients, the remaining two patients with objective response on CT did not have CA-125 levels available for analysis. The rates of objective response were significantly different in patients who were platinum resistant versus platinum sensitive (p= 0.015). Seventeen of 34 patients (50.0%) with platinum-sensitive disease experienced objective response. Only two of fifteen patients (13.3 %) defined as platinum resistant demonstrated objective response. One of these patients underwent 6 cycles of carbo/CsA as third line therapy, and experienced a partial objective response on CT after completion of treatment with a 10.9 month PFS before relapse. The second platinum-resistant responder had received five prior chemotherapeutic regimens. She did not have pretreatment CT imaging but had a documented Sister Mary Joseph nodule at the start of carbo/CsA. She remained on therapy for 10 cycles with a CA-125 response lasting for cycles two through ten. Repeat CT at the completion of her regimen indicated no gross disease and she experienced a PFS of 14.0 months.

Progression free survival was analyzed in those patients who did not discontinue the regimen due to toxicity or change in goals of care.[71] Progression was defined both by evidence of CT progression, increase in CA-125, or death. Mean progression free survival was available across 45 patients. Mean PFS was 7.7 months (SD 5.2), median 5.8 months, with a range of 1 to 25.4 months. There was no significant difference in PFS in patients who were platinum sensitive (mean 8.3 months) versus platinum resistant (mean 6.4 months). However, patients who experienced objective response experienced a significantly increased PFS than those who did not (mean 11.5 versus 5.4 months

respectively and a p= 0.0001). No difference in objective response, PFS, or OS was seen in patients who required desensitization dosing while on carbo/CsA. No significant difference was observed in objective response rate, PFS or OS in those patients who were dosed with carboplatin at an AUC of 5 versus 6.

We attempted to control for factors that may impact therapeutic response and survival, although our n was modest for multivariate statistics. In multivariate linear regression assessing objective response, there were no significant associations between prior number of previous chemotherapeutic regimens, high stage disease, serous vs non-serous histology, and age at treatment with objective response rate. Platinum resistance had an odds ratio of 0.23 for objective response, however the p-value was not significant p= 0.1. In multivariate linear regression controlling for high stage disease, platinum resistance and age at treatment, we found that objective response was associated with a 6.7-month increase in progression free survival ($p < 0.001$). When controlling for these factors as well as optimal cytoreduction, objective response was still associated with a significantly increased overall survival of 2.6 years.

Overall Survival and ensuing treatment

Overall survival was available in all 54 patients. Mean survival was 5.9 years (median 5.4 yrs, SD 2.8) ranging from 1.6 to 14.5 years. There was a statistically significant increase in overall survival in those patients who experienced objective response while undergoing carboplatin/CsA therapy. Mean overall survival in patients with objective response was 7.0 years and mean survival in nonresponders was 4.7 years (p= 0.0007). There was no statistically significant difference in overall survival based on whether

patients had attained optimal cytoreduction with initial staging surgery, had received neoadjuvant therapy, or had platinum-resistant disease.

Patients underwent a mean of 3.3 (median 3, SD 2.5) ensuing chemotherapy regimens after completion of carbo/CsA, with a range of 0 to 11 ensuing regimens. Of these ensuing treatments, 21 patients underwent further platinum-based chemotherapy, with 17 undergoing one course and 4 undergoing two courses. Of those patients who received further platinum-based therapy, 9 (52.9%) had experienced an objective response while on carbo/CsA. 6 patients who were platinum resistant at the time of receiving carbo/CsA received further platinum-based chemotherapy, only one of these patients had an objective response while on carbo/CsA.

Toxicities

Five patients discontinued the regimen due to toxicities. In these patients, the symptoms leading to regimen change were allergic reaction, hypertension, hypotension, hypothermia in one patient each, and nausea and vomiting in two patients. Two patients died while on therapy. One of these patients died one month after receiving cycle 5 of chemotherapy due to complications associated with disease progression. The second patient died within one month after receiving a single cycle of carboplatin/CsA in the context of a hospital admission for small bowel obstruction. Notes for neither patient indicated toxicity as a causal factor in their demise.

Cardiovascular toxicities were noted in seven patients (13%). One patient experienced grade 1 Atrial fibrillation. Three patients experienced hypertension with cyclosporin infusion, including one grade 3 hypertensive response. One patient experienced grade 4

hypotension. This hypotension was unexplained and not accompanied by other evidence of anaphylactic response. One patient experienced grade 3 acute cardiac syndrome, experiencing an NSTEMI with hemodynamic stability while on carbo/CsA. One complaint of grade 1 palpitations was noted. Renal complications were seen in three patients (5.5%), with grade 2 AKI seen in one patient and two patients with at grade 1 AKI. Seven patients (13%) experienced an allergic reaction, including six with grade 2 responses and one patient with a grade 3 reaction to cyclosporine infusion. Hematologic toxicities were documented in 8 patients including: grade 1 pancytopenia seen in a single patient, one case of grade 2 thrombocytopenia, grade 3 anemia was present in four patients, and grade 1 neutropenia seen in two patients, and grade 2 neutropenia in a single patient. Three patients experienced infection while on protocol, including one grade 4 urosepsis episode requiring ICU admission. The other infections included a grade 3 fever of unknown origin, and a grade 2 port cellulitis.

Headache was the most common toxicity reported. Fifteen patients (27.8%) experienced headache, with one patient reporting grade 3 intractable headache, four patients with grade 2 headaches and 9 with grade one headache. With regard to gastrointestinal complaints, Nausea and vomiting was experienced by thirteen patients (24.0%), including three instances of grade 3 nausea and vomiting. Grade 1 constipation was noted in one patient. Neurocognitive toxicities were noted in two patients (3.7%). Confusion was noted in one patient, and one episode of brief self-resolving hypomania was described in a patient who was taking dexamethasone on protocol. Nine patients (16.7%) experienced grade 1 fatigue. Remaining miscellaneous toxicities including: one patient with grade 2 hypothermia, one instance of grade one mucositis, and one instance of grade 1 peripheral neuropathy.

Discussion

The typical course of EOC is late presentation, with initial response to platinum-based therapy followed by high rates of relapse requiring multiple chemotherapeutic regimens, and often a conversion to platinum resistant disease. The patients described in this study predominantly had late stage serous disease, with 92.4% percent of patients diagnosed with stage IIIC or IV disease. They were also heavily pretreated, with a mean of 3.2 prior chemotherapeutic regimens. Our patients had, on average, received a greater number of prior chemotherapies than those patients included in clinical trials of carbo/CsA. In the Chambers Phase II trial, patients received CsA as a second to sixth line therapy, with a mean of 1.8 prior chemotherapies and in Morgan's 2007 Phase II trial, patients had a median of 2 prior therapies, ranging 1-5 prior chemotherapeutic courses.[60,63] While there were no differences in age, stage or histology we also found that platinum-resistant patients in this analysis had undergone more rounds of chemotherapy and more platinum-containing regimens prior to starting on carbo/CsA. Interval since diagnosis also trended towards significance when means were compared, and was significant when comparing median values. These differences were expected, given the tendency of platinum-resistance to increase with platinum exposure and number of prior therapies.

Our results showed a higher objective response rate than those found in prior clinical trials, with a 38% objective response rate, as compared with prior response rates of 4.3% to 24%. These earlier trials did not include PFS or OS analysis for comparison with our findings. There are three key differences in our study which may account for in increased objective response rate. Foremost, the sample described here is a mix of both

platinum-sensitive versus platinum-resistant patients, in roughly a two to one ratio. In 2004, Morgan and colleagues assessed outcomes exclusively in platinum resistant patients and showed the lowest rates of response a 4.3%.[62] Their 2007 study which added alpha-interferon to the regimen, included both resistant and sensitive patients with an overall response rate of 10.0%, with the resistant group demonstrating a 10% response rate and the platinum sensitive group indicating a 9.1% response rate.[63] Chambers and colleagues reported a 24% response rate, but subgroup comparison indicates that the higher response rate in our cohort is driven by a higher percentage of platinum-sensitive patients. While Chambers and colleagues included both platinum sensitive and resistant patients in their cohort the percentage of platinum resistance than found in our group was lower, at 74% versus 30.6%.[60] Of note, though Morgan and colleagues chose to exclude platinum-sensitive patients in their 2004 study, there is a theoretical benefit to using cyclosporin in platinum-sensitive patients: the same mechanisms which reverse platinum-resistance *in vitro* may prevent the development of platinum resistance and potential platinum sensitivity.[62] When comparing platinum-resistant patients only, we observed a 13% response rate. This was higher than the rates observed in the Morgan group trials, 4.7% in both 2004 and 10% in 2007, and comparable to the 14% response rate observed in Chambers.[60,62,63]

Another potential source of variability in outcomes was that our cohort experienced higher doses of both Carboplatin and Cyclosporin A than patients in the Morgan trials, with cyclosporin loading doses of 6 versus 10 mg/kg, and continuous infusions of 10 versus 11.6 mg/kg, and a starting dose of carboplatin at 4 AUC rather than 5.[58,62,63] The addition of alpha-interferon to the 2007 Morgan phase II trial adds another potentially contributing factor to the different response we observed.[63] Our dosing was similar to the

regimen used by developed the Chambers Phase I trial.[59] We used identical same cyclosporin dosing in all documented patients. Intended doses of carboplatin that ranged between 5 and 6 AUC, where the Chambers group began all patients at an AUC of 6.

Criteria for objective response was also defined differently across trials. In the Chambers phase II trial, a 75% reduction from CA-125 start of therapy was required.[60] Their trial was conducted prior to standardized guidelines for assessing CA-125 objective response to treatment. Morgan *et al* defined objective response by SWOG criteria in both phase II trials, requiring both evidence CA-125 and CT response in patients.[72] We defined our objective response criteria by what valid measures could be reproducible given available data points, using RECIST criteria for imaging and established criteria for CA-125 response. The absence of a required CT component in defining objective response in this sample may have led to a higher response rate, given that CA-125 is considered more sensitive than CT to disease response. It is reassuring, however, that studies comparing RECIST and SWOG criteria have shown comparable PFS and OS outcomes in other neoplasms.[73]

Our study showed objective response rates of 50% in platinum-sensitive patients and a mean PFS of 8.3 months. This is a robust rate of response, comparable to the 50% response rate seen in the Chamber's phase II trial.[60] Standard second line therapy in these platinum-sensitive patients is typically a return to platinum-based chemotherapy. Response rates of 30 to 67% are seen in platinum-sensitive patients with recurrent disease receiving combined platinum-based chemotherapy in clinical trials.[74,75] Thus, the carbo/CsA regimen here performs within the same range as more typical second line chemotherapies.

The response rate of 13.3% in platinum resistant disease is less encouraging than what was observed in platinum sensitive patients. However, two significant responses were documented. The response rate documented here falls within the 10-30% response range widely described in platinum-resistant patients receiving standard second line therapy, that is, a non-platinum single-agent chemotherapy such as paclitaxel or doxorubicin.[2] We could not find criteria contained within our dataset that could explain differences in platinum-resistant patients who responded to carbo/CsA versus who those who demonstrated a lack of response. In a larger dataset it would interesting to pursue such characteristics, including tumor genetics and more granular information on patient histories of platinum exposure, response, and platinum-free intervals than was available in our set.

To give greater context to carbo/CsA's efficacy in the heavily pretreated, late stage cohort included in the present analysis, we sought similar prior studies with more common salvage agents. While there is a broad literature on late-line chemotherapy generally, there has been only one prior study conducted in a similar cohort, with similar methodology to our own that was also conducted at Yale New Haven Hospital.[76] O'Malley and colleagues conducted a retrospective review of heavily pretreated EOC patients placed on weekly topotecan therapy at this institution in 2005.[77] Topotecan is a topoisomerase I inhibitor that inhibits DNA synthesis, which is frequently used as a salvage chemotherapy in recurrent EOC, however it is typically administered on days one through five of a 28 day regimen.[76,78,79] Response rates for topotecan in the second-line setting range from 19% to 33% for platinum-sensitive disease and 12 –18% for platinum-resistant disease.[80–82] O'Malley *et al.* assessed response to and tolerability of

a weekly topotecan regimen in 35 patients, 16 of whom were platinum sensitive and 16 were platinum resistant or refractory, and 3 had unknown platinum status. Patients received a total of 177 cycles (534 weeks). Patients in this cohort had received one to four prior chemotherapies regimens, with a median of 3 prior chemo regimens. They were also skewed towards late disease, with 33 of 36 patients documented as stage III or IV disease. These features are similar to the heavily pretreated, late stage cohort found in the present study. Response was assessed by CA-125 alone. Twenty-eight of 35 patients were evaluable for tumor response. One (6%) of the 16 patients with platinum-sensitive disease achieved a complete response and 3 (19%) achieved a partial response, for a total response rate of 25% in platinum-sensitive patients. In platinum resistant patients, one patient (6%) achieved a partial response. The median response duration was 28 weeks (range, 16 –44 weeks) for patients who achieved a partial response. Stable disease was reported in 13 (38%) patients, including 5 patients with platinum-resistant/refractory disease [77]. The responses in the carbo/CsA cohort, a 50% response rate seen in platinum-sensitive patients and 13% in platinum-resistant patients, compares favorably with response rates described in this cohort of patients treated with weekly topotecan.

It is of interest whether the immunomodulatory effects of CsA would provide benefit in the context of a platinum-based regimen combined with another cytotoxic agent, such as paclitaxel. A recent meta-analysis of single agent platinum versus combined platinum-based chemotherapy in platinum sensitive patients found significant improvements in both PFS and OS for those patients receiving combined therapy.[74] However, combined therapy is associated with increased toxicity and worsened quality of life measures in some clinical trials.[83,84] The survival benefit of dual therapy provides a potentially

interesting future direction for the assessment of cyclosporin's value in the treatment of EOC. It would be interesting to evaluate tolerability and survival in patients with recurrent EOC on a combination of carboplatin and either paclitaxel or doxorubicin with cyclosporin. In addition, it may be reasonable to consider the use of carboplatin/CsA in patients with recurrent EOC who are platinum sensitive but intolerant to common combined chemotherapies such as paclitaxel or doxorubicin.

When considering the impact of our regimen on survival, we found both significantly increased PFS and OS in those patients who experienced objective responses while on carboplatin/CsA. Objective response was associated with a 6.7 month increase in progression free survival when controlling for potentially contributing factors. This effect on PFS supports the clinical validity of the objective responses documented in our dataset. The significant increase in overall survival in patients with objective response was a more notable finding, as late line chemotherapy often does not yield overall survival benefit. This effect continued to be significant when controlling for age at treatment, stage of disease, histology, history of optimal cytoreduction, and neoadjuvant chemotherapy. It is possible that our sample size limited this analysis, and it is likely that factors not available in our dataset, such as patient functional status influenced outcomes. This effect does, however, support the clinical value of objective response to the regimen.

There are several subpopulations specific to this institution which are interesting to consider when analyzing chemotherapeutic outcomes. Neoadjuvant chemotherapy, although it has been demonstrated to have non-inferiority to initial surgery in appropriately selected patients, is available only through a subset of oncology providers,

including Yale New Haven Hospital. We have a small sample size within this study which may not have adequate power to reject a null hypothesis. However, our results, regarding objective response, PFS and OS showed no significant differences between outcomes in for those patients who received neoadjuvant therapy or immediate surgery in a population weighted towards late stage disease. These findings are consistent with the broader literature on survival outcomes in neoadjuvant chemotherapy recipients.[34]

This sample also included significant number of patients (31.5%) who underwent platinum desensitization while receiving carboplatin and cyclosporin. This dosing regimen is used in patients who are platinum sensitive but who experience platinum allergy. Allergic reactions in platinum allergic patients range from mild infusion-associated pruritic rash to true anaphylaxis. While desensitization rates are promising, with some studies showing success rates of 87%, there are a limited number of sites which offer desensitization to patients.[85] Desensitization dosing includes a period of premedication with dexamethasone and antihistamines, followed by exponentially increasing platinum challenge doses, beginning with a 1/1000 therapeutic dose infusion. Carboplatin infusion is stopped if allergic response is noted. This differs from a typical one-time infusion of carboplatin in patients who have no history of hypersensitivity. [86,87] Of the 17 patients here who underwent desensitization in this study, 16 (94.1 %) were able to continue the regimen. One patient was removed from the regimen for allergic reaction although it occurred during cyclosporin infusion prior to carboplatin dosing. Fisher's exact tests showed no difference in objective response rate, PFS, or OS between those patients who required desensitization dosing versus standard platinum dosing. We again caution that our sample is of modest size, so power issues could obscure alterations in therapeutic effects.

The overall toxicity profile of our sample compared favorably with prior studies of the carboplatin and CsA regimen Most common grade III or IV toxicities included anemia (7.4%) and nausea and vomiting (5.6%) with other grade III reactions including hypertension, headache, and pancytopenia, and allergic reaction. This profile indicates a lower incidence of similar serious side effects noted in prior prior trials. Most common serious toxicities included thrombocytopenia (22% patients), hypertension (18%), neutropenia (10%), anemia (8%), leukopenia (8%) in Chambers' 1996 study. Morgan 2004 evaluated toxicities by cycle of therapy, finding grade 3 or 4 granulocytopenia 29% courses of therapy, grade 3 anemia in 6.9% of cycles, and grade 4 nausea in 6.9% of cases. Most common low grade toxicities in this dataset included headache (24%), nausea and vomiting (18.5%), and fatigue (16.7%). In prior studies, low grade toxicities included thrombocytopenia, neutropenia, anemia, nausea and headache. Despite the higher rate of documented side effects, there were no documented patient withdrawals from these clinical trials. In contrast, this dataset had 9.3% drop out rate for for side effects. It is possible that this higher dropout rate may be attributable to a lower functional status of the patients in our set, or if patient preferences and quality of life concerns took higher priority in an off-trial setting.

Of note, this cohort of patients was collected over fifteen years while various supportive medications became more widely available. For example, the availability of colony stimulators, such as filgrastim and its analogs, likely altered the frequency of toxicities related to bone marrow suppression. Centrally-acting antiemetics, such as ondansetron, may have altered incidence of intractable nausea and vomiting. Further, we were limited to including those toxicities described in clinical notes, and mild toxicities or minor

medical intervention may not have been documented in charts available to us for the current analysis.

Our study contains several limitations. First, retrospective research is inherently limited. The patients included in this study were not randomized into groups designed to test the validity of our hypothesis. All findings are correlative rather than causative. Additionally, the data was not collected with our current study in mind, and may be susceptible to biases due to changes in data collecting methodology over time. We face a particular challenge in managing temporal change in our data set. Staging criteria has evolved over the course of time since our patients were placed on therapy, with accepted staging criteria for ovarian cancer changing several times during the time period represented by our data. We had to attempt to approximate staging according to FIGO 2000 criteria based on available operative notes and pathology reports. However, given the advanced disease found in our dataset we are confident that patients would not having substantive changes in their TNM classification with the new changes in staging. Another limitation is that the variables included in the data may not speak directly to our hypothesis, requiring the use of approximation and extrapolation for some of the available data. In particular, the lack of documentation of patient functional status may obscure an important factor contributing to patient outcomes. Finally, this data set is culled from a single institution, which may limit how our findings can be generalized to the broader ovarian cancer patient population. We believe, however, that the large number of patients and the rich dataset has yielded valuable information on the use of carbo/CSA in EOC despite the fact that our data come from experience at a single site.

Treatment options for refractory EOC have expanded since initial studies of Carboplatin/CsA. There is currently rich activity in the development of new treatment modalities for patients suffering from recurrent ovarian cancer.[37,76] Bevacizumab, which targetsthe VEGF-receptor and has demonstrated the ability to shrink tumors and prolong PFS in ovarian cancer, is just one of the angiogenesis pathways which may be targeted and exploited to improve survival.[88] The folate receptor, over-expressed in ovarian cancer and a likely component of cyclosporin's anti-resistance activity is also being targeted by novel agents. Farletuzamab is a humanized monoclonal antibody to folate receptor alpha and is being evaluated in a phase III placebo controlled trial with second-line carboplatin–paclitaxel in patients with platinum-sensitive relapsed ovarian cancer.[89] Vintafolide is a cytotoxic agent, consisting of folate linked to a potent vinca alkaloid chemotherapy agent, desacetylvinblastine monohydrazide (DAVLBH). It is currently being evaluated in a phase III clinical trial for platinum-resistant ovarian cancer.[90] Several new therapies focus on individualized therapy for subsets of disease. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in base excision repair. PARP inhibitors, such as olaparib, have shown promising activity in BRCA-mutation positive disease. BRCA 1 and 2 are key for homologous recombination. Mutations in BRCA can be exploited by inhibiting PARP, and thereby by inhibiting DNA-base excision repair, ultimately yielding chromosomal instability and apoptosis.[91,92]

It is interesting to consider that Cyclosporin A, while functioning in a less well-understood pathway in potentiating response to platinum, shares potential mechanisms with some of these novel agents. In particular, CsA is thought to impair the folate pathway and interfere with DNA- repair. Given that cyclosporin and carboplatin are both widely

available, relatively inexpensive, and well-tolerated, the combination offers an intriguing option in the armamentarium for recurrent EOC.[93,94]

Conclusion

We reviewed the outcomes and tolerability of carboplatin and cyclosporin therapy in 54 patients with recurrent, heavily-pretreated and predominantly late-stage EOC treated at a single institution over the last ten years. Patients received a total of 265 cycles of carbo/CsA with a mean of 4.9 cycles per patient (range 1-10). Mean PFS was 7.7 months (SD 5.2), median 5.8 months, with a range of 1 to 25.4 months. By measure of either CT or Ca-125 a total of 19 patients exhibited objective response for an overall response rate of 38.8%. The rates of objective response were significantly different in patients who were platinum resistant versus platinum sensitive (p= 0.015), with a 50% response rate in platinum-sensitive patients and 13.3% objective response rate observed in platinum resistant patients. Both PFS and OS were significantly higher in patients that demonstrated objective response on carboplatin/CsA than those who did not. These effects remained significant when controlling for age at treatment, number of prior chemotherapies, platinum resistance, and disease stage and histology. Toxicity profile compared favorably with prior studies of carboplatin/CsA, with most common grade 3 to 4 side effects including nausea and vomiting, and anemia. There was, however, a 9.3% drop out rate for side effects. Our outcomes are limited by their retrospective nature, but have comparable findings to encouraging phase II trials of carboplatin and CsA. We consider the therapeutic effects and toxicity profile documented here adequately encouraging for further study of this regimen. Given that cyclosporin is widely available, relatively inexpensive, and well-tolerated, its combination with carboplatin offers a valid potential treatment option for recurrent epithelial ovarian

cancer, particularly for those patients who may be intolerant of, or who have already failed, other combined chemotherapeutic regimens.

Table 1

Patient Demographics				
	Overall (n=54)	**Platinum Sensitive (n= 36)**	**Platinum Resistant (n=17)**	p-value
Mean Age at Dx	61.8 (33-87)	62.4 (SD 9.6)	60.6 (SD 10.8)	p=0.53
Mean Age at Tx	66.12 ,(35.1-89.3)	66.0 (SD 1.6)	66.3 (2.7)	p= 0.92
Mean Interval since diagnosis (wks)	474.13 (57-5577)	303.6 (SD 876.7)	845.3 (SD 1727.4)	p=0.13
Median interval since diagnosis (wks)	150	133	295	***p= 0.02***
Mean Prior chemos	3.2 (1-9)	2.5 (SD 1.3)	4.8 (SD 2.4)	**p < 0.0001**
Mean Prior platinum chemos	1.7 (1-4)	1.4 (SD 0.5)	2.3 (SD 1.1)	**p= 0.0001**
Ethnicity (n)				p=0.582
Caucasian	50	34	16	
AA	1	0	1	
Hispanic	2	2	0	
Asian	1	1	0	
Staging (n)				p=0.158
IIC	1	0	1	
IIIA	2	1	1	
IIIB	0	0	0	
IIIC	34	23	11	
IV	16	13	3	
Unkown	1		1	
Histology (n)				
Serous	37	13	24	
Non-Serous	14	3	11	
Unknown	3	0	3	

Table 2

Incidence of toxicities documented on Carboplatin and Cyclosporin A (n=54)				
Toxicity	Grade 1 or 2		Grade 3 or 4	
	n	%	n	%
Headache	13	24.07	1	1.85
Fatigue	9	16.67	0	0.00
Nausea-Vomiting	10	18.52	3	5.56
Constipation	1	1.85	0	0.00
Cardiovascular				
Palpitations	1	1.85	0	0.00
Acute Coronary Syndrome		0.00	3	5.56
Hypertension	2	3.70	1	1.85
Atrial Fibrillation	1	1.85	0	0.00
Hypotension		0.00	1	1.85
Hypothermia	1	1.85	0	0.00
Hyperglycemia	1	1.85	0	0.00
Hematologic				
Neutropenia	3	5.56	0	0.00
Anemia	0	0.00	4	7.41
Thrombocytopenia	1	1.85	0	0.00
Pancytopenia	0	0.00	1	1.85
AKI	3	5.56	0	0.00
Allergic Reaction	6	11.11	1	1.85
Neurocog	2	3.70	0	0.00
Infection	1	1.85	2	3.70
Mucositis	1	1.85	0	0.00
Peripheral Neuropathy	1	1.85	0	0.00

Table 3

Treatment Outcomes				
Outcomes	Overall	Platinum Sensitive	Platinum Resistant	p value
PFS (mths)	7.7 (SD 1-25.4)	8.3 (SD 5.6)	6.4 (SD 1.1)	p=0.25
Objective Response (n=38)	38.80%	50%	13%	**p= 0.001**
Overall Survival (yrs) (n=41)	5.93 (SD 2.8)	5.6 (SD 2.7)	6.6 (SD 3.2)	p=0.26

Table 4

Comparison with Prior Studies of Carbo/CsA				
	Current Study	**Chambers 1996**	**Morgan 2004**	**Morgan 2007***
Demos				
Total N	54	51	23	30
Platinum Sensitive	37		0	11
Platinum Resistant	17		23	19
cycles	265	234	58	86
cycles/patient	mean 4.9, med 5.5, range1-10	mean not listed, range "1 to greater than 6"cycles	median 2, range 1–7	median 2, range 1–9
Dosing				
Initial Carbo Dosing	AUC 5-6	AUC 6	AUC 4	AUC 4
CsA				
loading	10 mg/kg over 5 h	10 mg/kg over 5 h	6 mg/kg over 2 h	6 mg/kg over 2 h
continuous	24-h continuous infusion of 11.6 mg/kg CsA	24-h continuous infusion of 11.6 mg/kg CsA	24-h continuous infusion of 9 mg/kg	24-h continuous infusion of 9 mg/kg
objective response rate (%)				
overall	38	24	N/A	10
platinum sensitive	50	50	N/A	10.5
platinum resistant	13	14	4.3	9.4
				interferon

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