A PHASE II EVALUATION OF CARBOPLATIN/PACLITAXEL/BEVACIZUMAB IN THE TREATMENT OF ADVANCED STAGE ENDOMETRIAL CARCINOMA

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STUDY FLOW CHART/SCHEMA

Compressively surgically staged patients with advanced endometrial cancer (stage III-IV) with non-measurable or measurable disease

Regimen

Carboplatin (AUC 5) plus Paclitaxel (175 mg/m^2 over 3 hours) plus Bevacizumab (15 mg/kg) every three weeks for 6 cycles

Follow-up

Measurable disease: CT-scan/MRI/US Following cycle 3 and 6

Non-measurable disease: CT after 6 cycles

All patients: Clinical exam every 3 months for 2 years then 6 months for an additional three years and annually thereafter

1.0 BACKGROUND

1.1 DISEASE BACKGROUND

In the United States, adenocarcinoma of the endometrium remains the most common malignancy of the female genital tract. Approximately 40,880 new cases were diagnosed in 2005, resulting in about 7,310 deaths. Five-year survival rates exceed 77% largely due to the ability to diagnose women with endometrial cancer at an early stage. Seventy-five percent of women are Stage I (tumor confined to the uterus) at the time of diagnosis. However, when the disease is diagnosed at more advanced stages or if the disease recurs, prognosis is very poor with cure rates less than 15%.[1] Clearly improvements are needed in primary therapeutic strategies.

After initial surgical diagnosis, the standard primary systemic chemotherapy for women with advanced endometrial cancer consists of chemotherapy. The optimal regimen remains under investigation. When endometrial cancer has spread beyond the uterine cavity and the draining lymph node basins, the regions at risk for recurrence expand greatly. Patients with positive pelvic washings or adnexal involvement and women with Stage IV or recurrent disease are at risk for tumor spread in a pattern similar to that of women with ovarian cancer. These patients are at significant risk for the development of intraabdominal carcinomatosis as well as hepatic, pulmonary, bone, and central nervous system metastases. Treatment strategies have therefore, focused on this extended area of risk and include: whole abdominal radiotherapy with or without sensitizing chemotherapy, hormonal therapy, and systemic cytotoxic chemotherapy. Prior to 2002, the control arm of collaborative group trials studying the treatment of women with measurable Stage III, Stage IV, and recurrent disease was cytotoxic chemotherapy utilizing cisplatin (50 mg/m_2) and doxorubicin (60 mg/m_2) for seven cycles. This was based on several clinical observations. First, the results of whole abdominal radiotherapy in women with measurable disease has been disappointing, particularly in women with residual tumor size greater than two centimeters.[2,3] Secondly, the results of Gynecologic Oncology Group

(GOG) Protocols #107 & #163 have suggested that the combination of cisplatin/doxorubicin is superior to single-agent therapy and not worse than all other combinations tested. In these two GOG chemotherapy trials, the combination of cisplatin/doxorubicin had a favorable toxicity profile with a response rate and PFS superior to doxorubicin as a single agent and similar to the combination of doxorubicin/paclitaxel. In GOG Protocol #107, the overall response rate to cisplatin/doxorubicin was 42% with a complete response rate of 19%. Median progression-free survival (PFS) was 4.8 months.[4] In GOG Protocol #163, complete response to cisplatin/doxorubicin was 15% with an overall response rate of 40%.This was not significantly different from the experimental arm of doxorubicin/paclitaxel. Median PFS was 7.2 months. [5,6]

The most recently completed GOG trial in advanced and recurrent endometrial cancer (GOG 177) was published in 2004.[7] In this trial, cisplatin/doxorubicin was compared to the triplet of cisplatin/doxorubicin/paclitaxel (TAP). The conclusions of this trial were that TAP improved progression-free survival as well as overall survival in patients with advanced and recurrent endometrial cancer compared to treatment with cisplatin/doxorubicin. The improvement in median progression free survival and median survival was 3 months in the TAP arm. In summary, the best expected result with current cytotoxic chemotherapies is a complete response rate of 22% and a median PFS of approximately nine months in patients with measurable and recurrent disease. In patients with advanced endometrial cancer without measurable disease (GOG 122) the regimen of cisplatin/ doxorubicin was compared to whole abdomen radiation. Chemotherapy was superior to radiation with PFS and overall survival improved approximately 30%. The PFS at 24 months for the entire population studied was approximately 50%. [7.5]

Simultaneous to the evolution of treatment for patients with advanced and recurrent endometrial cancer in the GOG trials described above, a significant segment of the community has adopted a treatment regimen consisting of carboplatin and paclitaxel. Use of this combination is largely based on physicians' vast experience with this regimen's tolerability and efficacy in ovarian cancer. Although the regimen has not been tested in a

phase III setting in endometrial cancer patients, its efficacy has been addressed in phase II studies.[8-10] Hoskins and co-workers reported a 50% to 78% response rate in patients stratified by risk.[8] In this study, toxicity was minimal and median failure-free interval varied by risk group (6 – 23 months). Nakamura and coworkers reported similar results in their phase II trial.[9] Their series of 18 patients with advanced endometrial carcinoma treated with carboplatin (AUC = 5 – 6) and paclitaxel (180 mg/m_2), a complete response rate of 45% was achieved. Finally, Price and coworkers reported a 63% response rate in their series of 20 patients treated with carboplatin (AUC = 5) and paclitaxel (135 – 175 mg/m_2).[10]

Currently the GOG is conducting a randomized phase III trial evaluating doxorubicin/ cisplatin/paclitaxel versus carboplatin/paclitaxel in patients with stage III and IV or recurrent endometrial cancer. In addition, the GOG has supported a randomized prospective trial using carboplatin/paclitaxel as the standard therapy in high risk endometrial histological subtypes (three versus six cycles).

Rationale for Angiogenesis -Targeted Therapeutics:

Angiogenesis is one of the cardinal processes leading to invasion and metastasis of solid tumors. The angiogenic-signaling pathway may be triggered by the release of angiogenic promoters such as vascular endothelial growth factor (VEGF) from tumor cells into the local microenvironment. There is evidence that angiogenesis plays a role in endometrial cancer disease progression and prognosis.[11-16] Given that a relationship has been demonstrated between the expression of biomarkers of angiogenesis and the behavior of endometrial cancer, it is logical to test pharmacological inhibitors of angiogenesis. Neutralizing anti-VEGF monoclonal antibodies have demonstrated therapeutic activity in a variety of pre-clinical solid tumor models.[17, 18] The GOG has supported this concept with the design of phase II trial evaluating Bevacizumab in the treatment of recurrent or persistent endometrial carcinoma.

Given the poor outcomes of advanced endometrial cancer patients with standard chemotherapy regimens and the pre-clinical evidence that angiogenesis plays a role in prognosis we have designed a Phase II

protocol evaluating the regimen of Carboplatin (AUC 5) and paclitaxel (175 mg/m2 over 3 hrs) combined with bevacizumab (15 mg/kg) given every three weeks in patients with advanced stage endometrial cancer.

1.2 BEVACIZUMAB CLINICAL EXPERIENCE

Bevacizumab has been studied in a multitude of Phase I, II, and III clinical trials in more than 5000 patients and in multiple tumor types. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial.). Approximately 130,000 patients have been exposed to bevacizumab as a marketed product or in clinical trials. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Please refer to the bevacizumab Investigator Brochure for descriptions of all completed Phase I, II, and III trials reported to date. (Bevacizumab Investigator Brochure, October 2005)

In a large phase III study (AVF2107g) in patients with metastatic colorectal cancer, the addition of bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF), to irinotecan/5-fluorouracil/leucovorin (IFL) chemotherapy resulted in a clinically and statistically significant increase in duration of survival, with a hazard ratio of death of 0.67 (median survival 15.6 vs. 20.3 months; $p < 0.001$). Similar increases were seen in progression-free survival (6.2 vs. 10.6 months; $p < 0.001$), overall response rate (35% vs. 45%; $p < 0.01$) and duration of response (7.1 vs. 10.4 months; $p < 0.01$) for the combination arm versus the chemotherapy only arm (bevacizumab Investigator Brochure, October 2005).

Based on the survival advantage demonstrated in Study AVF2107g, bevacizumab was designated for priority review and was approved on 26 February 2004 in the United States for first-line treatment in combination with IV 5-FU–based chemotherapy for subjects with metastatic colorectal cancer. (http://www.fda.gov/cder/foi/nda/2004/STN-125085_Avastin.htm)

Additional data from Phase III trials in metastatic CRC (E3200), non–small cell lung cancer (NSCLC; E4599), and metastatic breast cancer (E2100) have also demonstrated clinical benefit from bevacizumab when added to chemotherapy. In Study E3200, the addition of bevacizumab to FOLFOX chemotherapy resulted in improved overall survival compared with FOLFOX alone (13.0 vs. 10.8 months, respectively, HR = 0.75; $p < 0.01$) in a population of previously treated CRC patients.

There was also improved overall survival in first-line NSCLC patients (E4599) treated with carboplatin/paclitaxel + bevacizumab compared with chemotherapy alone (12.3 vs. 10.3 months, respectively; HR = 0.80; p = 0.003). The results from this trial were the basis for FDA approval of bevacizumab for use in combination with carboplatin + paclitaxel as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic, non-squamous NSCLC in October 2006. Finally, patients with untreated metastatic breast cancer (E2100) who received bevacizumab in combination with weekly paclitaxel had a marked improvement in PFS compared with chemotherapy alone (13.3 vs. 6.7 months, respectively; HR = 0.48; $p < 0.0001$) (see the Bevacizumab Investigator Brochure for additional details).

1.3 Translational Research Related to Anti-VEGF Therapy

Markers of Angiogenesis

Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are among the most well studied angiogenic growth factors. In tumors, angiogenesis has been studied by quantifying the tumor blood micro-vessel density (MVD) determined immunohistochemically using antibodies to CD31, a protein expressed on the surface of vascular endothelial cells. MVD has been shown to predict the response of gastric adenocarcinomas to taxane-based therapy.[20] In addition to MVD, most angiogenesis studies also evaluate VEGF, which has been shown to promote neovascularization and stimulate endothelial cell survival. [21] VEGF levels were also found to correlate with MVD in endometrial and cervical, but not ovarian cancers. [22,24] In ovarian cancer, higher VEGF

levels, but not MVD, were found to significantly correlate with decreased patient survival. [23] Multivariate analysis demonstrated that VEGF was an independent prognostic indicator of overall survival. [23] Additionally, resting and activated circulating endothelial cells (CECs) were found to be elevated in newly diagnosed cancer patients (lymphoma) and declined with curative therapy.[25] Though this has not been studied in endometrial cancer patients, this is a potential novel surrogate marker for angiogenesis. [25]

Immunohistochemistry will be utilized to evaluate the expression of CD-31 and VEGF in previously untreated primary or metastatic tumor tissue. Expression of these angiogenic markers will be examined in conventional unstained tumor sections compared with tissue microarrays (TMAs). An analysis will be undertaken to assess the relationship between tumor tissue expression of angiogenic markers and clinical outcome including tumor response, progression free survival and overall survival in this patient population in the TMAs created for this trial if appropriate. If not, conventional unstained tissue sections will be used to examine the relationship between the angiogenic markers and clinical outcome in patients participating in this treatment protocol. The exact choice of biomarkers to be evaluated and assays to be performed in tumor and/or serum specimens will be reevaluated based on evolving data in the field. Serum and plasma will be obtained prior to therapy and prior to each cycle of chemotherapy to evaluate if levels of angiogenin, bFGF, VEGF, and VCAM-1 can predict over time if these levels are related to therapy or response to therapy. This is an exploratory analysis to attempt to generate further hypothesis.

Genomic Analysis: Possible role of tumor suppressor genes (TSGs) in endometrial cancer.

Several TSGs have already been implicated in endometrial cancer, either in germ line or sporadic, such as pTEN, p53, MSH2, MLH1, and p16 (Appendix H). Polymorphisms that reduce expression of these genes could lead to increased risk. Commonly, one assumes that both alleles must be lost in somatic cells before a TSG contributes to tumor

transformation or progression. However, with one allele yielding reduced levels, loss of the high-expressing allele may suffice for progression. Such amber mutations are difficult to detect, but in the case of adenomatous polyposis coli tumor suppressor gene (APC) [26], this concept has been validated in colon cancer. Therefore Yan et al. [26] had called for the development of a rapid assay to measure such allelic expression imbalance (AEI), as a potential contributor to disease risk. We have successfully developed such an assay, measuring the allelic ratios for genomic DNA and mRNA in tissue samples. This has already led to the characterization of the functional polymorphisms in two genes, namely MDR1 and OPRM1. [27,28] In this project we will use AEI assays for 18 TSGs – already fully developed and validated. This has already revealed the presence of relatively frequent AEI for three genes in normal subjects (transformed B lymphocytes), one of which being p16 (5% frequency of AEI in normals). Even where no AEI is detectable in normal subjects (only 15-30 subjects have been tested thus far for each gene), we expect germ line AEI to more frequent in endometrial cancer patients if the TSG plays a role. Moreover, we expect to find the high-expressing gene to be deleted in the tumor tissues as reported for APC.[26] This specific set of predictions provides a powerful means of sorting out those functional polymorphisms and genes with an immediate role in endometrial cancer.

Endometrial cancer is a monoclonal disease and is characterized by a high degree of genetic damage that is manifest both at the karyotypic and the molecular levels. It is unclear whether the severity of these alterations reflects the need to inactivate multiple genes or is the result of wide spread loss of genomic stability. Specific genes that are altered during the development of endometrial cancers are illustrated in Appendix H

Since only 5% of endometrial cancers are hereditary, most of the molecular alterations involved in endometrial carcinogenesis are thought to be associated with sporadic tumors. Alteration of the PTEN and p53 tumor suppressor gene is the most frequent genetic event described thus far in endometrial cancers and has been associated a worse prognosis

[30,31] Extensive genotyping in both normal and tumor tissues can yield additional information on a patient's tumor characteristics and prognosis.

The availability of extensive genotyping panels at the Pharmacogenomics Laboratory affords the opportunity to search broadly for genetic variants affecting disease risk and treatment response. More than 180 polymorphisms can be measured for pharmacogenetic studies, involving drug metabolizing enzymes, transporters, and drug targets. Moreover the panels currently include 70 genes involved in tumor biology. While we will broadly run these panels for most samples, the present project focuses on 18 TSGs, a rich target implicated in all cancers but in need of novel approaches capable of revealing their true role in cancer and treatment. This TSG list does not include p53, because this gene harbors no germ line SNP in the transcribed region of sufficient frequency that can serve as a marker for AEI. Also, MLH1 is as yet missing (Appendix H), but will be added for this project. Focus on TSGs in this context promises maximal return, and provides the basis for more extensive studies in the future.

1.4 STUDY RATIONALE

Limited numbers of patients have been treated previously on phase II trials with carboplatin/paclitaxel therefore consideration was made to performing a randomized phase II to further characterize the efficacy of this treatment regimen. However, there is an ongoing GOG Phase III trial evaluating paclitaxel/cisplatin/doxorubicin compared to carboplatin/paclitaxel which will provide a contemporary treatment group that will permit a reasonable estimate of the contribution of bevacizumab to the treatment outcomes (eligibility criteria will be matched in the current trial to that ongoing phase III trial). Given the poor outcomes of advanced endometrial cancer patients with standard chemotherapy regimens and the pre-clinical evidence that angiogenesis plays a role in prognosis we have designed a Phase II protocol evaluating the regimen of Carboplatin (AUC 5) and paclitaxel (175 mg/m2 over 3 hrs) combined with bevacizumab (15 mg/kg) given every three weeks in patients with advanced stage endometrial cancer.

2.0 OBJECTIVES

2.1 Primary

2.11 To assess the progression free survival at 24 months after initiating treatment for advanced endometrial cancer treated with carboplatin/paclitaxel/bevacizumab.

2.12 To determine the toxicity profile of carboplatin/ paclitaxel/bevacizumab as assessed by NCI Common Toxicity Criteria for Adverse Events Version 3.0 (CTCAE) in this cohort of patients.

2.2 Secondary

2.21 To estimate overall survival for patients treated with this regimen.

2.22 To assess objective tumor response using modified RECIST criteria (see section 3.2.2.1).

2.3 Translational Research Objectives (see Appendix G,H,I for description)

2.31 Determine the levels of specific biomarkers

2.311 Use immunohistochemistry assays to evaluate the levels of vascular endothelial growth factor (VEGF), CD-31, thrombospodin-1 (TSP-1), and CD-105 (endoglin) in pre-treatment tumor tissue and correlate with clinical response in pre-treatment tissue (from recent surgery).

2.312 Use enzyme-linked immunosorbent assays (ELISA) to quantify the level of angiogenin, basic fibroblast growth factor (bFGF), VEGF, and soluble vascular cell adhesion molecule-I (VCAM-I) in serum obtained prior to initiation of therapy, prior to each additional cycle of chemotherapy and through follow up (every 3 months until recurrence or 3 years). This will be used to evaluate the changes in these levels over time.

2.313 Use ELISA to measure the level of VEGF in plasma obtained prior to initiation of therapy prior to each additional cycle of chemotherapy and through follow up. This will be used to evaluate the changes in these levels over time.

2.32 Perform exploratory data analysis

2.321 Exploratory analysis to develop and test a high through-put system for the identification of genetic polymorphisms in endometrial cancer tissue and blood. (see Appendix I)

3.0 STUDY DESIGN

3.1 Description of the Study

This is a single center open label single arm Phase II clinical trial with no control group. A regimen of Carboplatin (AUC 5 IV over 30 minutes) and paclitaxel (175 mg/m2 over 3 hrs) combined with bevacizumab (15 mg/kg IV) given every 21 days in patients with advanced stage endometrial cancer for a maximum of 6 cycles.

3.2 Outcome Measures

3.2.1 Primary Outcome Measures:

3.2.1.1 Progression-Free Survival is the period from study entry until disease progression or date of last contact.

3.2.1.2 Toxicity profile of the triple drug combination therapy as assessed by CTCAE v3.(Appendix C)

3.2.2 Secondary Outcome Measures

3.2.2.1 Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest dimension to be recorded). Patients with measurable disease must have at least one "target lesion" to be used to assess response on this protocol as defined by RECIST (target lesion must be greater than 1 cm by spiral CT). Measurable disease, baseline documentation of "target" and "non-target"

lesions, follow-up documentation of “target” and “non-target” lesions and documentation of the best response achieved (complete response, partial response, stable disease) or of progression of disease will be required.

3.2.2.1.1 Complete Response (CR) is disappearance of gross evidence of disease with confirmation at least 4 weeks later.

3.2.2.1.2 Partial Response (PR) is a 30% or greater reduction in measurement of longest dimension of each lesion with confirmation at least 4 weeks later..

3.2.2.1.3 Progressive Disease (PD) at least a 20% increase in the sum of the longest dimension of target lesions, taking as reference the smallest sum of the longest dimension recorded since the treatment start or the appearance of one or more new lesions.

3.2.2.1.4 Stable Disease is any condition not meeting the above criteria.

3.2.2.3 Overall Survival is the duration of time from initiation of treatment to death or the date of last contact.

Evaluation of best overall response will be evaluated as follows:

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD

Any	PD*	No	PD
Any	Any	Yes	PD

*Unequivocal progression of existing non-target lesions, other than pleural effusions without cytological proof of neoplastic origin, in the opinion of the treating physician (in this circumstance an explanation must be provided)

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

4.0 SAFETY PLAN

See Section 4.1 for complete details of the safety evaluation for this study.

Frequency and severity of adverse effects as assessed by CTCAE v3 for the triple drug combination. The frequency of all toxicities will be tabulated from submitted case report forms and summarized for review. All serious and/or unexpected events will be communicated to FDA, Genentech, Inc and to the OSU Cancer IRB at the James Cancer Hospital and Solove Research Institute. See section 12.2 for details.

4.1 GENERAL PLAN TO MANAGE SAFETY

a. Bevacizumab-Specific

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria (see Section 5.3) and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording

of adverse events, physical examinations, blood pressure, and laboratory. Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28–42 days) after the decision to discontinue treatment (see Section 7.1.3).

Specific monitoring procedures are as follows:

- Hypertension will be monitored through routine evaluation of blood pressure prior to each bevacizumab treatment. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab.
- Proteinuria will be monitored by urine protein:creatinine (UPC) ratio.See section 9.0 Clinical and Laboratory Evaluations and Appendix E: Procedure for Obtaining a urine protein/creatinine ratio.

- If patients on treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4-8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin/restart bevacizumab until 4 weeks after that procedure (in the case of high risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 wk and bevacizumab no earlier than 8 wk after surgery).

Please see Section 8.0 for detailed instructions for the management of study drug–related toxicities.

5.0 STUDY SUBJECTS

5.1 Subject Selection

5.1.1 All patients with advanced endometrial carcinoma, of any histology including clear cell and serous papillary carcinomas. Surgical stage III and IV disease, including those patients with positive adnexa, tumor invading the serosa, positive pelvic and/or para-aortic nodes, involvement of bowel mucosa, intra-abdominal metastases, positive pelvic washings or vaginal involvement.

5.1.2 Surgery must have included a hysterectomy, and bilateral salpingo-oophorectomy. Selective pelvic and para-aortic lymph node sampling is optional however strongly encouraged. Chemotherapy will be initiated within 12 weeks after surgery and the patient must have fully recovered from surgery.

5.1.3 All patients and/or guardians must have signed an informed consent prior to entry into the study.

5.2 Inclusion Criteria

5.2.1 All patients with advanced endometrial carcinoma, of any histology including clear cell and serous papillary carcinomas. Surgical stage III and IV disease, including those patients with positive adnexa, tumor invading the serosa, positive pelvic and/or para-aortic nodes, involvement of bowel mucosa, intra-abdominal metastases, positive pelvic washings or vaginal involvement.

5.2.2 Patients must have adequate end-organ function as follows:

5.2.2.1 Bone marrow function: Absolute neutrophil count (ANC) greater than or equal to 1,500/mcl and WBC ≥ 3.0, equivalent to Common Terminology Criteria for Adverse Events (CTCAE v3.0) grade 1. Platelets greater than or equal to 100,000/mcl.

5.2.2.2 Renal function: creatinine less than or equal to 1.5 x institutional upper limit normal (ULN), CTCAE v3.0 grade 1.

5.2.2.3 Hepatic function: Bilirubin less than or equal to 1.5 x ULN (CTCAE v3.0 grade 1). SGOT and alkaline phosphatase less than or equal to 2.5 x ULN (CTCAE v3.0 grade 1).

5.2.2.4 Urine Protein Creatinine: Urine protein creatinine (UPC) ratio must be < 1.0 gm. If UPC ratio > 1, collection of 24-hour urine measurement of urine protein is recommended.

UPC ratio of spot urine is an estimation of the 24 urine protein excretion – a UPC ratio of 1 is roughly equivalent to a 24-hour urine protein of 1 gm. UPC ratio is calculated using one of the following formulas:

1. [urine protein]/[urine creatinine] – if both protein and creatinine are reported in mg/dL

2. [(urine protein) x0.088]/[urine creatinine] – if urine creatinine is reported in mmol/L

5.2.2.5 Blood coagulation parameters: Blood coagulation parameters: PT such that international normalized ratio (INR) is < 1.5 (or an in-range INR, usually between 2 and 3, if a patient is on a stable dose of therapeutic warfarin) and a PTT < 1.5 times the institutional upper limit of normal.

5.2.3 Patients must have a GOG Performance Status of 0, 1, or 2.

5.2.4 Patients must have signed an approved informed consent and authorization

5.2.5 Patients must be 18 years of age or older.

5.2.6 Patients may have received radiation for the treatment of endometrial cancer. At least four weeks should have elapsed since completion of RT involving the whole pelvis or over 50% of the spine.

5.2.7 Patients must be enrolled between 1 and 12 weeks after initial surgery performed for the combined purpose of diagnosis, staging and cytoreduction (not to receive chemotherapy for at least 4 weeks following surgery).

5.2.8 Patients with measurable and non-measurable disease are eligible. Patients may or may not have cancer-related symptoms.

5.3 Exclusion Criteria

a. Disease-Specific Exclusions

- Patients with a concomitant malignancy other than non-melanoma skin cancer. Patients with a prior malignancy who have been disease-free for less than 5 years will be excluded.
- Patients with concomitant medical illness such as serious uncontrolled infection, uncontrolled angina, or serious peripheral neuropathy, which, in the opinion of the treating physician, make the treatments prescribed on this study unreasonably hazardous for the patient.
- Patients whose circumstances will not permit study completion or adequate follow-up.
- Patients who have received prior cytotoxic chemotherapy for the treatment of endometrial cancer, including chemotherapy used for radiation sensitization. Patients may have received prior hormonal therapy or therapy with biologic agents, but such therapies must be discontinued prior to entry on this study and prior to registration.

b. General Medical Exclusions

Subjects meeting any of the following criteria are **ineligible** for study entry:

- Inability to comply with study and/or follow-up procedures
- Current, recent (within 4 weeks of the first infusion of this study), or planned participation in an experimental drug study other than a Genentech Sponsored bevacizumab cancer study.
- Life expectancy of less than 12 weeks.
- Active malignancy, other than superficial basal cell and superficial squamous (skin) cell.

c. Bevacizumab-Specific Exclusions

- Inadequately controlled hypertension (defined as systolic blood pressure >150 and/or diastolic blood pressure > 100 mmHg)
- Any prior history of hypertensive crisis or hypertensive encephalopathy
- New York Heart Association (NYHA) Grade II or greater congestive heart failure (see Appendix E)
- Known CNS disease, except for treated brain metastasis

  Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 3 months prior to Day 1 will be excluded
- Significant vascular disease (e.g., aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day 1.
- History of hemoptysis (>1/2 teaspoon of bright red blood per episode) within 1 month prior to Day 1.
- Symptomatic peripheral vascular disease
- Evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation)
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 1 or anticipation of need for major surgical procedure during the course of the study (Enrollment is

acceptable however chemotherapy should not be initiated earlier than 28 days following surgery)

- Core biopsy or other minor surgical procedure within 7 days prior to study enrollment (patients may have had minor surgical procedures but will need to allow for 7 days prior to enrollment). excluding placement of a vascular access device (no delay needed)
- History of abdominal fistula, gastrointestinal perforation, or intra-abdominal abscess within 6 months prior to Day 1.
- Serious, non-healing wound, ulcer, or bone fracture
- Proteinuria at screening as demonstrated by either
  - Urine protein:creatinine (UPC) ratio $\geq$ 1.0 at screening OR
  - Urine dipstick for proteinuria ≥ 2+ (patients discovered to have ≥2+ proteinuria on dipstick urinalysis at baseline should undergo a 24 hour urine collection and must demonstrate ≤ 1g of protein in 24 hours to be eligible).
- Known hypersensitivity to any component of bevacizumab
- any history of stroke or transient ischemic attack at any time
- Pregnant (positive pregnancy test) or lactating. Use of effective means of contraception (men and women) in subjects of child-bearing potential is required.
- history of myocardial infarction or unstable angina within 12 months of study enrollment
- Patients who have received prior therapy with any anti-VEGF drug, including Bevacizumab.

6.0 TREATMENT PLAN

6.1 Treatment Plan

This is a single center open label single arm Phase II clinical trial with no control group. A regimen of Carboplatin (AUC 5 IV over 30 minutes) and paclitaxel (175 mg/m2 over 3 hrs) combined with bevacizumab (15 mg/kg IV) given every 21

days in patients with advanced stage endometrial cancer for a maximum of 6 cycles

6.2 Accrual

Thirty-eight patients will be treated on protocol (inevaluable patients to be replaced at one to one). There are currently five full-time gynecologic oncologists at our institution performing over five hundred major oncology surgeries annually. Approximately 30-40% are endometrial malignancy cases, yielding about 150-200 primary endometrial cancer patients annually. Approximately 20-30 (15%) of these patients will be diagnosed with stage III-IV endometrial cancer. In addition, two other academic gynecologic practices with high clinical volumes have committed to the current protocol if single institution recruitment does not meet goals (minimum of 12 patients annually). We estimate recruitment to be completed in approximately 2 years. Patients will be followed for a maximum of 5 years or until death. Approximate date of completion is 2013, but mature data should be available by 2010.

7.0 STUDY MEDICATION

7.1 BEVACIZUMAB DOSAGE AND FORMULATION

Bevacizumab is to be given at 15 mg/kg every 3 weeks for 6 cycles.

Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for intravenous (IV) infusion. Bevacizumab may be supplied in 5-cc (100-mg), 20-cc (400-mg), and 50-cc (1000-mg) glass vials containing 4 mL, 16 mL, or 40 mL of bevacizumab, respectively (all at 25 mg/mL). Vials contain bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection (SWFI), USP. Vials contain no preservative and are suitable for single use only.

a. Safety Profile

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in phase III trials include congestive heart failure (CHF), gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE). These and other safety signals are described in further detail as follows and in the bevacizumab Investigator Brochure.

Hypertension: An increased incidence of hypertension has been observed in patients treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequelae of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) (Ozcan et al., 2006; Glusker et al., 2006). RPLS may include signs and symptoms of headache, altered mental function, seizures, and visual disturbances / cortical blindness and requires treatment, which should include control of hypertension, management of specific symptoms, and discontinuation of bevacizumab.

There is no information on the effect of bevacizumab in patients with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiating bevacizumab therapy in these patients. Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for patients on treatment with or without bevacizumab.

Temporary interruption of bevacizumab therapy is recommended in patients with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued. Bevacizumab should be permanently discontinued in patients who develop hypertensive crisis or hypertensive encephalopathy.

Proteinuria: An increased incidence of proteinuria has been observed in patients treated with bevacizumab compared with control arm patients. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g). The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated patients, and Grade 4 in up to 1.4% of bevacizumab-treated patients. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy. Bevacizumab should be discontinued in patients who develop Grade 4 proteinuria (nephrotic syndrome)

Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose.

Proteinuria will be monitored by urine protein:creatinine (UPC) ratio at least every 6 weeks. If the UPC ratio is not available, a dipstick urinalysis may be used to allow treatment to proceed.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in patients treated with bevacizumab in the colorectal cancer trials and, to a lesser extent, in patients treated with bevacizumab in NSCLC and breast cancer trials.

Venous thromboembolism (including deep venous thrombosis, pulmonary embolism, and thrombophlebitis): In the phase III pivotal trial in metastatic CRC, there was a slightly higher rate of **venous TE** events that was not statistically significant in patients treated with bevacizumab plus chemotherapy compared with chemotherapy alone (19% vs. 16%).

In Study AVF2107g, a Phase III, pivotal trial in metastatic CRC, VTE events, including deep venous thrombosis, pulmonary embolism, and thrombophlebitis, occurred in 15.2% of patients receiving chemotherapy alone and 16.6% of patients receiving chemotherapy + bevacizumab.

The incidence of NCI-CTC Grade ≥ 3 venous VTE events in one NSCLC trial (E4599) was higher in the bevacizumab-containing arm compared to the chemotherapy control arm (5.6% vs. 3.2%). One event (0.2%) was fatal in the bevacizumab-containing arm; not fatal events were reported in the carboplatin/paclitaxel arm (see Bevacizumab Investigator Brochure). In metastatic CRC clinical trials, the incidence of VTE events was similar in patients receiving chemotherapy + bevacizumab and those receiving the control chemotherapy alone.

In clinical trials across all indications the overall incidence of VTE events was 2.8%–17.3% in the bevacizumab-containing arms compared with 3.2%–15.6% in the chemotherapy control arms. The use of bevacizumab with chemotherapy does not substantially increase the risk of VTE event compared with chemotherapy alone. However, patients with metastatic CRC who receive bevacizumab and experienced a VTE event may be at higher risk for recurrence of VTE event.

Arterial Thromboembotic Events: **:** An increased incidence of ATE events was observed in patients treated with bevacizumab compared with those receiving control treatment. ATE events include cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE events. In a pooled analysis of data from five randomized Phase II and III trials (mCRC [AVF2107g, AVF2192g, AVF0780g]; locally advanced or metastatic NSCLC [AVF0757g]; metastatic breast cancer [AVF2119g]), the incidence rate of ATE events was 3.8% (37 of 963) in

patients who received chemotherapy + bevacizumab compared with 1.7% (13 of 782) in patients treated with chemotherapy alone. ATE events led to a fatal outcome in 0.8% (8 of 963) of patients treated with chemotherapy + bevacizumab and 0.5% (4 of 782) of patients treated with chemotherapy alone. Cerebrovascular accidents (including TIAs) occurred in 2.3% of patients treated with chemotherapy + bevacizumab and 0.5% of patients treated with chemotherapy alone. Myocardial infarction occurred in 1.4% of patients treated with chemotherapy + bevacizumab compared with 0.7% of patients treated with chemotherapy alone (see the Bevacizumab Investigator Brochure for additional details).

Aspirin is a standard therapy for primary and secondary prophylaxis of arterial thromboembolic events in patients at high risk of such events, and the use of aspirin ≤ 325 mg daily was allowed in the five randomized studies discussed above. Use of aspirin was assessed routinely as a baseline or concomitant medication in these trials, though safety analyses specifically regarding aspirin use were not preplanned. Due to the relatively small numbers of aspirin users and arterial thromboembolic events, retrospective analyses of the ability of aspirin to affect the risk of such events were inconclusive. However, similarly retrospective analyses suggested that the use of up to 325 mg of aspirin daily does not increase the risk of grade 1-2 or grade 3-4 bleeding events, and similar data with respect to metastatic colorectal cancer patients were presented at ASCO 2005 (Hambleton et al., 2005). Further analyses of the effects of concomitant use of bevacizumab and aspirin in colorectal and other tumor types are ongoing.

Gastrointestinal perforation Patients with metastatic carcinoma may be at increased risk for the development of gastrointestinal perforation when treated with bevacizumab and chemotherapy. Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory process and gastrointestinal perforation to bevacizumab has not been established. Nevertheless, caution should be exercised when treating patients with

intra-abdominal inflammatory processes with bevacizumab. Gastrointestinal perforation has been reported in other trials in non-colorectal cancer populations (e.g., ovarian, renal cell, pancreas, and breast, and NSCLC) and may be higher in incidence in some tumor types.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in patients receiving bevacizumab. In an analysis of pooled data from two trials in metastatic colorectal cancer, patients undergoing surgery 28-60 days before study treatment with 5-FU/LV plus bevacizumab did not appear to have an increased risk of wound healing complications compared to those treated with chemotherapy alone (Scappaticci et al., 2005). Surgery in patients currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in patients receiving elective surgery; however, the estimated half life of bevacizumab is 20 days. Bevacizumab should be discontinued in patients with severe wound healing complications.

If patients receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Patients undergoing a major surgical procedure should not begin or restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that chemotherapy be restarted no earlier than 6 weeks and bevacizumab no earlier than 8 weeks after surgery).

Hemorrhage: Overall, grade 3 and 4 bleeding events were observed in 4.0% of 1132 patients treated with bevacizumab in a pooled database from eight phase I, II, and III clinical trials in multiple tumor types (bevacizumab Investigator Brochure, October 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (see below) and minor mucocutaneous hemorrhage.

Tumor-associated hemorrhage – Major or massive pulmonary hemorrhage or hemoptysis has been observed primarily in patients with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. Among the possible risk factors evaluated (including squamous cell histology, treatment with anti-rheumatic/anti-inflammatory drugs, treatment with anticoagulants, prior radiotherapy, bevacizumab therapy, previous medical history of atherosclerosis, central tumor location, and cavitation of tumors during therapy), the only variables that showed statistically significant correlations with bleeding were bevacizumab therapy and squamous cell histology.

Of patients experiencing pulmonary hemorrhages requiring medical intervention, many had cavitation and/or necrosis of the tumor, either preexisting or developing during bevacizumab therapy. Patients developing lung cavitation on treatment should be assessed by the treating physician for risk-benefit.

In Study E4599, in which squamous cell carcinoma was excluded, the rate of any type of Grade ≥ 3 hemorrhage was 1.0% in the control arm (carboplatin and paclitaxel) versus 4.1% in the carboplatin and paclitaxel + bevacizumab arm (Sandler et al. 2006).

GI hemorrhages, including rectal bleeding and melena have been reported in patients with CRC, and have been assessed as tumor-associated hemorrhages.

Tumor-associated hemorrhages were also seen rarely in other tumor types and locations, including a case of CNS bleeding in a patient with hepatoma with occult CNS metastases and a patient who developed continuous oozing of blood from a thigh sarcoma with necrosis.

Mucocutaneus Hemorrage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of patients treated with bevacizumab. These were most commonly NCI-CTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical

intervention and did not require any changes in bevacizumab treatment regimen.

There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of bevacizumab-treated patients developing signs and symptoms that are consistent with RPLS, a rare neurologic disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In patients who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of bevacizumab. The safety of reinitiating bevacizumab therapy in patients previously experiencing RPLS is not known (Glusker et al. 2006; Ozcan et al. 2006).

Congestive heart failure: In clinical trials CHF was observed in all cancer indications studied to date, but predominantly in patients with metastatic breast cancer. In the Phase III clinical trial of metastatic breast cancer (AVF2119g), 7 (3%) bevacizumab-treated patients experienced CHF, compared with two (1%) control arm patients. These events varied in severity from asymptomatic declines in left ventricular ejection fraction (LVEF) to symptomatic CHF requiring hospitalization and treatment. All the patients treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose of 240–360 mg/m^2). Many of these patients also had prior radiotherapy to the left chest wall. Most of these patients showed improved symptoms and/or left ventricular function following appropriate medical therapy (Miller et al. 2005).

In a randomized, Phase III trial of patients with previously untreated metastatic breast cancer (E2100), the incidence of LVEF decrease (defined as NCI-CTC Grade 3 or 4) in the paclitaxel + bevacizumab arm was 0.3% versus 0% for the paclitaxel alone arm

No information is available on patients with preexisting CHF of New York Heart Association (NYHA) Class II–IV at the time of initiating bevacizumab therapy, as these patients were excluded from clinical trials.

Prior anthracyclines exposure and/or prior radiotherapy to the chest wall may be possible risk factors for the development of CHF. Caution should be exercised before initiating bevacizumab therapy in patients with these risk factors.

A Phase II trial in patients with refractory acute myelogenous leukemia reported 5 cases of cardiac dysfunction (CHF or LVEF decrease to $< 40\%$) among 48 patients treated with sequential cytarabine, mitoxantrone, and bevacizumab. All but 1 of these patients had significant prior exposure to anthracyclines as well (Karp et al. 2004).

Two additional studies investigated concurrent administration of anthracyclines and bevacizumab. In 21 patients with inflammatory breast cancer treated with neoadjuvant docetaxel, doxorubicin, and bevacizumab, no patients developed clinically apparent CHF; however, patients had asymptomatic decreases in LVEF to $< 40\%$ (Wedam et al. 2004). In a small Phase II study in patients with soft tissue sarcoma, 2 of the 17 patients treated with bevacizumab and high-dose doxorubicin (75 mg/m^2) developed CHF (one Grade 3 event after a cumulative doxorubicin dose of 591 mg/m^2, one Grade 4 event after a cumulative doxorubicin dose of 420 mg/m^2); an additional 4 patients had asymptomatic decreases in LVEF (D'Adamo et al. 2004).

Other studies in patients with various tumor types and either a history of anthracycline exposure or concomitant use with bevacizumab are ongoing.

Patients receiving concomitant anthracyclines or with prior exposure to anthracyclines should have a baseline MUGA scans or echocardiograms (ECHOs) with a normal LVEF.

Neutropenia: Increased rates of severe neutropenia, febrile neutropenia, or infection with severe neutropenia (including some fatalities) have been

observed in patients treated with some myelotoxic chemotherapy regimens plus bevacizumab in comparison to chemotherapy alone (Sandler et al. 2006).

Additional Adverse Events: See the bevacizumab Investigator Brochure for additional details regarding the safety experience with bevacizumab.

7.1.1 Bevacizumab Administration

Bevacizumab will be diluted in 100ml of 0.9% Sodium Chloride Injection.. Administration will be as a continuous IV infusion. Anaphylaxis precautions should be observed during study drug administration. It is not necessary to correct dosing based on ideal weight.

The initial dose will be delivered over 90±15 minutes. If the first infusion is tolerated without infusion-associated adverse events (fever and/or chills), the second infusion may be delivered over 60±10 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be delivered over 30±10 minutes.

If a subject experiences an infusion–associated adverse event, she may be premedicated for the next study drug infusion; however, the infusion time may not be decreased for the subsequent infusion. If the next infusion is well tolerated with premedication, the subsequent infusion time may then be decreased by 30±10 minutes as long as the subject continues to be premedicated. If a subject experiences an infusion-associated adverse event with the 60-minute infusion, all subsequent doses should be given over 90±15 minutes. Similarly, if a subject experiences an infusion-associated adverse event with the 30-minute infusion, all subsequent doses should be given over 60±10 minutes.

7.1.2 Bevacizumab Storage

Upon receipt of the study drug, vials are to be refrigerated at 2°C–8°C (36°F–46°F) and should remain refrigerated until just prior to use. DO NOT FREEZE. DO NOT SHAKE. Vials should be protected from light.

Opened vials must be used within 8 hours. VIALS ARE FOR SINGLE USE ONLY. Vials used for 1 subject may not be used for any other subject. Once study drug has been added to a bag of sterile saline, the solution must be administered within 8 hours.

7.2 PACLITAXEL (NSC #673089)

Formulation: Paclitaxel is a poorly soluble plant product from western yew, Taxus brevifolia. Improved solubility requires a mixed solvent system with further dilutions of either 0.9% sodium chloride or 5% dextrose in water.

Supplier/How Supplied: Commercially available. A sterile solution concentrate, 6 mg/ml in 5 ml vials (30 mg/vial) in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol, USP, 50%. The contents of the vial must be diluted just prior to clinical use. It is also available in 100 and 300 mg vials.

Solution Preparation: Paclitaxel, at the appropriate dose, will be diluted in 500-1000 cc of 0.9% Sodium Chloride injection, USP or 5% Dextrose injection, USP (D5W) (500 cc's is adequate if paclitaxel is a single agent). Paclitaxel must be prepared in glass or polyolefin containers due to leaching of diethylhexlphthalate (DEHP) plasticizer from polyvinyl chloride (PVC) bags and intravenous tubing by the Cremophor vehicle in which paclitaxel is solubilized.

NOTE: Formation of a small number of fibers in solution (within acceptable limits established by the USP Particulate Matter Test for LVPs) have been observed after preparation of taxol. Therefore, in-line filtration is necessary for administration of paclitaxel solutions. In-line filtration should be accomplished by incorporating a hydrophilic, microporous filter of pore size not greater than 0.22 microns (e.g.: IVEX-II, IVEX-HP or equivalent) into the IV fluid pathway distal to the infusion pump

Although particulate formation does not indicate loss of drug potency, solutions exhibiting excessive particulate matter formation should not be used.

Storage: The intact vials can be stored in a temperature range between 2-25^{o}C (36-77°F).

Stability: Commercially available paclitaxel will be labeled with an expiration date. All solutions of paclitaxel exhibit a slight haziness directly proportional to the concentration of drug and the time elapsed after preparation, although when prepared as described above, solutions of paclitaxel (0.3-1.2 mg/ml) are physically and chemically stable for 48 hours. 24

Administration of Paclitaxel: Paclitaxel, at the appropriate dose and dilution, will be given as a 3-hour continuous IV infusion. Paclitaxel will be administered via an infusion control device (pump) using non-PVC tubing and connectors, such as the IV administration sets (polyethylene or polyolefin) which are used to infuse parenteral Nitroglycerin. Nothing else is to be infused through the line where paclitaxel is being administered.

Adverse Effects:

Hematologic: Myelosuppression

Gastrointestinal: Nausea and vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis, ischemic colitis, neutropenic enterocolitis

Heart: arrhythmia, heart block, ventricular tachycardia, myocardial infarction (MI), bradycardia, atrial arrhythmia

Pulmonary: Pneumonitis

Blood Pressure: Hypotension, hypertension (possibly related to concomitant medication--Dexamethasone)

Neurologic: Sensory (taste), peripheral neuropathy, seizures, mood swings, hepatic encephalopathy, encephalopathy

Skin: Infiltration: erythema, induration, tenderness, rarely ulceration, radiation recall reactions

Allergy: Anaphylactoid and urticarial reactions (acute), flushing, rash, pruritus

Liver: Increased SGOT, SGPT, bilirubin and alkaline phosphatase, hepatic failure, hepatic necrosis

Other: Alopecia, fatigue, arthralgia, myalgia, light-headedness, myopathy

Other, Vision: Sensation of flashing lights, blurred vision, scintillating scotomata

*Refer to Package Insert for additional information Paclitaxel is a poorly soluble plant product from western yew, Taxus brevifolia. Improved solubility requires a mixed solvent system with further dilutions of either 0.9% sodium chloride or 5% dextrose in water.

7.3 CARBOPLATIN (Paraplatin®, NSC # 241240)

Formulation: Carboplatin is supplied as a sterile lyophilized powder available in single-dose vials containing 50 mg, 150 mg and 450 mg of carboplatin for administration by intravenous infusion. Each vial contains equal parts by weight of carboplatin and mannitol.

Solution Preparation: Immediately before use, the content of each vial must be reconstituted with either sterile water for injection, USP, 5% dextrose in water, or 0.9% sodium chloride injection, USP, according to the following schedule:

Vial Strength	Diluent Volume
50 mg	5 ml
150 mg	15 ml
450 mg	45 ml

These dilutions all produce a carboplatin concentration of 10 mg/ml.

NOTE: Aluminum reacts with carboplatin causing precipitate formation and loss of potency. Therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

Storage: Unopened vials of carboplatin are stable for the life indicated on the package when stored at controlled room temperature and protected from light.

Stability: When prepared as directed, carboplatin solutions are stable for eight hours at room temperature. Since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded eight hours after dilution.

Supplier: Commercially available from Bristol-Myers Squibb Company.

Administration: Carboplatin will be administered as a 30 minute infusion.

Adverse effects:

Hematologic: Myelosuppression

Gastrointestinal: Nausea, vomiting, diarrhea, abdominal pain, constipation

Neurologic: Peripheral neuropathy, ototoxicity, visual disturbances, change in taste, central nervous system symptoms

Renal: Abnormal renal function test results including serum creatinine, blood urea nitrogen, and creatinine clearance

Hepatic: Abnormal liver function tests including bilirubin, SGOT, and alkaline phosphatase

Electrolyte Changes: Abnormally decreased serum electrolyte values reported for sodium, potassium, calcium, and magnesium

Allergic Reactions: Rash, urticaria, erythema, pruritus, and rarely bronchospasm and hypotension

Injection Site Reactions: Redness, swelling, pain; necrosis associated with extravasation has been reported.

Other: Pain, asthenia, alopecia. Cardiovascular, respiratory, genitourinary, and mucosal side effects have occurred in 6% or less of the patients. Cardiovascular events (cardiac failure, embolism, cerebrovascular accidents) were fatal in less than 1% of patients and did not appear to be related to chemotherapy. Cancer associated hemolytic-uremic syndrome has been reported rarely. Malaise, anorexia, and hypertension have been reported as part of post-marketing surveillance.

*See FDA-approved package insert for a comprehensive list of adverse events associated with carboplatin.

7.4 Methods of Chemotherapy Administration

Biometric considerations in dose calculation

Maximum body surface area used for Carboplatin, Paclitaxel and dose calculations will be 2.0 m2.

Bevacizumab will be dosed at 15 mg/kg, with no maximum to total mg.

7.4.1 Sequence and timing of drug administration:

- Paclitaxel will be infused over 3 hours. Due to the risk of immediate hypersensitivity reaction, paclitaxel should always be the first drug to be infused during any combination.
- Carboplatin will be administered as a 30 minute infusion, following paclitaxel administration.
- Bevacizumab administration will be as a continuous intravenous infusion following carboplatin infusion. Anaphylaxis precautions should be observed during Bevacizumab administration DO NOT ADMINISTER AS AN IV PUSH OR BOLUS. The initial dose should be delivered over 90 minutes as an IV infusion. If the first infusion is well tolerated, the second infusion may be administered over 60 minutes. If the 60- minute infusion is well tolerated, all subsequent infusions may be administered over 30 minutes.

7.4.2 Pre-Medication:

7.4.2.1 Paclitaxel

For all courses where paclitaxel is to be administered, it is recommended that a preparative regimen be employed one hour prior to the treatment regimen on that day, to reduce the risk associated with hypersensitivity reactions to these drugs. This regimen should include a standard dose of Dexamethasone (either IV or PO), an anti-histamine H1 (diphenhydramine 25-50 mg IVP or orally, or an equivalent dose of an alternate H1 blocker such as loratadine or fexofenadine), and a standard dose of antihistamine H2 IVP (such as cimetidine, ranitidine, or famotidine).

7.4.2.2 In the event of a prior Bevacizumab hypersensitivity reaction, the prophylactic regimen should be modified as suggested in 7.4.2.2.1 below.

7.4.2.2.1 Suggested Prophylaxis in Event of Prior Bevacizumab Infusion Reaction

In the event of a prior Bevacizumab hypersensitivity reaction, the following recommended prophylactic regimen is recommended upon re-exposure:

- H1 blocker (diphenhydramine 25-50 mg IVP or orally one hour prior to injection; or an equivalent dose of an alternate H1 blocker such as loratadine 10 mg or fexofenadine 60 mg).
- H2 blocker (famotidine 20 mg IVP or orally one hour prior to injection; or an equivalent dose of an alternate H2 blocker).
- Dexamethasone (10 mg administered PO 12 and 6 hours prior to Bevacizumab injection).

7.4.2.3 Antiemetic Regimens

It is anticipated that nausea and vomiting may be a significant side effect. The following representative antiemetic regimens are suggested:

• Ondansetron 8-32 mg IV 30 minutes prior to administration of chemotherapy and dexamethasone 10-20 mg IV 30 minutes prior to drug administration or,

• Granisetron 10 mcg/kg IV (or 2 mg PO) 30 minutes prior to chemotherapy, with or without lorazepam 0.5-2.0 mg IV 30 minutes prior to chemotherapy.

7.4.2.4 Dosing of Carboplatin

The carboplatin dose will be calculated to reach a target area under the curve (AUC) of concentration x time according to the Calvert formula using an estimated glomerular filtration rate (GFR) from the Jelliffe formula.

The initial dose of carboplatin must be calculated using GFR. In the absence of new renal obstruction or other renal toxicity greater than or equal to CTC Grade 2 (serum creatinine >1.5 x ULN), the dose of carboplatin will not be recalculated for subsequent cycles, but will be subject to dose modification as noted.

In patients with an abnormally low serum creatinine (less than or equal to 0.6 mg/dl), due to reduced protein intake and/or low muscle mass, the creatinine clearance should be estimated using a minimum value of 0.6 mg/dl. If a more appropriate baseline creatinine value is available within 4 weeks of treatment,which may also be used for the initial estimation of GFR.

CALVERT FORMULA:

Carboplatin dose (mg) = target AUC x (GFR + 25)

For the purposes of this protocol, the GFR is considered to be equivalent to the creatinine clearance. The creatinine clearance is calculated by the method of Jelliffe using the following formula:

$$Ccr = 0.9 \times \frac{\{98 - [0.8\ (age - 20)]\}}{Scr}$$

Where: Ccr = creatinine clearance in ml/min

Age = patient's age in years (from 20-80)

Scr = serum creatinine in mg/dl

7.4.2.5 Dosing of Bevacizumab

Bevacizumab will be administered at 15 mg/kg IV. Patient weight prior to each cycle will be used to determine the Bevacizumab dose.

8.0 TREATMENT MODIFICATIONS

In order to maintain dose-intensity and cumulative dose-delivery on this study, reasonable efforts will be made to minimize dose reduction and treatment delays as specified. No dose escalation is planned for this study.

8.1 Individual Dose Modification Levels

All modifications are relative to the actual starting doses for the specific Regimen. For application of individual dose modifications, see specific guidelines below. Allowable drug dose levels and instructions are summarized in Tables A, B, and C.

• General Guidelines for Hematologic Toxicity (Section 8.2)

• Hematologic Nadirs, Table A (Section 8.3)

• Delayed Hematologic Recovery, Table B (Section 8.4)

• Non-Hematologic Toxicity Table C (Section 8.5)

8.2 General Guidelines for Hematologic Toxicity

8.21 Initial treatment modifications will consist of cycle delay and/or dose reduction as directed.

8.22 Treatment decisions will be based on the absolute neutrophil count (ANC) rather than the total white cell count (WBC).

8.23 Subsequent cycles of therapy will not begin until the ANC is ≥ 1500 cells/mm3 (CTCAE Grade 1) and the platelet count is ≥ 100,000/mm3.

Therapy may be delayed for a maximum of three weeks until these values are achieved. Patients who fail to recover adequate counts within a three-week delay will no longer receive protocol directed cytotoxic chemotherapy

8.24 Use of Hematopoietic Cytokines and Protective Agents

The use of hematopoietic cytokines and protective reagents are restricted as noted:

8.241 In general, patients will NOT receive prophylactic filgrastim (G-CSF), PEG-filgrastim (Neulasta), or sargramostim (GM-CSF) unless they experience treatment delays or recurrent neutropenic complications after treatment modifications as specified. However, this is not disallowed in the current protocol. Patients may also receive growth factors for management of neutropenic complications in accordance with clinical treatment guidelines. If required, it is recommended that growth factors be initiated the day after the last dose of chemotherapy and typically continuing for a minimum of 10 days or until the ANC is sustained above >1000/mm3. Growth factors should be discontinued if the ANC exceeds 10,000/ mm3 and should not be used within 72 hours of a subsequent dose of chemotherapy.

8.242 Patients will NOT receive prophylactic thrombopoietic agents unless they experience recurrent Grade 4 thrombocytopenia after treatment modifications as specified below.

8.243 Patients may receive erythropoietin (EPO), iron supplements, and/or transfusions as clinically indicated for management of anemia.

8.244 Patients may receive amifostine or other protective reagents.

8.25 Dose Modifications for Bevacizumab

There will be no dose reduction for Bevacizumab. Treatment should be interrupted or discontinued for certain adverse events, as described above.

8.3 Modifications for Hematologic Toxicity (Nadirs)

8.31 Initial occurrence of dose-limiting neutropenia (defined in 8.32) and dose limiting thrombocytopenia (defined in 8.33) will be performed according to Table A.

8.32 Dose-Limiting Neutropenia (DLT-ANC) is defined by the occurrence of febrile neutropenia or prolonged Grade 4 neutropenia persisting ≥ 7 days. There will be no modifications for uncomplicated Grade 4 neutropenia lasting less than 7 days. Febrile neutropenia is defined within the CTCAE as fever with or without clinically or microbiologically documented infection with ANC less than 1,000 /mm3 and fever greater than or equal to 38.5°C.

8.33 Dose-limiting thrombocytopenia (DLT-PLT) is defined by any occurrence of Grade 4 thrombocytopenia (<25,000/mm3) or bleeding associated with Grade 3 thrombocytopenia (25,000 to <50,000/mm3). There will be no modifications for uncomplicated Grade 3 thrombocytopenia.

Table A: Modification Instructions for Dose-Limiting Hematologic Toxicity				
DLT ANC	**DLT PLT**	**First Occurrence**	**Second Occurrence**	**Third Occurrence**
Yes	No	Reduce carboplatin one AUC unit	Add G-CSF and maintain all current drug doses	Off Study
Yes	Yes	Reduce carboplatin one AUC unit	Add G-CSF and decrease carboplatin one AUC	Off Study
No	Yes	Reduce carboplatin one AUC unit	decrease carboplatin one AUC	Off Study

8.4 Modifications for Delayed Hematologic Recovery:

8.41 Delay on the basis of neutropenia (Delay-ANC) is defined if the ANC is less than 1,500 cells/mm3 (CTCAE Grade 2 or worse) within 24 hours prior to scheduled therapy, or less than 1,000 cells/ mm3, if the patient received G-CSF during the previous cycle.

8.42 Delay on the basis of thrombocytopenia (Delay-PLT) is defined if the platelet count is less than 100,000/ mm^3 within 24 hours prior to scheduled therapy.

8.43 Modifications noted below are only required for management of delays in the absence of dose reductions stipulated by nadir DLT-ANC and/or DLT-PLT (as noted above). In other words, if the patient experiences DLT-ANC and Delay- ANC, make the modifications as indicated for the nadir counts without additional modifications based on delayed recovery.

Table B. Modifications for Delayed Hematologic Recovery		
Category	Delay (days)	Modification
Delay-ANC	1-7	No Change
	8-21	Add G-CSF with next cycle and decrease carboplatin one AUC
	>21	Off Study (see Section 8.7)
Delay-PLT	1-7	No Change
	8-21	Decrease carboplatin one AUC
	>21	Off study

8.5 Adjustments for Non-Hematologic Toxicity

Table C: Modifications for Non-Hematologic Toxicity			
Drug	**Regimen -2 Level**	**Regimen -1 Level**	**Regimen Starting Dose**
Paclitaxel	110 mg/m^2	135 mg/m^2	175 mg/m^2
Carboplatin (AUC)	4.0	5.0	6.0
Bevacizumab	Not applicable	Not applicable	Not applicable

8.5.1 Peripheral neuropathy Grade 2 (or greater) requires reduction of one dose level in paclitaxel and delay in subsequent therapy for a maximum of three weeks until recovered to Grade 1. If peripheral neuropathy fails to recover to Grade ≤ 2 by a maximum delay of three weeks from time therapy is due, then paclitaxel only should be reduced 1 treatment level.

8.5.2 Hypertension. Patients receiving Bevacizumab should be monitored prior to each dose with measurement of blood pressure. Medication classes used for management of patients with Grade 3 hypertension receiving Bevacizumab included angiotensin-converting enzyme inhibitors, beta blockers, diuretics, and calcium channel blockers. The goal for blood pressure control should be consistent with general medical practice guidelines (i.e. < 140/90 mmHg in general and < 130/80 mmHg for patients with diabetes).

- For controlled hypertension, defined as systolic ≤ 150 mm Hg and diastolic ≤ 90 mm Hg, continue Bevacizumab therapy.
- For uncontrolled hypertension (systolic > 150 mm Hg or diastolic > 90) or symptomatic hypertension less than CTCAE Grade 4, hold Bevacizumab treatment, with anti-hypertensive therapy initiated or continued, as in 8.52.
- During the period of combination chemotherapy with Bevacizumab, if hypertension is controlled and symptomatic hypertension has resolved by one week after holding treatment, continue all therapy.
- During the period of combination chemotherapy with Bevacizumab, if hypertension remains uncontrolled or symptomatic hypertension, less than CTCAE Grade 4, persists one week after holding treatment, the next treatment cycle should contain paclitaxel and Carboplatin only, if applicable, as otherwise indicated in the protocol, with Bevacizumab omitted.
- Bevacizumab should be discontinued for the remainder of the study in any patient developing CTCAE Grade 4 hypertension.

8.5.3 Proteinuria. Patients receiving Bevacizumab should be monitored by urine analysis for urine protein:creatinine (UPC) ratio prior to every other dose of Bevacizumab:

UPC ratio < 3.5	Continue Bevacizumab
UPC ratio ≥ 3.5	Hold Bevacizumab until UPC ratio recovers to < 3.5. If therapy is held for > 2 months due to proteinuria, discontinue Bevacizumab.
Grade 4 or nephrotic syndrome	Discontinue Bevacizumab

8.5.4 Hemorrhage. Bevacizumab will be discontinued in patients with CTCAE Grade 3 hemorrhage and receiving full-dose anticoagulation. For all other patients with CTCAE Grade 3 hemorrhage, Bevacizumab should be held until ALL of the following criteria are met:

8.541 bleeding has resolved

8.542 blood hemoglobin level is stable

8.543 there is no bleeding diathesis that would increase the risk of therapy

8.544 there is no anatomical or pathologic condition that can increase the risk of hemorrhage recurrence.

Patients who experience delay of resolution according to the above criteria for >3 weeks, recurrence of Grade 3 hemorrhage, or any CTCAE Grade 4 hemorrhage will be taken off Bevacizumab therapy.

8.5.5 Thrombosis.

8.5.51 Arterial Thrombosis

Bevacizumab will be discontinued for ≥ CTCAE Grade 3 arterial thrombotic events (including cerebrovascular ischemia, transient ischemic attack, cardiac ischemia/infarction, peripheral or visceral

arterial ischemia) or CTCAE Grade 2 arterial thrombotic events new or worsened since beginning Bevacizumab therapy.

8.5.52 Venous Thrombosis

Treatment with Bevacizumab will be held for CTCAE Grade 3 or asymptomatic CTCAE Grade 4 venous thrombosis. For patients on therapeutic anticoagulation, PT INR or PTT (whichever appropriate) should be monitored closely during Bevacizumab therapy. If the planned duration of full-dose anticoagulation is ≤ 3 weeks, Bevacizumab should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is > 3 weeks, Bevacizumab may be resumed during the period of full dose anticoagulation if ALL of the following criteria are met (otherwise such patients will be taken off Bevacizumab therapy):

8.5.52.1 The patient must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) or on stable dose of heparin prior to restarting treatment.

8.5.52.2 The subject must not have pathological conditions that carry high risk of bleeding (e.g. tumor involving major vessels).

8.5.52.3 The subject must not have had hemorrhagic events while on study.

8.5.52.4 The patient is benefiting from treatment (no evidence of disease progression).

Patients with symptomatic CTCAE Grade 4, or recurrent/worsening venous thromboembolic events after resumption of Bevacizumab treatment, will be taken off Bevacizumab therapy.

8.5.6 Coagulopathy. For CTCAE Grade 3 or 4 coagulopathy: hold treatment, until PT/PTT resolve to Grade 1. For patients with PT/INR greater than the intended therapeutic range while on therapeutic warfarin, treatment with Bevacizumab will be held at the discretion of the treating physician in discussion with the principal investigator. Patients

experiencing treatment delay greater than three weeks because of failure to meet the above criteria will be taken off Bevacizumab therapy.

8.5.7 Wound Disruption/Bowel Perforation, Fistula, or GI Leak:

Bevacizumab will be discontinued in the event of wound disruption requiring surgical intervention. Bevacizumab will be discontinued in the event of new development of bowel perforation, fistula, or GI leak

8.5.8 Renal toxicity (associated with reduction in GFR) is not expected as a direct complication of chemotherapy in this previously untreated patient population using the prescribed dose and schedule of each regimen. As such, there are no specific dose modifications for renal toxicity. However, the target AUC dose of carboplatin must be recalculated each cycle in any patient who develops renal toxicity, defined by serum creatinine greater than 1.5 x institutional upper limit normal (ULN), CTCAE Grade ≥ 2.

8.5.9 Intestinal obstruction. Bevacizumab will be held for occurrence of CTCAE Grade 3 toxicity, until resolution to ≤CTCAE Grade 1 and will be permanently discontinued for occurrence of CTCAE Grade 4 toxicity.

8.5.10 Hepatic toxicity is not expected as a direct complication of chemotherapy in this untreated patient population using the prescribed dose and schedule for each regimen. However, the development of Grade 3 (or greater) elevations in SGOT (AST), SGPT (ALT), alkaline phosphatase or bilirubin requires reduction of one dose level in paclitaxel and withholding of Bevacizumab, and delay in subsequent therapy for a maximum of three weeks until recovered to Grade 1.

8.5.11 There will be no dose modifications for **alopecia, nausea, constipation,** or **diarrhea**. It is recommended that routine medical measures be employed to manage nausea, constipation, and diarrhea.

8.5.12 In general, with the occurrence of a **hypersensitivity** reaction to paclitaxel, carboplatin, Bevacizumab is not considered a dose-limiting toxicity. Patients may be retreated at full doses after administration of medication to prevent hypersensitivity reactions, and adjustments in infusion rates should be made. However, if despite these safety measures repeat attempt at infusion of the inciting drug results in a recurrent

hypersensitivity reaction, the inciting drug should be discontinued for the remainder of the study. In the event of any CTCAE Grade 3 or 4 allergic or infusional reaction to Bevacizumab, Bevacizumab will be permanently discontinued.

Also, please see Appendix J for management of suspected hypersensitivity reactions to Bevacizumab.

8.5.13 Potential modifications for other non-hematologic toxicities with an impact on organ function of Grade 2 (or greater) require discussion with the principal investigator except where noted below in Section 8.631.

8.5.14 Special Modifications in Study Treatment

8.6311 For any CTCAE Grade 3 non-hematologic adverse event (except controllable nausea/emesis), Bevacizumab should be held until symptoms resolve to ≤ CTCAE Grade 1. If a CTCAE Grade 3 adverse event persists for > three weeks or recurs after resumption of Bevacizumab, the patient will be taken off all protocol therapy.

8.6312 For any CTCAE Grade 4 non-hematologic adverse event (except controllable nausea/emesis), the patient will be taken off all protocol therapy.

8.6 Bevacizumab Dose Modification and Toxicity Management

There are no reductions in the bevacizumab dose. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes.

Any toxicities associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice. Bevacizumab has a terminal half-life of 2 to 3 weeks; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued.

Infusion Reaction: Infusion of bevacizumab should be interrupted for subjects who develop dyspnea or clinically significant hypotension. Subjects who

experience a NCI CTCAE v. 3.0 Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from bevacizumab treatment.

The infusion should be slowed to 50% or less or interrupted for subjects who experience any infusion-associated symptoms not specified above. When the subject's symptoms have completely resolved, the infusion may be continued at no more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.

Adverse events requiring delays or permanent discontinuation of bevacizumab are listed in Table 1

Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 2 months (for events not treatment related). The maximum allowable length for treatment interruptions secondary to treatment related toxicities 3 weeks as described in Section 8.0

Table1: Bevacizumab Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension	
No dose modifications for grade 1/2 events	
Grade 3	If not controlled to 150/100 mmHg with medication, hold bevacizumab.
Grade 4 (including RPLS (confirmed by MRI) or hypertensive encephalopathy)	Discontinue bevacizumab.
Hemorrhage	
No dose modifications for grade 1/2 nonpulmonary and non-CNS events	
Grade ≥ 2 pulmonary or CNS hemorrhage	Discontinue bevacizumab.
Grade 3 nonpulmonary and non-CNS hemorrhage	Subjects who are also receiving full-dose anticoagulation will be discontinued from receiving bevacizumab. All other subjects will have study treatment held until all of the following criteria are met: • The bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence. Subjects who experience a repeat Grade 3 hemorrhagic event will be discontinued from receiving bevacizumab.
Grade 4 Non pulmonary or non-CNS hemorrhage	Discontinue bevacizumab.
Venous Thrombosis	
[Note: Subjects with lung cancer placed on anticoagulant therapy for a thrombotic event should be discontinued from receiving bevacizumab]	
No dose modifications for grade 1/2 events	

Grade 3/ Asymptomatic Grade 4	Hold study drug treatment. If the planned duration of full-dose anticoagulation is <2 weeks, study drug should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is >2 weeks, study drug may be resumed during the period of full-dose anticoagulation if all of the following criteria are met: • The subject must have an in-range INR (usually between 2 and 3) on a stable dose of warfarin (or other anticoagulant) prior to restarting study drug treatment. • The subject must not have had a Grade 3 or 4 hemorrhagic event while on anticoagulation. • The subject must not have had evidence of tumor involving major blood vessels on any prior CT scan.
Symptomatic Grade 4	Discontinue bevacizumab.

Table 1
Bevacizumab Dose Management due to Adverse Events (continued)

Arterial Thromboembolic event (Angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, pulmonary embolus and any other arterial thromboembolic event)	
Any grade	Discontinue bevacizumab.
Congestive Heart Failure (Left ventricular systolic dysfunction) No dose modifications for grade 1/2 events	
Grade 3	Hold bevacizumab until resolution to Grade ≤ 1.
Grade 4	Discontinue bevacizumab.
Proteinuria No dose modifications for grade 1/2 events	
Grade 3 (UPC> 3.5, urine collection > 3.5 g/24 hr, or dipstick 4+)	Hold bevacizumab treatment until ≤ Grade 2, as determined by either UPC ratio ≤ 3.5 or 24 hr collection ≤ 3.5 g
Grade 4 (nephrotic syndrome)	Discontinue bevacizumab
GI Perforation	Discontinue bevacizumab.
Bowel Obstruction	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.
Grade 2	Hold bevacizumab for partial obstruction requiring medical intervention. Patient may restart upon complete resolution.
Grade 3/4	Hold bevacizumab for complete obstruction. If surgery is necessary, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion.
Wound dehiscence requiring medical or surgical therapy	Hold bevacizumab at the discretion of the treating physician and principle investigator.
Other Unspecified Bevacizumab-Related Adverse Events	
Grade 3	Hold bevacizumab until recovery to ≤ Grade 1
Grade 4	Discontinue bevacizumab.
Any Grade	**Reversible Posterior Leukoencephalopathy** Discontinue Bevacizumab

8.7 CONCOMITANT MEDICATIONS

Low-dose aspirin (≤ 325 mg/d) may be continued in subjects at higher risk for arterial thromboembolic disease. Subjects developing signs of arterial ischemia or bleeding on study should be evaluated for possible bevacizumab discontinuation per Table 1, Bevacizumab Dose Management Due To Adverse Events.

9.0 CLINICAL AND LABORATORY EVALUATIONS

9.1 **Pretreatment/During Treatment/Post-Treatment Evaluations:**

The following observations and tests are to be performed and recorded on the appropriate form(s).

The following observations and tests are to be performed and recorded:

Parameter	**pre-treat**	**Nadir Labs**	**Prior to every cycle**	**prior to every other cycle**	**Prior to cycle 4 after cycle 6**	**After Treatment and every 3 months for 2 years than every 6 months x 3 yrs**
History and Physical	X[4]		X			X
Blood pressure	X[4]		X			X
Toxicity Assessment	X[4]		X			X
Clin. Tumor Measurement	X[4]				X	X
CT-scan/MRI/US	X [2]				X	X
CBC/Diff./ Platelets	X [4]	X[6, 7]	X [5,6]			X [8]
Electrolytes, BUN Creat, Ca, Mg, PO4,	X [4]		X [5]			X [8]
Bilirubin, SGOT, Alkaline Phosphatase	X [4]			X		
PT/INR,PTT	X [4]		X			
Chest x-ray	X [1]					
CA-125	X [4]		X [3]			X[3]
Serum for Translational studies	X 4				X	X
UPC ratio	X			X		

Notes:

1. Repeat chest x-ray if initially abnormal or if required to monitor tumor response.
2. CT scan, ultrasound, or MRI as appropriate for consistent measurement of tumor response at the physician discretion within 28 days of initial therapy.
3. To be followed as an adjuvant marker.
4. Must be obtained within 14 days prior to initiating protocol therapy.
5. CBC/Diff./Platelets and UPC ratio must be obtained within 4 days of re-treatment with protocol therapy

6. If grade 4 neutropenia is documented (ANC<500/mm3), obtain twice weekly to weekly until resolved to grade 3.
7. Nadir Labs to be obtained approximately 10 days after chemotherapy (+/- 2 days)
8. Post-Treatment visit only (at the completion of chemotherapy) should be completed 30days after the last treatment date.

10.0 SUBJECT DISCONTINUATION

Subjects who meet the following criteria should be discontinued from study treatment:

- Grade 4 hypertension or reversible posterior leukoencephalopathy syndrome (RPLS)
- Nephrotic syndrome
- Grade ≥ 2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
- Symptomatic Grade 4 venous thromboembolic event (for lung protocols: any venous thromboembolic event requiring full dose warfarin or equivalent (i.e., unfractionated or low molecular weight heparin)
- Any grade arterial thromboembolic event
- Grade 4 congestive heart failure
- Gastrointestinal perforation
- Tracheoesophageal fistula (any grade) or Grade 4 fistula
- Wound dehiscence requiring medical or surgical intervention
- Bowel obstruction that has not fully recovered despite medical or surgical intervention.
- Wound dehiscence requiring medical or surgical intervention
- Inability of subject to comply with study requirements
- Determination by the investigator that it is no longer safe for the subject to continue therapy
- All Grade 4 events thought to be related to bevacizumab by the investigator
- Inability to tolerate the lowest doses (Carboplatin AUC4 or paclitaxel 110 mg/m^2) because of toxicity.

- The patient may withdraw from the study at any time for any reason.
- Patients with evidence of progressive disease or patients with significant side effects or deterioration of performance status may be removed from study at the investigator's discretion.
- All the patients will be followed until death or maximum of 5 years after receiving first treatment.

Patients who have an ongoing bevacizumab-related Grade 4 or serious adverse event at the time of discontinuation from study treatment will continue to be followed until resolution of the event or until the event is considered irreversible

11.0 STATISTICAL METHODS

A Fleming single stage Phase II design will be used to assess whether the 24 month failure rate is at most 50% against the alternative that the 24 month failure rate is at least 70% (alpha of 0.1, Beta of 0.1, power of 90%). Data will be reported for all patients treated under the protocol. Toxicity, overall survival, objective tumor response, correlative endpoints will be examined. All patients will who start treatment will be included in the analysis. If a patient enrolls in the study and does not receive any of the protocol chemotherapy will be declared "cancelled" and not included in the analysis. These patients will be replaced at a one to one basis.

11.1 Interim Analysis:

No planned interim analysis planned of efficacy data. However, annual review will be performed to assure annual recruitment goals are met (minimum of 12 patients annually). Safety will be continually evaluated (see Safety section)

11.2 Planned Efficacy Evaluations

Efficacy Endpoints: Parameters employed to evaluate treatment efficacy and toxicity are:

11.2.1 Primary Efficacy Variables

Primary Endpoints:

- 24 month failure-free rate (events are defined as disease progression, death without progression, or discontinue due to progression, death, or unacceptable toxicity) is calculated by the Kaplan Meier method. Patients without a defined event will be censored at the last tumor assessment date.

- Frequency and severity of adverse effects as assessed by CTCAE v3.

11.2.2 Secondary Efficacy Variables

11.2.2.1 Progression-free survival and overall survival

11.2.2.2 Time to progression will be reported using Kaplan Meier method.

11.2.3 Translational Research Endpoints (Analysis to be exploratory as hypothesis generating):

11.2.3.1 Levels of VEGF, CD-31, TSP-1, and CD-105 in tumor tissue as determined by IHC at baseline will be reported in frequency tables with 24 month failure free rate as an end point to attempt to determine if the levels predict response to the study drug.

11.2.3.2 Levels of angiogenin, bFGF, VEGF, and VCAM-I in serum as determined with ELISA. These values will be evaluated over time in a time series plot to evaluate if these change over time with treatment or with recurrence. If deemed appropriate further statistical analysis will be performed.

11.2.3.3 ELISA measures of VEGF in plasma will be performed. These values will be evaluated over time in a time series plot to evaluate if these change over time with treatment or with recurrence. If deemed appropriate further statistical analysis will be performed.

11.2.3.4. Gene Analysis Statistics and sample cohorts needed.(see appendix I) In genes where we do not see any AEI in normal subjects (such as APC; our results are identical to those of Yan et al.), the presence of even a single AEI patient can be followed mechanistically by comparing allelic ratios in normal and tumor tissues (as done by Yan et al[32].

11.3 Sample Size Considerations

A sample size of 38 patients, a one sample test of proportions will have a power of 90% and a one-sided alpha=0.10 to detect that the 24 month Failure free rate is greater than 50% when the true 24 month is at leas 70%. A 90% confidence interval will be constructed using properties of binomial distribution.

11.4 Methods of Analysis

Progression-free survival and overall survival will be estimated using Kaplan-Meier method. Estimates of rates of clinical response (defined as complete or partial response) will be calculated along with corresponding 95% confidence intervals. Safety analyses will include listing and tabulating frequencies of adverse events (AE) and serious adverse events (SAE) resulting from both clinical observation and laboratory tests. Each AE will be graded from 1 to 4 based on criteria established by NCI CTCAE v3. Abnormal laboratory parameters in data listings will be flagged. Reports will present tables of all AEs as well as only those related to the treatment regimen. .

12.0 SAFETY REPORTING OF ADVERSE EVENTS

12.1 ADVERSE EVENT REPORTING AND DEFINITIONS

In the event of an adverse event the first concern will be for the safety of the subject.

Investigators are required to report to Genentech Drug Safety ANY serious treatment emergent adverse event (STEAE) as soon as possible.

A STEAE is any sign, symptom or medical condition that emerges during Bevacizumab treatment or during a post-treatment follow-up period that (1) was not present at the start of Bevacizumab treatment and it is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of Bevacizumab treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy, AND that meets any of the following regulatory serious criteria:

- Results in death
- Is life-threatening
- Requires or prolongs inpatient hospitalization
- Is disabling
- Is a congenital anomaly/birth defect

- Is medically significant or requires medical or surgical intervention to prevent one of the outcomes listed above.

Serious adverse events occurring after a patient is discontinued from the study will NOT be reported unless the investigator feels that the event may have been caused by the study drug or a protocol procedure. Study-specific clinical outcomes of death because of disease progression are exempt from serious adverse event reporting, unless the investigator deems them related to use of the study drug. Hospitalization for study drug administration is not a serious adverse event.

In general, serious adverse events assessed as clearly being due to disease progression and not due to study drug(s) should be excluded from adverse event reporting. However, in cases where the specificity or severity of an event is not consistent with the risk information, the event should be reported.

If clearly related to the commercial agent(s), adverse effects that are either severe or for which the cause is unknown must be reported using the rules listed below.

The study will utilize the NCI Common Terminology for Adverse Reporting Version 3.0.

Toxicity Grade	**Type (a)**
4,5	Unknown
5	Known
2,3	Unknown
4 (non-myelo)	Known
4 (myelo b)	Known

(a)Type is based on toxicities included in the NCI list of known toxicities included in the known toxicities associated with the study drug(s).

(b)Myelosoppression, which includes neutropenia, anemia, and thrombocytopenia.

12.2 Reporting of Serious Treatment Emergent Adverse Events

All STEAEs should be recorded on a MedWatch 3500 Form and faxed to:

Genentech Drug Safety

Fax: (650) 225-4682 or (650) 225-4683

(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

AND:

David O'Malley, MD (Principal Investigator)

Lois Dial, RN fax #614-293-0058(Research Center)

Bobbi Cobb, RN, OCN, CCRP. fax # 614-2933078 (Study Coordination Center)

AND:

Ohio State University IRB

Beth Wiley, Tel. 614-292-0243. fax # 614-688-0366

MedWatch 3500 Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500 form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500 report and submitting it as follow-up

- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500 form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported.

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that bevacizumab caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to bevacizumab administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

12.3 Safety Reporting Requirements for IND Exempt Studies

For **Investigator Sponsored IND Exempt Studies**, there are some reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 314.80.

Postmarketing 15-Day "Alert Report":
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is **unexpected and assessed by the investigator to be possibly related to the use of Bevacizumab**. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.

All Postmarketing 15-Day "Alert Reports" submitted to the FDA by the Sponsor-Investigator must also be faxed to: Genentech Drug Safety

Fax: (650) 225-4682 or (650) 225-5288 (Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

For questions related to safety reporting, contact:

Genentech Drug Safety
Tel: 1-888-835-2555

or
Fax: (650) 225-4682 or (650) 225-5288
(Please use the safety reporting fax cover sheet attached to this document for your fax transmission)

13.0 DATA AND SAFETY MONITORING

Overview:
Data Safety Monitoring Board is to determine the safe and effective conduct of this clinical trial. This committee will recommend conclusions of the clinical trial. Risk associated with participation in research must be minimized to the extent practical. Monitoring will be conducted in toxicity, dose-finding studies, efficacy, effectiveness and comparative trials involving greater than minimal risk to participants to assure the safety and welfare of the research subjects.

Data Safety Monitoring Plan

1. Summary of main findings,
2. Discussion of issues or problems
3. List of participants of the trial, data personal and data centers.
4. Report and review accrual and multi site participation.
5. Review unanticipated problems, adverse events, and serious adverse events as they are reported in the data. Ensure proper reporting of each event (ex. FDA and sponsor)
6. Review data quality, timeliness, patient recruitment, accrual and retention
7. Review protocol violations-deviations from protocol treatment, monitoring or study calendar
8. Discuss protocol amendments as they would relate to the data collection results.
9. Written report will document the results of the meeting.

Data Safety Monitoring Committee for Gyn Oncology
Gyn Oncology Data Safety Monitoring Committee will include the principal investigator, data manager, clinical trials manager, research nurse, and disease specific committee director. A written report of the Disease Specific Committee's recommendations of the Data Safety Monitoring Monthly report for this clinical trial will be signed by the principle investigator and Disease Specific Committee Director. Multi site participation of principal investigators can be accomplished by teleconference. Sub investigators will be sent the written report on a monthly basis. All data safety monitoring committee reports will be sent to the IRB with the continuing review submission.

14.0 RETENTION OF RECORDS

The Federal Privacy Act protects the confidentiality of OSUMC medical records. Clinical records are kept in the office of the Division of Gynecologic Oncology (M 210 Starling-loving Hall).Division of Gynecologic Oncology clinical trial personnel is the only people with the authority to have access to these records. The Act does allow release of some information from the medical records without

permission, for example if the Food and Drug Administration (FDA) or the Sponsor of this study (Genentech) requires it. When the results of the research study are reported in medical journals or at scientific meetings the people who take part are not named or identified.

All documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence will be retained for at least 2 years after the investigation is completed.

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APPENDIX A

STUDY FLOW CHART/SCHEMA

Compressively surgically staged patients with advanced endometrial cancer (stage III-IV) with non-measurable or measurable disease

Regimen

Carboplatin (AUC 5) plus Paclitaxel (175 mg/m^2 over 3 hours) plus Bevacizumab (15 mg/kg) every three weeks for 6 cycles

Follow-up

Measurable disease: CT-scan/MRI/US Following cycle 3 and 6

Non-measurable disease: CT after 6 cycles

All patients: Clinical exam every 3 months for 2 years then 6 months for an additional three years and annually thereafter

APPENDIX B

NCI COMMON TOXICITY CRITERIA

APPENDIX C

FDA MEDWATCH FORM 3500a

APPENDIX D

NYHA GUIDELINES

NYHA Classification

Excerpted from Oxford Textbook of Medicine. Vol 2, p.2228. Oxford Press.1997.

Class	Description
I	Subjects with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Subjects with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Subjects with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Subjects with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present event at rest. If any physical activity is undertaken, discomfort is increased.

APPENDIX E

PROCEDURE FOR OBTAINING A URINE PROTEIN / CREATININE RATIO

1) Obtain at least 4 ml of a random urine sample (does not have to be a 24 hour urine)
2) Determine protein concentration (mg/dL)
3) Determine creatinine concentration (mg/dL)
4) Divide #2 by #3 above: urine protein / creatinine ratio = protein concentration (mg /dL) / creatinine concentration (mg /dL)

The UPC directly correlates with the amount of protein excreted in the urine per 24 hrs (i.e. a UPC of 1 should be equivalent to 1g protein in a 24hr urine collection)

Protein and creatinine concentrations should be available on standard reports of urinalyses, not dipsticks. If protein and creatinine concentrations are not routinely reported at an Institution, their measurements and reports may need to be requested.

APPENDIX F

COLLECTION AND PROCESSES OF SAMPLES

1. Blood Samples: Following patient counseling and consent, patients will have approximately 5 ml of blood collected into a red top and 5 ml of blood into a purple top Vacutainer tube for serum and DNA processing. The total amount of blood taken for research purposes will be approximately 15 ml. The blood will be drawn during the patients pre-operative office visit or in the pre-operative area prior to surgery at the James Cancer Hospital Surgical Area.

2. Tissue Specimens: Tissue specimens will be harvested by Ohio State University pathologists following removal of the specimen from the patient in surgery. Only tissues that are removed as part of the scheduled operative procedure will be considered for sampling. In the case of cancer, metastatic specimens will be obtained for comparison with primary tumors when there is gross evidence of extended spread. Normal appearing portions of the surgical specimen may also be sampled for comparison with cancer-involved areas. In the cases of benign gynecologic disease, the portion of the diseased tissue as well as normal tissue will be sampled from the excised surgical specimen. All specimens will be frozen within 60 minutes of removal from the patient in order to maximize the quality of molecular composition. The specimens will be temporarily stored at –80 until the final diagnosis has been confirmed by The Department of Pathology at The Ohio State University (OSU) for purposes of patient management. If there is any question regarding diagnostic issues, the tissue collected for research purposes can be recalled to further delineate problems in making the clinical diagnosis. Once the formal pathology report has been rendered as part of the patient's care, each "research specimen" will be processed for permanent storage in The Ohio State University Gynecologic Oncology Tissue and Serum Bank, maintained by The Department of Pathology at The Ohio State University. The patient's diagnosis and determination of stage will not be affected by the acquisition of tissue for our research purposes. Only remnant tissue samples not needed for diagnosis will be selected for the tissue bank. The Ohio State University Gynecologic Oncology Tissue and Serum Bank will be maintained by the Human Tissue Resource Network a division in the Department of Pathology at OSU.

Approximately 25% of each tissue specimen will be embedded in OCT and stored at –80°C to avoid artifact that can result from storage of OCT embedded at lower temperatures. The remainder of the tissue specimen will be stored in cryotubes at -180°C in a vapor phase nitrogen freezer (MVE chart). Each OCT embedded specimen will have a hematoxylin and eosin stained slide prepared that will be reviewed by a board certified gynecologic pathologist. This microscopic image will be photographed using the Aperio Scanscope and stored into database for future use. In the case of a large volume tumor, tissue specimens from the primary tumor will be minced into fragments and placed into separate cryotubes.

In cases in which there is less than 75% of the tissue specimen composed of tumor, specimens may also be processed using laser capture microscopy to isolate focal aspects of a particular tissue specimen. Molecular extracts (i.e. DNA, RNA, and protein) will be prepared according to the techniques described below and stored in the molecular library maintained by the Human Tissue Resource Network a division in the Department of Pathology at OSU.

Refrigeration and freezer units will be equipped with continuous chart recording of temperature. The laboratory technicians for the Human Tissue Resource Network a division in the Department of Pathology at OSU will handle the quality assurance management of the equipment. The refrigerator/freezer unit will be locked for security and keys will only be distributed to the laboratory technicians.

3. Dissection and Acquisition of Cancer Cells: Specimens of ascitic fluid will be centrifuged for collection of tumor cells prior to extraction of DNA/RNA or preparation of cell cultures. The white blood cell buffy coat will be similarly collected after centrifuging of blood specimens, with collection of genomic DNA. Both gross and laser capture microdissection (LCM) will be performed on the frozen tissue specimens in order to maximize the yield of tumor cells prior to DNA and RNA extraction. Morphologically defined normal and tumor epithelial cells will be dissected out by laser capture micro-dissection. In instances in which blood was not collected from a patient, genomic DNA will be extracted from paraffin embedded normal tissue specimens. If paraffin tissue is used, sections (5 μm) will be treated with xylene initially to remove paraffin and continued as per frozen sections. All tissue microdissection will be performed in a laser capture microscopy core facility located in the Department of Pathology at OSU. Microdissected specimens will undergo DNA, RNA, and protein extraction according to procedures listed below. All “extracts” will be stored in the

Ohio State University Gynecologic Oncology Tissue and Serum Bank using bar codes that correspond with those that are used to label the tissue specimens that are permanently maintained by The Ohio State University.

4. Sample preparation. Genomic DNA and RNA will be prepared from 100 mg of frozen tissues. For tissue extractions, frozen tissue samples are pulverized under liquid nitrogen. The frozen powder is portioned into aliquots for DNA and RNA extractions. DNA is prepared by digestion of the pellet or frozen powder with SDS and proteinase K followed by NaCl "salting out" precipitation of proteins. The DNA in the supernatant is further purified and recovered by ethanol precipitation. For RNA preparation, the starting material is homogenized in Trizol ™ reagent. Chloroform is added to partition the solution into an organic phase containing the proteins, a DNA interface, and an aqueous layer containing the RNA. The RNA is recovered by precipitation with isopropanol followed by centrifugation. For additional purification, the RNA precipitate is dissolved in RNase free water or Qiagen buffer, then extracted using Qiagen RNeasy ™ columns according to the manufacturers instructions. Complementary DNA (cDNA) is generated from the mRNA by Superscript II reverse transcriptase (Invitrogen) employing oligo dT as a primer. This ensures that all polyA gene transcripts are represented at a rate that reflects the original abundance of each gene product. However, the oligo dT priming often fails in autopsy brain tissues, particularly where the marker SNP for AEI analysis resides far upstream of the polyadenylation site. Therefore we use gene specific oligos targeting a region immediately 3' of the marker SNP to prime cDNA synthesis. Since tissue samples are often in limited supply, we have multiplexed up to 25 primers to permit 25 RT-PCR or AEI assays per cDNA preparation. Comparisons between single and multiple primers showed no significant differences for the mRNA levels and AEI ratios. We have been successful in extracting DNA and RNA from small frozen brain sections on slides, under protocols we have established for optimal results with SNaPshot, SNPlex, and other genotyping assays. Since most of our assays are highly multiplexed, minimum requirements for DNA and RNA are in the order of 100 ng for DNA and 1 µg for RNA (depending upon the quality). However, for multiple and diverse assays as proposed here we need several micrograms of DNA and RNA. Where needed we apply whole-genome preamplification, with phi-29 DNA polymerase with random hexamer primers, using as little as 10 ng DNA. This however does introduce additional error and is not useful for quantitation or methylation analysis.

5. Serum extraction: Blood samples will be allowed to clot for 30-45 minute prior to centrifuge at 1000 x 10 min under refrigeration at 4°C x 2. The supernatent will be removed for storage at -180°C in a vapor phase nitrogen freezer

6. DNA extraction: DNA will be extract from white blood cell buffy coat and/or tissue using the Qiagen nucleic acid isolation kits for DNA using the robotic M48 equipment. This technology uses a magnetic bead system. The integrity and concentration of DNA from tissue will be determined spectrophotometrically. The DNA will be dissolved in pure DNase free and stored at -180°C in a vapor phase nitrogen freezer

7. RNA extraction: Tissues will be homogenized in RNAzol B followed by chloroform/isopropanol extraction and ethanol precipitation. The RNA will be dissolved in filtered DEPC water. Contamination of any genomic DNA will be removed by the treatment of RNA preparation with RNase free DNase. Blood collected in PaxGene tubes will be processed for RNA using the RNA extraction kit that is commercially available. Quality and quantity of RNA will be checked on formaldehyde-agarose gels and there will be a spectrophotometric determination of concentration prior to storage at -180°C in a vapor phase nitrogen freezer.

8 ELISA: Vascular endothelial growth factor (VEGF), CD-31, thrombospodin-1 (TSP-1), and CD-105 (endoglin) levels will be obtained by ELISA. ELISA will be performed using standard practices and commercially available kits.

9. Recording. All tissue specimens as well as extracted components will be bar coded to maintain confidentiality. The exact location of the sample in the freezer will be stored in database (i.e. rack 1, box 3, row 4, 6^{th} sample left-right) using am MS SQL database program for specimen inventory. The amount of tissue as well as extracted components will also be listed within the database. For example, there may 1 vial of tissue, as well as 1 DNA sample, 1 RNA sample and 1 serum sample that were broken into 25% aliquots. This would result in 13 tubes of material that would be recorded within the bank. Samples (and quantity) added or removed from the bank will be recorded. The audit trail will record aliquot changes and deletions as well. Security levels and passwords that protect the data meet HIPPA and HHS requirements.

10. Security. The tissues will remain in the bank maintained by The Ohio State University Gynecologic Oncology Tissue and Serum Bank until they have been depleted as a result of use in research studies. The director of tissue procurement will be in charge of maintaining the security of the tissue for The Ohio State University Gynecologic Oncology Tissue and Serum Bank. Future studies that utilize the molecular extracts must undergo approval by

The Ohio State University Gynecologic Oncology Tissue and Serum Bank Oversight Committee. Access into the laboratory in The Ohio State University Gynecologic Oncology Tissue and Serum Bank will be obtained through a locked door that is only accessible to the laboratory technician and authorized personnel that work in The Ohio State University Gynecologic Oncology Tissue and Serum Bank. All molecular extracts will be maintained in a locked freezer with keys only available to the laboratory technician/honest broker for the GDP. In addition, video surveillance of the building will guarantee the security of the tissue bank as well as the equipment located there.

11 Patient Confidentiality: All data along with an inventory of tissue and serum specimens and the master link between patient identities band the patient's code numbers will be maintained at The Ohio State Gynecologic Oncology Tissue and Serum Bank. The identity code will not include patient's social security number. All information will be gathered by the research staff (principal investigators, data managers, research assistants, research administrators, research nurses) of the Division of Gynecologic Oncology. Only the honest broker, specified by the principal investigator, will have access to the link between patient identity and coded number used to label tissue specimens and data forms. Documents will be destroyed 20 years after the close of the study. Retrospective data will be destroyed immediately upon completion of the databases for that year. Agencies receiving de-identified data will be governed by memorandums of understanding, which outline confidentiality requirements are necessary. Institutions are also bound by federal guidelines regarding this data. All patients will be asked to sign HIPAA consent form. Patients can withdraw from the study at anytime. Patients will be instructed during time of consent to contact the principal investigator (David O'Malley, MD at 614-293-8737) if they wish to withdraw from the study. At this time the patient may request that any samples that they have donated by destroyed or that their identification be removed without destroying the samples. Data collected before the patients withdraw from the study will be kept private and will not affect the patient's medical care.

APPENDIX G

GENETIC ALTERATIONS IN ENDOMETRIAL CANCERS

GENETIC ALTERATIONS IN ENDOMETRIAL CANCERS

	CLASS	ACTIVATION	FREQUENCY
Hereditary			
BRCA1	tumor suppressor	mutation/deletion	1%
BRCA2	tumor suppressor	mutation/deletion	1%
MSH2	DNA repair	mutation	1-3%
Sporadic			
PTEN	tumor suppressor	mutation/allelic loss	50%
K-*ras*	G protein	mutation	10-30%
BRAF	G protein	mutation	20%
B-catenin	signal transduction	overexpression	20%
p53	tumor suppressor transcription factor	mutation/deletion overexpression	Type I: 10-20% Type II: 80-90%
HER-2/*neu*	tyrosine kinase	amplification/ Overexpression	10-30%
RB1	transcription factor	allelic loss	20%
p16	tumor suppressor cdk inhibitor	homozygous deletion	10-15%
c-*myc*	transcription factor	overexpression	10-15%

APPENDIX H

GENOTYPING AND ANALYSIS FOR ALLELIC EXPRESSION IMBALANCE OF 18 TUMOR SUPPRESSOR GENES

Genotyping and analysis for allelic expression imbalance of 18 tumor suppressor genes.

Approach. Using SNPlex, we measure 3-7 SNPs per gene to assess genetic variability (including haplotype information) and to determine which samples are heterozygous for the marker SNP(s) in the transcribed region of the 18 TSG genes. We then perform SNaPshot analysis for those samples heterozygous for the marker SNPs, measuring allelic ratio in genomic DNA and mRNA. The presence of an allelic expression imbalance is detected by a difference in the allelic ratios for DNA and mRNA. AEI analysis will be performed in PBLs (germ line) and cancer plus adjacent normal tissues. The pattern of AEI in these samples alerts us to a possible role of the respective gene in ovarian cancer. This may also predict treatment outcome. Once we have established the pattern and frequency of AEI, we can use AEI as the phenotype to search for the function polymorphism (23,24). However this exceeds the scope of the present grant application, but rather can form the basis for R01 applications.

Methods. **Selection of marker SNPs.** Marker SNPs must be in the transcribed region and sufficiently abundant (because we can perform allelic assays only in heterozygous individuals). Table 2 contains the marker SNPs we have selected. For two genes the marker SNPs were of unexpected low abundance and other SNPs will need to be used.

Table 1. Eighteen tumor suppressor genes included with this study. Also provided are the marker SNPs we have selected for developing the AEI assays. For some genes more than one marker are used to increase the number of heterozygous samples and provide internal controls. These SNPs are also in the rapid genotyping panels, so that we can rapidly identify heterozygous carriers for each gene.

APC_rs42427	**HPC1_rs486907**	**TP73_rs1801174**	**MLH1_rs1799977**
APC_rs465899	**RB1_rs4151539**	**TP53_rs1042522**	**RAD51_rs1801174**
ATM_rs1801516	**p16_rs11515**	**VHL_rs1642742**	**XPC_rs2228000**
BRCA1_rs1799966	**p16_3088440**	**NF1_rs1801052**	**WRN_ rs1346044**
BRCA2_rs144848	**p27_rs2066827**	**NF2_rs1008515**	**WRN_rs1800392**
BLM_rs11852361	**p27_rs34330**	**NF2_rs1034880**	
	p21_rs1801270		

Genotyping. We use either SNPlex (ABI) or SNaPshot (ABI) for genotyping. SNPlex permit the simultaneous analysis of 48 SNPs per run, with a throughput of 5-10,000 SNPs per hour. (We have already determined over 150,000 genotypes with these methods, and have controlled for quality with SNaPshot. Panels for the tumor suppressor genes are available,

with frequent htSNPs, any known functional SNPs, and the marker SBPs in Table 2. Genotypes not amenable to SNPlex analysis are done by SNaPshot or other methods.

SNaPshot methodology for allele-specific mRNA assays**.** The method has been described by us in detail (23, 24) It is understood that this type of analysis is challenging. Therefore, each AEI assay requires rigorous validation and quality control, and every single step in the process must be optimized. Main causes of analytical variability include cDNA formation and PCR amplification (see alternative methods below). In contrast to absolute levels of mRNA which are also measured in all samples by real-time RT-PCR, allelic ratio analysis takes advantage of the fact that one allele serves as the control for the other. We determine whether the DNA and mRNA ratios differ statistically for each single sample (requiring independent repeats, 3 repeats each measured twice). In this fashion we can use AEI as the individual phenotype to scan the gene locus for the functional polymorphisms. For most genes, we can reliably detect a 10-20% difference in mRNA levels generated from each of the two alleles present. The level of AEI required for functional consequences is likely to vary from one gene to the next. We have selected SNaPshot (ABI) as the assay system, involving PCR and fluorescence primer extension analysis.

SNaPshot assay procedure. The details of this assay for detecting AEI (measuring bot DNA and mRNA) have been published (23,24). Briefly, a stretch of genomic DNA (~70 bp's), or cDNA made from the same tissue, is amplified by polymerase chain reaction (PCR), and the polymorphism is measured by primer extension using fluorescently labeled terminator nucleotides. The products are analyzed using an ABI 3730 capillary electrophoresis DNA instrument, and calculated with Gene Mapper ^{TM} 3.0 (ABI) software. This permits injection of 48 samples every 20 min. The data for each incorporated fluorescently labeled nucleotide is measured as a peak area. Success rate for assay development is >90%, and alternative SNPs can be selected to overcome problems. For maximum precision, we dilute samples as needed so that DNA and mRNA thresholds are similar. Using cloned DNA, we establish the number of mRNA molecules present (1,000 or greater for optimal results) using standard curves. S.D.s for heterozygous genomic DNA samples range from 3-7%. mRNA/cDNA ratios (triplicate samples each assayed twice) show higher standard deviations (5-15%), so that detectability of AEI varies with the quality of the sample and the SNP analyzed. We have developed numerous controls, such as standard curves using cloned gene fragments containing the marker SNP, the use of more than one marker SNP per gene, use of extension primers in both directions, 3 independent cDNA preparations, and use of gene specific primers for cDNA preparation.

Potential problems. AEI can also be caused by epigenetic factors. If we suspect such factors to play a role, CpG island methylation is measured using methylation-sensitive restriction enzymes and PCR. In tumor samples the allelic DNA ratios can vary (LOH, gene amplification). This is addressed by normalizing the mRNA ratios to the DNA ratios, set as 1. However, significantly different DNA ratios in some samples indicate the presence of chromosomal instability, which is also to be expected if one allele (the high-expressing one) is selectively deleted thereby yielding growth advantage to the cancer cells. We also expect the tumor samples not to be homogenous; this problem is partially overcome by the precision of the AEI analysis, whereby we can detect even small differences in allelic ratios (assuming a mixture of tumor and stroma). Additionally, the use of microdissection techniques (Appendix A-section 3) will be used were applicable.

Statistics and sample cohorts needed. In genes where we do not see any AEI in normal subjects (such as APC; our results are identical to those of Yan et al.), the presence of even a single AEI patient can be followed mechanistically by comparing allelic ratios in normal and tumor tissues (as done by Yan et al.). In the case of p16, with 5% AEI frequency in normal subjects, regular power analyses can yield the number of cases needed to show a statistical association with ovarian cancer. More importantly however, we can use each patient separately by comparing AEI in PBL/normal tissue versus tumor tissues. The requirement that the high-expressing allele be deleted in tumors is highly specific, thus reducing the number of subject needed drastically.