

Philip J. DiSaia, M.D.
President

Administrative Office
Four Penn Center
1600 John F. Kennedy Boulevard, Suite 1020
Philadelphia, Pennsylvania 19103
Phone: 215-854-0770 Fax: 215-854-0716

Laura L. Reese
Executive Director of Operations



Larry J. Copeland, M.D.
Vice President

Finance/Development Office
2127 Espey Court
Suite 100
Crofton, Maryland 21114
Phone: 410-721-7126 Fax: 301-261-3972

Mary C. Sharp
Chief Financial Officer

SUMMARY OF CHANGES

For Protocol Amendment #6

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#	Section	Page(s)	Comments
1.	Title Page	1	<u>NCI Version Date has been updated.</u> <u>Includes Amendments 1-6 has been added.</u> <u>Jennifer Love Isaac has been replaced by Sarah Reese as the Study Nurse Contact.</u> <u>Revised has been added to the footer</u>
2	7.31	45	<u>Blood collection has been updated in the specimen collection requirements table.</u>
3	10.2	62	<u>The Translational Research folder has been updated in the Data Management Forms Table.</u>
4	App V	101-108	<u>The Translational Research Appendix has been updated.</u>
5	ICD		NCI Version Date has been updated.

PROTOCOL GOG-0286B

A RANDOMIZED PHASE II/III STUDY OF PACLITAXEL/CARBOPLATIN/METFORMIN
(NSC#91485) VERSUS PACLITAXEL/CARBOPLATIN/PLACEBO AS INITIAL THERAPY
FOR MEASURABLE STAGE III OR IVA, STAGE IVB, OR RECURRENT ENDOMETRIAL
CANCER NCT #02065687

IND EXEMPT STUDY

POINTS:

PER CAPITA - 20

MEMBERSHIP –6

NCI Version Date: June 21, 2017

Includes Amendments 1-6

TR PER CAPITA – Award based on specimen submission with 1 point for each FFPE and 1 point for whole blood
(MAX = 5 points).

Lead Organization: NRG / NRG Oncology (08/18/14)

Participating Organizations (08/18/14)

ALLIANCE / Alliance for Clinical Trials in Oncology
ECOG-ACRIN / ECOG-ACRIN Cancer Research Group
SWOG / SWOG

<p><u>STUDY CHAIR</u> VICTORIA BAE-JUMP, M.D., PH.D. THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL DIV. OF GYNECOLOGIC ONCOLOGY DEPT. OF OB & GYN CB# 7572, B105 PHYSICIANS OFFICE BUILDING CHAPEL HILL, NC 27599-7572 PHONE: 919-843-4899 FAX: 919-966-2646 EMAIL: vbae@unch.unc.edu</p>	<p><u>STUDY CO-CHAIR</u> PAOLA GEHRIG, M.D. SEE GOG WEBSITE DIRECTORY</p> <p><u>MEDICAL ONCOLOGY CO-CHAIR</u> CAROL AGHAJANIAN, MD SEE GOG WEBSITE DIRECTORY</p> <p><u>STUDY STATISTICIAN</u> MICHAEL SILL, PH.D. SEE GOG WEBSITE DIRECTORY</p>
<p><u>NURSE CONTACT</u> SARAH REESE THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL DIV. OF GYNECOLOGIC ONCOLOGY DEPT. OF OB & GYN CB# 7572, B180 PHYSICIANS OFFICE BUILDING CHAPEL HILL, NC 27599-7572 PHONE: 919-966-7676 FAX: 919-966-2646 EMAIL: sarah_reese@med.unc.edu</p>	<p><u>TRANSLATIONAL RES. SCIENTIST</u> HEATHER A. LANKES, PH.D., MPH SEE GOG WEBSITE DIRECTORY</p> <p><u>STUDY PATHOLOGIST</u> WILLIAM H. RODGERS, MD, PH.D. SEE GOG WEBSITE DIRECTORY</p>
<p><u>QUALITY OF LIFE CO-CHAIRS</u> KAREN BASEN-ENGQUIST, PH.D, MPH SEE GOG WEBSITE DIRECTORY LARI WENZEL, PH.D SEE GOG WEBSITE DIRECTORY</p>	

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SCHEMA (04/28/15)

ELIGIBILITY**Stage III or IVA endometrial cancer with measurable disease****Stage IVB endometrial cancer (whether there is measurable disease or not)****Recurrent endometrial cancer (whether there is measurable disease or not)****AND****NO PRIOR CHEMOTHERAPY****Randomization:****Arm 1:**Paclitaxel 175 mg/m² IV over 3 hours day 1

Carboplatin AUC = 5 IV day 1

Metformin 850 mg oral QD, beginning on day 1. If tolerated for 4 weeks, the dose will be increased to Metformin 850 mg BID.

Every 21 days x 6 cycles

Maintenance regimen (for patients in complete response, partial response or stable disease) – Metformin 850 mg oral BID (one cycle of maintenance therapy = 21 days)

Patients continue to receive maintenance treatment until disease progression or until adverse events prohibit further therapy.

Arm 2:Paclitaxel 175 mg/m² IV over 3 hours day 1

Carboplatin AUC = 5 IV day 1

Placebo for metformin 850 mg oral QD, beginning on day 1. If tolerated for 4 weeks, the dose will be increased to placebo for metformin 850 mg BID.

Every 21 days x 6 cycles

Maintenance regimen – (for patients in complete response, partial response or stable disease) – Matched placebo oral BID (one cycle of maintenance therapy = 21 days)

Patients continue to receive maintenance treatment until disease progression or until adverse events prohibit further therapy.

NOTE: Patients with stable disease (SD) or partial response (PR) who still have measurable disease at the completion of cycle 6 may continue to receive paclitaxel and carboplatin (with metformin or placebo) up to a total of 10 cycles (if deemed necessary by the treating investigator). Patients who continue with cycles 7-10 will continue with all study assessments as described for Prior to each cycle (cycles 1-6) (see Section 7, Study Parameters).TABLE OF CONTENTS

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1.0 OBJECTIVES

1.1 Primary Objective:

- 1.11 To determine if the addition of metformin to the standard regimen of carboplatin and paclitaxel prolongs progression-free survival (PFS) in women with advanced or recurrent endometrial cancer (Phase II). To determine if the addition of metformin to the standard regimen of carboplatin and paclitaxel prolongs overall survival (OS) in the same population if a phase III study is conducted. Both clinical trials (Phase II and III) will utilize OS as a primary endpoint if a phase III trial is opened.

1.2 Secondary Objectives:

- 1.21 To estimate the proportion of patients with objective response (RR) in the population of patients with measurable disease by treatment.
- 1.22 To estimate the duration of response in the population of patients with measurable disease who respond by treatment.
- 1.23 To estimate overall survival (OS) and relative hazards of death for each treatment arm if the study stops after the phase II trial is completed. If the study continues with a phase III clinical trial, then PFS will be a secondary endpoint.
- 1.24 To determine the nature, frequency and degree of toxicity as assessed by CTCAE for each treatment arm.
- 1.25 To estimate possible differences in RR, PFS, OS, and toxicity rates for the treatment regimens by the patients' level of obesity.

1.3 Translational Research Objectives (12/05/16)

1.31 Integrated Biomarker Research Objectives

- 1.311 To test whether PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations have a lower hazard of progression or death (PFS endpoint) among patients who are treated with metformin.
- 1.312 To test whether higher expression of MATE 2 is associated with a lower hazard of progression or death (PFS endpoint) among patients who are treated with metformin.

1.32 Exploratory Biomarker Research Objectives

- 1.321 To explore the association of metabolic factors (i.e., BMI, hip-to-waist

ratio, diabetes status, HgbA1C, fasting insulin and glucose levels, HOMA scores) with treatment response to metformin/paclitaxel/carboplatin, PFS, and OS.

- 1.322 To test whether genomic profiles (i.e. PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations) differ between the tumors of obese and non-obese EC patients.
- 1.323 To correlate expression of key targets of the insulin/IGF-1/mTOR signaling pathway (p-IGF1R, p-S6 and p-4EBP-1) with treatment response to metformin/paclitaxel/carboplatin, PFS, OS and obesity status.
- 1.324 To determine if the genetic variants of the metformin transporters correspond with treatment response to metformin/paclitaxel/carboplatin, PFS and OS.

1.4 Patient-Reported Outcome Objectives

- 1.41 To estimate differences in physical functioning, physical activity, and fatigue between treatment arms. *Hypothesis:* Patients assigned to the metformin treatment arm will report better physical functioning and physical activity and less fatigue compared to patients assigned to the placebo treatment arm. This between-arm difference will be most prominent in the obese patients.
- 1.42 To explore the association between metabolic factors (i.e., BMI, hip-to-waist ratio, diabetes status, HgbA1C, fasting insulin and glucose levels, HOMA scores) and physical functioning, physical activity, and fatigue. *Hypothesis:* We postulate that patients with increased BMI, hip-to-waist ratio, diabetes, HgbA1C, fasting insulin and glucose levels, and HOMA scores will report higher rates of health status decline, as measured by physical functioning, physical activity, and fatigue self-report scores.

2.0 BACKGROUND AND RATIONALE

Obesity and diabetes have been linked to poorer survival and increased recurrence rates in endometrial cancer. We postulate that the metabolic and endocrine effects of obesity likely play a role in the pathogenesis of endometrial cancer and invariably lead to biologically different cancers than those that arise in leaner women, possibly necessitating different treatment approaches. The anti-diabetic medication, metformin, has been shown to have anti-tumorigenic effects *in vitro* and *in vivo*. We aim to explore whether metformin is broadly useful as a chemotherapeutic agent for all women with endometrial cancer or more efficacious in the obese population. Thus, we will conduct a two arm, randomized, placebo-controlled phase II/III trial designed to assess the efficacy and safety of metformin in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin alone in women with advanced and recurrent endometrial carcinoma. We predict that the addition of metformin will improve the efficacy of carboplatin and paclitaxel in advanced and recurrent endometrial cancer patients and have minimal added toxicity. In addition, we hypothesize that metformin/carboplatin/paclitaxel will be more efficacious in obese women as compared to their non-obese counterparts. The primary endpoint of the randomized phase II trial is efficacy (progression-free survival). The primary endpoint of the phase III trial is efficacy (overall survival). Data will be collected on the patients' level of obesity (BMI and hip-to-waist ratio). The trial will be stratified by BMI (<30 or ≥30). We will determine presence of diabetes (yes/no by baseline medical history and by baseline HgbA1C) and insulin resistance (fasting glucose and insulin levels at baseline and prior to cycle 3).

Endometrial cancer is the fourth most common cancer among women in the United States and has been increasing in frequency secondary to an aging female population and changes in dietary and hormonal factors, with obesity as a major culprit. In 2012, approximately 47,130 new cases were diagnosed, and 8,010 women will succumb to this disease¹. Obesity, diabetes and insulin resistance are well-known risk factors that drive the development of endometrial cancers². Obesity is not only a risk factor for developing endometrial cancer, but is associated with an increased risk of death³⁻⁵. Obese women with endometrial cancer have a 6.25 fold increased risk of death from this disease as compared to their non-obese counterparts⁴. Metformin is an anti-diabetic medication from the biguanide class that is widely used as the first line treatment of type II diabetes. Recent epidemiological evidence suggests that metformin use lowers cancer risk and reduces cancer deaths among diabetic patients⁶⁻⁸. It is not known whether the underlying mechanism behind metformin's potential anti-neoplastic effects relates to the systemic action of this drug, by reducing circulating insulin levels, or a direct action on cancer cells.

Metformin's immediate downstream target is AMP-activated protein kinase (AMPK), and its activation leads to regulation of multiple signaling pathways involved in the control of cellular proliferation, including inhibition of the mammalian target of rapamycin (mTOR) pathway (Figure 1). Alterations in the mTOR pathway have previously been implicated in endometrial cancer carcinogenesis. PTEN is a negative regulator of this pathway, and loss of PTEN expression is one of the most prevalent

molecular abnormalities associated with endometrial cancers⁹⁻¹¹. Mutations and amplifications of the catalytic subunit of PI3K (i.e. PIK3CA) are also commonly seen and result in hyperactivation of the mTOR pathway¹²⁻¹⁶. Our preliminary data finds that metformin is a potent inhibitor of cell proliferation in endometrial cancer cell lines, and that this effect is partially mediated through inhibition of the mTOR pathway¹⁷. In addition, treatment with metformin in combination with paclitaxel resulted in a synergistic anti-proliferative effect in these cell lines¹⁸. Thus, metformin may have important therapeutic implications for endometrial cancer, a disease strongly influenced by obesity and insulin resistance, with potential mechanisms of action including mTOR inhibition and chemo-sensitization.

As previously stated, obesity and diabetes have been linked to an increased risk of mortality from endometrial cancer making metformin a particularly innovative treatment strategy for this disease. However, the important biological question remains whether metformin will be universally effective in endometrial cancer

treatment or more efficacious in the obese population. We postulate that the interaction between the tumor and its host environment (i.e., the obese state) may be critical in endometrial cancer development and progression, and ultimately, in response to a therapy such as metformin. In addition, metformin is not normally given in normal weight, non-diabetic individuals, raising the issue of tolerability between the obese and non-obese patient populations. Thus, the **overall objective** of this concept is to compare the efficacy and tolerability of metformin in combination with paclitaxel and carboplatin versus paclitaxel and carboplatin in women with advanced or recurrent endometrial cancer. We recognize that metformin is not likely to produce numerous untoward toxicities; however, if our prediction that the addition of metformin will improve the efficacy of carboplatin and paclitaxel in advanced and recurrent endometrial cancer patients and have minimal added toxicity, patients may experience less disease burden and thus report better reported health outcomes and functioning than those without metformin. Further, if, as

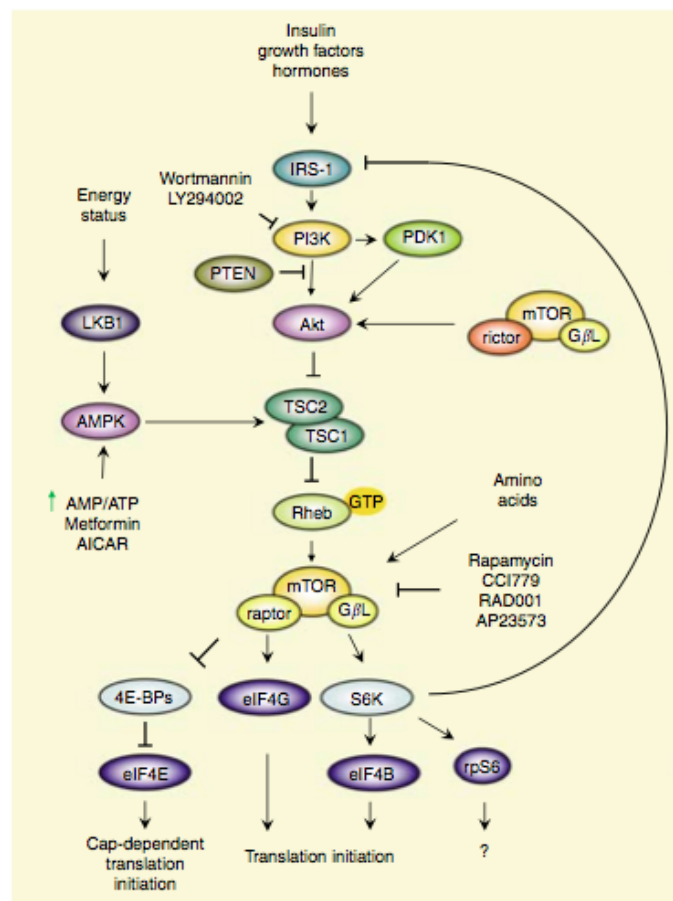


Figure 1. A schematic of the relationship between metformin and the mTOR pathway. (From Hartford, CM, and Ratain MJ, Rapamycin: Something Old, Something New, Sometimes Borrowed and Now Renewed, *Clinical Pharmacology & Therapeutics* (2007) 82, 381–388)

hypothesized, metformin/carboplatin/paclitaxel is more efficacious in obese women as compared to their non-obese counterparts, this benefit may also be observed in obese patients in this arm reporting of less fatigue, better physical functioning, and more physical activity compared to their non-obese counterparts. Therefore, we will also estimate the efficacy, patient-reported outcomes and toxicity rates of the metformin/paclitaxel/carboplatin treatment regimen for obese versus non-obese patients.

2.1 Current treatment of Endometrial Cancer

Women with early stage endometrial cancer have a relatively good prognosis with surgery alone or surgery plus radiation¹⁹. However, 10-15% of patients are diagnosed with stage III disease at the time of surgery, and have an estimated 5 year survival rate of 40-70%²⁰. Lastly, the remaining 10-15% of patients are diagnosed with stage IV disease and have a very poor 5 year survival of 0-10%²¹. Those patients with advanced disease are unlikely to be cured by surgery or radiation alone. In addition, the prognosis for recurrent disease is even more dismal, with expected overall survival of only 14-15 months.

Much work needs to be done with regards to managing patients with advanced stage or recurrent disease. The current standard is systemic treatment with paclitaxel and carboplatin. A large phase III non-inferiority study performed by the Gynecologic Oncology Group (GOG) compared paclitaxel/carboplatin versus a three drug regimen of paclitaxel, doxorubicin and cisplatin (TAP) and showed that paclitaxel/carboplatin was not inferior to TAP in terms of PFS and OS. Overall, paclitaxel and carboplatin also had a favorable toxicity profile²².

Second-line chemotherapy options for endometrial cancer are even less effective²², with Megace being the only FDA approved agent in this setting. The GOG has conducted multiple phase II trials of single-agent chemotherapy in the second-line treatment setting, with response rates all < 15% (except for paclitaxel in taxane naïve patients that had a response rate of 27%). Clinical trials of therapies that target specific molecular abnormalities for endometrial cancer have shown some promise in the second line setting, such as mTOR inhibitors and vascular epidermal growth factor inhibitors (VEGF).

2.2 Metformin and the mTOR Pathway

Metformin is a biguanide drug that is widely used for the treatment of type II diabetes. Recent epidemiological evidence suggests that metformin lowers all cancer risk and reduces cancer incidence and deaths among diabetic patients, including mortality from endometrial cancer^{6-8, 23}. Furthermore, a recent retrospective cohort study of diabetic patients with early stage breast cancer found that those women receiving metformin and adjuvant chemotherapy had a higher response rate²⁴. This has led to the idea that metformin may have a role in cancer treatment and prevention and multiple Phase I-III clinical trials are ongoing, most notably in breast and prostate cancer, to further test metformin's anti-neoplastic

effects^{25, 26}. A Phase I study of temsirolimus and metformin in advanced solid tumors has already been completed and demonstrated acceptable toxicity and promising response rates in a heavily pre-treated group of patients²⁷.

Metformin is commonly thought of as an insulin sensitizer because it enhances signaling through the insulin receptor, leading to an improvement in insulin resistance, followed by a reduction in circulating insulin levels. More recently, evidence suggests that metformin's key target of action is the inhibition of hepatic gluconeogenesis²⁸, resulting in a secondary decline in insulin levels. Although the molecular mechanism of metformin has been well-studied in liver, muscle and fat, little is known about its effects on epithelial tissues, including the endometrium. Metformin inhibits complex I activity in the mitochondria. This leads to activation of its downstream target, AMPK, which regulates multiple signaling pathways controlling cellular proliferation, including inhibition of the mTOR pathway (Figure 1)³⁰. AMPK regulates energy metabolism and is activated in response to cellular stresses that deplete cellular energy levels and increase the AMP/ATP ratio³⁰. AMPK functions to detect cellular energy and ensure that cell division only proceeds if there are sufficient metabolic resources to support proliferation. Once activated, AMPK restores cellular energy levels by stimulating catabolic pathways, such as glucose uptake, glycolysis and fatty acid oxidation and halting ATP-consuming processes such as fatty acid, cholesterol and protein synthesis. LKB1 is the kinase responsible for phosphorylating and activating AMPK²⁸.

AMPK activation through LKB1 leads to regulation of multiple downstream pathways involved in the control of cellular proliferation, including inhibition of the mTOR pathway. Given the interrelationship between these two pathways, metformin is thought to behave as a novel mTOR inhibitor and has been shown to dramatically decrease proliferation in a number of different human cancer cell lines *in vitro*^{17, 31-33}. As demonstrated in our previous work in endometrial cancer cell lines, metformin-mediated AMPK activation decreases cell growth through inhibition of mTOR and a decrease in phosphorylation of its downstream target, S6¹⁷. This ultimately results in the inhibition of translation and critical mRNAs involved in cell cycle progression^{32, 34}. Treatment with metformin has also been shown to effectively repress tumor growth in xenograft animal models of breast, prostate and colon cancer³⁵⁻³⁷. Some preclinical data in animal models also suggests that the anti-tumorigenic efficacy of metformin is dependent on the metabolic composition of its host (i.e. obese and insulin resistant). Metformin has been found to inhibit the growth of Lewis lung LLC1 carcinoma cells in diet-induced obese, insulin-resistant C57BL/6J mice but not in mice fed a control diet³⁸. Similar results were also found using breast tumor cells in lean and obese Balb/c mice³⁹.

2.3 Metformin as a Chemosensitizer

mTOR inhibitors are thought to be potent chemotherapeutic chemosensitizers in many types of cancer cells, including our previous work in endometrial cancer

cell lines^{40, 41}. Given the parallels in the effects of metformin and mTOR inhibitors on the mTOR signaling cascade, it is not surprising that metformin may also behave as a chemosensitizer when used in combination with cytotoxic agents. Paclitaxel is commonly used in the treatment of endometrial cancer; and thus, we examined the effects of metformin used in combination with paclitaxel in human endometrial cancer cell lines. We found a synergistic relationship between paclitaxel and metformin in regards to inhibition of cell proliferation and induction of apoptosis in endometrial cancer cells¹⁸. Treatment with metformin and paclitaxel also resulted in decreased phosphorylation of S6, a critical downstream target of the mTOR pathway¹⁸. These findings suggest that the combination of metformin and paclitaxel may be an effective treatment strategy in the management of endometrial cancer patients.

Recent studies in breast, prostate and lung cancer cell lines confirm our work that the combination of metformin and paclitaxel shows great promise in the management of these various cancers^{42, 43}. The combination of metformin with paclitaxel, carboplatin or doxorubicin has been shown to prolong relapse rate in prostate and lung xenograft models⁴². Most importantly from this study, metformin had comparable effects on tumor regression and prevention of relapse even when combined with a reduced dose of doxorubicin that is not usually effective as a monotherapy⁴². A subsequent study found similar results in that metformin and paclitaxel resulted in decreased proliferation via cell cycle arrest in G2 phase in lung and breast cancer cell lines⁴³. In this same study, the combination of paclitaxel and metformin was also found to more effectively decrease tumor growth and induce apoptosis in a xenograft model of lung cancer as compared to the individual drug treatments alone⁴³. Thus, it seems logical that the addition of metformin to standard paclitaxel and carboplatin treatment for endometrial cancer will lead to improved efficacy with a minimal increase in toxicity.

Metformin has many advantages over using a specific mTOR inhibitor for cancer treatment, including low cost, oral route of administration and low toxicity. Common side effects for mTOR inhibitors (occurring in >30% of patients) include weakness and fatigue, anemia, thrombocytopenia, rash, mouth sores, nausea, poor appetite, peripheral edema, hyperglycemia, hypercholesterolemia, hypophosphatemia, increased liver enzymes and increased blood creatinine levels. In contrast, metformin has a good safety profile and is well-tolerated in patients. The main side effect of metformin is gastrointestinal distress, manifested generally as transient nausea and diarrhea that rarely requires discontinuation of the drug. A rare but serious risk of metformin is fatal and non-fatal lactic acidosis, which is usually associated with predisposing risk factors such as uncontrolled congestive heart failure, liver disease, renal failure or alcohol abuse. The cost of metformin is approximately a dollar a day as compared to weekly infusions of an mTOR inhibitor such as temsirolimus at \$4,000 per infusion. Lastly, metformin is an oral agent, potentially providing an improved quality of life benefit for cancer patients.

Thus, the overall goal of this proposal is to compare the response rates and tolerability of metformin in combination with carboplatin and paclitaxel versus carboplatin and paclitaxel alone in patients with advanced and recurrent endometrial cancer. Given that metformin is considered cytostatic and relatively non-toxic, metformin treatment may be extended beyond the cytotoxic therapy in patients with a complete clinical response (CR), partial clinical response (PR) or stable disease (SD) and be given until evidence of disease progression. A secondary goal is to estimate the outcomes and toxicities between obese and non-obese patients who are on the arm receiving metformin/paclitaxel/carboplatin. We hypothesize that the metabolic composition of the patient may have a bearing on which chemotherapeutic strategies would be the most beneficial and if this is true, metformin may logically be a more effective treatment in the obese population. Carboplatin and paclitaxel were chosen as therapeutic partners for metformin based on our preclinical work demonstrating synergy between metformin and paclitaxel as well as the standard use of carboplatin and paclitaxel as first line treatment in this patient population. This trial will offer the potential benefit of a targeted therapy (metformin) to this traditional regimen which should be appealing to both patients and their oncologists. Potential biomarkers of response to treatment will be explored. This proposal is innovative in that it will be the first clinical trial of metformin for endometrial cancer treatment. Most importantly, this clinical trial will not only evaluate the utility of metformin as a chemotherapeutic agent but will also explore whether metformin is more beneficial and more tolerable in obese versus non-obese endometrial cancer patients. The findings from this study may lead to the individualization of endometrial cancer treatment based on both tumor biology and the metabolic composition of the patient.

As summarized above, epidemiological and preclinical studies suggest that metformin has potential as a chemotherapeutic agent for a variety of cancers, including endometrial cancer. Given this, multiple phase I-III clinical trials are ongoing to further test metformin's anti-neoplastic effects^{25, 26}. In fact, there are 36 clinical trials listed on "www.clinicaltrials.gov" for metformin in regards to cancer, including translational and pre-operative window studies, chemotherapeutic trials, prevention trials and survivorship studies. Although there are many ongoing trials for metformin in regards to cancer treatment and prevention, the results of only four clinical trials have been reported in the literature. In a pre-operative window study in operable breast cancer patients, the percentage of cells staining for Ki-67 fell significantly after only 2 weeks of treatment with metformin, with parallel beneficial effects on cell signaling pathways such as the mTOR pathway⁴⁴. This was followed by a randomized control trial of metformin versus placebo for 4 weeks in a similar group of operable breast cancer patients⁴⁵. In contrast to the previous study, metformin before surgery did not significantly affect Ki-67 staining overall⁴⁵. However, metformin did show a significant benefit in patients with insulin resistance or high BMI⁴⁵, suggesting that metformin's anti-tumorigenic activity may be more related

to its systemic effects on improving the metabolic milieu as opposed to its direct effects on the tumor cells. Another short term clinical trial demonstrated that a one month treatment of metformin resulted in a decrease in proliferation and the size and number of colorectal aberrant crypt foci, an endoscopic surrogate marker of colorectal cancer⁴⁶. Lastly, a phase I study of temsirolimus and metformin in advanced solid tumors has been completed that demonstrated acceptable toxicity and promising response rates in a heavily pre-treated group of patients²⁷.

Carboplatin and paclitaxel were chosen as therapeutic partners for metformin based on our preclinical work demonstrating synergy between metformin and paclitaxel as well as the standard use of carboplatin and paclitaxel as first line treatment in this patient population.

For this proposed clinical trial, we plan to enroll the following advanced endometrial cancer patients:

- Stage III or IVA with measurable disease
- Stage IVB endometrial cancer (whether measurable disease is present or not)
- Recurrent endometrial cancer (whether measurable disease is present or not)

This is the same patient population as enrolled in the recently completed GOG study in this population (GOG-0086P, A three arm randomized phase II study of paclitaxel/carboplatin/bevacizumab, paclitaxel/carboplatin/temsirolimus and ixabepilone/carboplatin/bevacizumab as initial therapy for measurable stage III or IVA, stage IVB, or recurrent endometrial cancer). GOG-0086P accrued 334 evaluable patients. As of the most recent GOG statistical report (January 2013), the histologic types on central review are endometrioid grade 1 (12%), endometrioid grade 2 (25%), endometrioid grade 3 (24%), serous (21%), clear cell (5%), mixed epithelial (4%), undifferentiated (2%), adenocarcinoma, NOS (3%), and other/pending review (3%).

We propose limiting eligibility in this study to endometrioid grade 1, endometrioid grade 2, endometrioid grade 3, serous, clear cell, mixed epithelial, undifferentiated and adenocarcinoma, NOS. Mucinous and squamous cell cancers are rare and represent a different biology, and therefore will not be eligible for this study. From the above GOG-0086P data, we would expect only 12% of the patients to have low grade tumors (endometrioid grade 1).

The system used to grade endometrial cancer is the FIGO grading system. This grading system requires evaluation of histologic features that are difficult to access reproducibly. Furthermore, the current classification system for subtypes, in particular the distinction between endometrioid grade 3 and serous carcinomas is also limited by its reproducibility. In regards to obesity and histologic subtypes, traditional dogma for endometrial cancer has been that serous cancers occur more often in thin, African American women versus endometrioid cancers

that occur more often in Caucasian, obese women. We performed a retrospective look at 881 patients with endometrial cancer treated in the last five years at UNC². Of these patients, 691 of these tumors were of endometrioid histology and 190 were of serous histology. As we would expect, 17% of the endometrioid tumors and 30% of the serous tumors were from African American women. Of the endometrioid and serous endometrial cancers, 84% and 78% were overweight or obese (p=NS) respectively and 62% and 51% were obese (p=0.007). 95% of the African American patients were overweight or obese compared to 80% of the Caucasian patients (p<0.001). African Americans had a higher mean BMI than Caucasians for endometrioid (37.0 v 33.9, p<0.005) and serous (34.9 v 30.2, p<0.001) cancers. For serous endometrial cancers, African Americans had 6.62 times the odds of being overweight or obese (95CI 1.93, 22.6) as Caucasians. The proportion of diabetes did not differ between endometrioid and serous endometrial cancer patients (23.1 v 20.5%, p=0.456). So as we expected, obesity and diabetes seem to be equally important in serous as endometrioid endometrial cancers. In a recently reported pooled analysis of 25 studies in the *Epidemiology of Endometrial Cancer Consortium*, BMI was also positively associated with both endometrioid and serous endometrial cancers⁴⁷.

Preliminary review of the data derived from GOG-0210 also suggests that obesity is associated with both low grade and high grade disease. Of the 2,244 women with G1-2 endometrioid tumors, 20% were overweight and 52% were obese. For the 354 women with G3 endometrioid tumors, 25% were overweight and 45% were obese. This is comparable to the women with serous tumors in this study (n=321) of which 25% were overweight and 40% were obese.

Lastly, although our hypothesis is that metformin may be more efficacious in the obese population, we do not know this for sure. Although there are many ongoing trials for metformin in regards to cancer treatment and prevention, only four clinical trials have been reported in the literature^{44-46, 48}. These clinical trials were not limited to obese patients but did show beneficial effects of metformin treatment. In my laboratory, we have also tested human uterine serous cell lines and found metformin to be equally effective for inhibiting cell growth as in the endometrioid cell lines (unpublished data). Given that obesity may be related to all endometrial cancers and that the impact of obesity on the efficacy of metformin is not clear, it seems reasonable to include all biological subtypes on this trial.

2.4 Translational Research Background

The correlation between obesity and diabetes and risk of endometrial cancer growth will be examined. The ability of metformin to influence that risk will be assessed. Baseline BMI, hip-to-waist ratio, HgBA1C, and diabetes status will be documented. Fasting insulin and glucose levels will be obtained prior to cycles 1 and 3. The Homeostasis Model Assessment Score (HOMA) (fasting insulin (microunits.ml) X fasting glucose (mmol/22.5)) will be calculated for each

patient, which is a measurement of insulin resistance.

PI3K/AKT/MTOR pathway mutations and amplification will be analyzed. DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor and whole blood will be used for targeted sequencing using a hybrid capture approach in conjunction with next generation sequencing. PIK3CA mutations/amplifications and PIK3R1/PIK3R2 mutations will be specifically assessed. FFPE will be used for metformin transporter proteins (OCT 1-3, MATE 1/2 and PMAT), PTEN, AMPK, and LKB1 immunohistochemistry.

OBESITY, THE MTOR PATHWAY AND ENDOMETRIAL CANCER

Obesity is a risk factor and a poor prognostic indicator for many cancers, including endometrial cancer.

Obesity has reached epidemic proportions in the United States, with over 30% of adults considered obese and 65% considered overweight based on their body mass index (BMI). Impaired glucose regulation and insulin resistance are consequences of obesity, often culminating in type 2 diabetes⁵⁹. It is postulated that hyperglycemia and hyperinsulinemia resulting from over-nutrition in obese patients may provide abundant nutrients and growth factors to cancer cells, resulting in the ideal environment for tumor initiation and promotion. The mTOR pathway is critical for the regulation of cell proliferation and metabolism and is often hyperactivated in both obesity and endometrial cancer, suggesting a possible link between these two disease processes. We hypothesize that the metabolic and endocrine effects of obesity play a role in the pathogenesis of endometrial cancer and invariably lead to biologically different cancers than those that arise in leaner women, possibly through aberrant modulation of mTOR signaling. Thus, obese endometrial cancer patients may derive increased benefit from chemotherapeutic agents related to inhibition of this pathway, such as metformin.

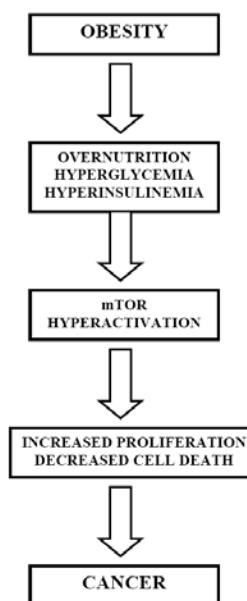


Figure 5. Diagram of the relationship between obesity, mTOR hyperactivation and cancer. The biological question remains whether obesity-driven cancers will be more susceptible to treatment with inhibitors of the mTOR pathway, such as metformin.

ELEVATED IGF/PI3K/AKT/MTOR SIGNALING, SERUM GLUCOSE AND METABOLISM OF GLUCOSE MAY MEDIATE INITIATION AND/OR PROMOTION OF ENDOMETRIAL CANCER

Overweight and obese states may be linked to endometrial cancer through nutrient-sensitive signaling cascades, such as the insulin/insulin growth factor (IGF) and PI3K/Akt/mTOR pathways. Hyperinsulinemia, IGF-1, and IGF-1

receptor (IGF-1R) levels are important in endometrial cancer development and progression^{60, 61}. Signaling through IGF-1R leads to activation of the PI3K/Akt/mTOR pathway, and components of this pathway are often mutated, amplified or aberrantly expressed in endometrial cancers^{62, 63}. Activation of the PI3K/Akt/mTOR pathway through PIK3CA amplifications and mutations and PTEN loss of function has been linked to more aggressive behavior, especially in early stage disease^{12, 63}. Thus, mTOR inhibitors as a targeted therapy for endometrial cancer are already being actively investigated in Phase 1, 2 and 3 clinical trials^{62, 63}.

Glucose metabolism and growth control are tightly linked in proliferating cells and involve signaling pathways including the PI3K/Akt/mTOR pathway. Elevated serum glucose and glucose metabolism by the tumor, termed the “Warburg effect,” also play a role in endometrial cancer pathogenesis^{64, 65}; facilitative glucose transporters (GLUTs) are strongly expressed in endometrial cancer, and their expression correlates with clinical stage of disease⁶⁶⁻⁷¹. Taken together, obesity is a high-energy, pro-inflammatory condition that culminates in increased growth factor signaling via the insulin/IGF axis, as well as a saturating nutrient environment via increased glucose (and other nutrients), ultimately resulting in excessive stimulation of the PI3K/Akt/mTOR pathway (Figure 5)^{25, 26, 72, 73}. In experimental animal models, diet-induced obesity leads to activation of Akt and mTOR in a variety of epithelial tissues^{74, 75}. Conversely, calorie restriction has the opposite effect and represses signaling through this pathway^{74, 75}. Therefore, obesity may create a unique environment in which a therapeutic approach could take advantage of as a strategy to improve endometrial cancer outcomes. Thus, it is logical that a targeted agent, such as metformin, that indirectly decreases circulating glucose and insulin levels and specifically disrupts the PI3K/Akt/mTOR pathway may break the link between obesity and cancer and be particularly useful in obesity-driven cancers. Traditional mTOR inhibitors have been found to upregulate gluconeogenesis, resulting in detrimental metabolic side effects such as insulin resistance and hyperglycemia⁷⁶. The great advantage of metformin over traditional mTOR inhibitors is its ability to inhibit mTOR without inducing hyperglycemia, due to its concomitant effects on inducing energy stress.

PROPOSED MECHANISMS OF THE ANTI-TUMORIGENIC EFFECTS OF METFORMIN

Metformin is believed to have both indirect and direct effects on tumor growth (Figure 6)⁷⁷. Its indirect effects include inhibition of hepatic gluconeogenesis, resulting in an improvement in insulin sensitivity and a reduction in blood glucose and circulating insulin levels which may lead to decreased growth factor stimulation to tumor cells. On a more cellular or direct level, metformin inhibits respiratory complex 1 in the mitochondria, interfering with oxidative phosphorylation and resulting in decreased ATP production and energetic stress⁷⁷. In tumor cells that are unable to cope with energetic stress, energetic crisis and ultimately cell death will occur⁷⁷. Genetic defects that may predispose a tumor

cell to increased sensitivity to energy stress are loss of function of AMPK or of LKB1, the major kinase that phosphorylates and activates AMPK⁷⁷.

In tumor cells that can adequately respond to energetic stress, AMPK is subsequently activated after exposure to metformin which leads to the regulation of multiple signaling pathways that control cellular proliferation, including inhibition of the mammalian target of rapamycin (mTOR) pathway (i.e. specifically mTORC1 inhibition). Metformin has also been found to inhibit the mTOR pathway via AMPK-independent mechanisms through its effects on the Ragulator complex (Rag GTPase) and REDD1 upregulation⁷⁸.

Alterations in the mTOR pathway, involving LKB1 and PTEN, have been implicated in endometrial carcinogenesis in up to 83% of endometrial cancers^{9-11, 79}. Unlike most other tumor types, loss of PTEN expression is observed in premalignant hyperplastic lesions of the endometrium, suggesting that PTEN loss may be a potential initiator of endometrial cancer development⁹. Mutations and amplifications of the catalytic subunit of PI3K (i.e. PIK3CA) are also commonly seen in endometrial cancer and result in hyperactivation of the mTOR pathway¹²⁻¹⁶. PIK3R1 and PIK3R2 code for the p85 α and p85 β inhibitory subunits of PI3K and have also been found to be frequently mutated in endometrial cancers⁸⁰. Thus, we hypothesize that hyperactivation of the mTOR pathway in tumor cells may lead to increased susceptibility to the anti-tumorigenic effects of metformin. One exception may be activating mutations in PIK3CA which have been linked with insulin insensitivity in tumor cells and theoretically may render tumor cells less responsive to metformin⁸¹.

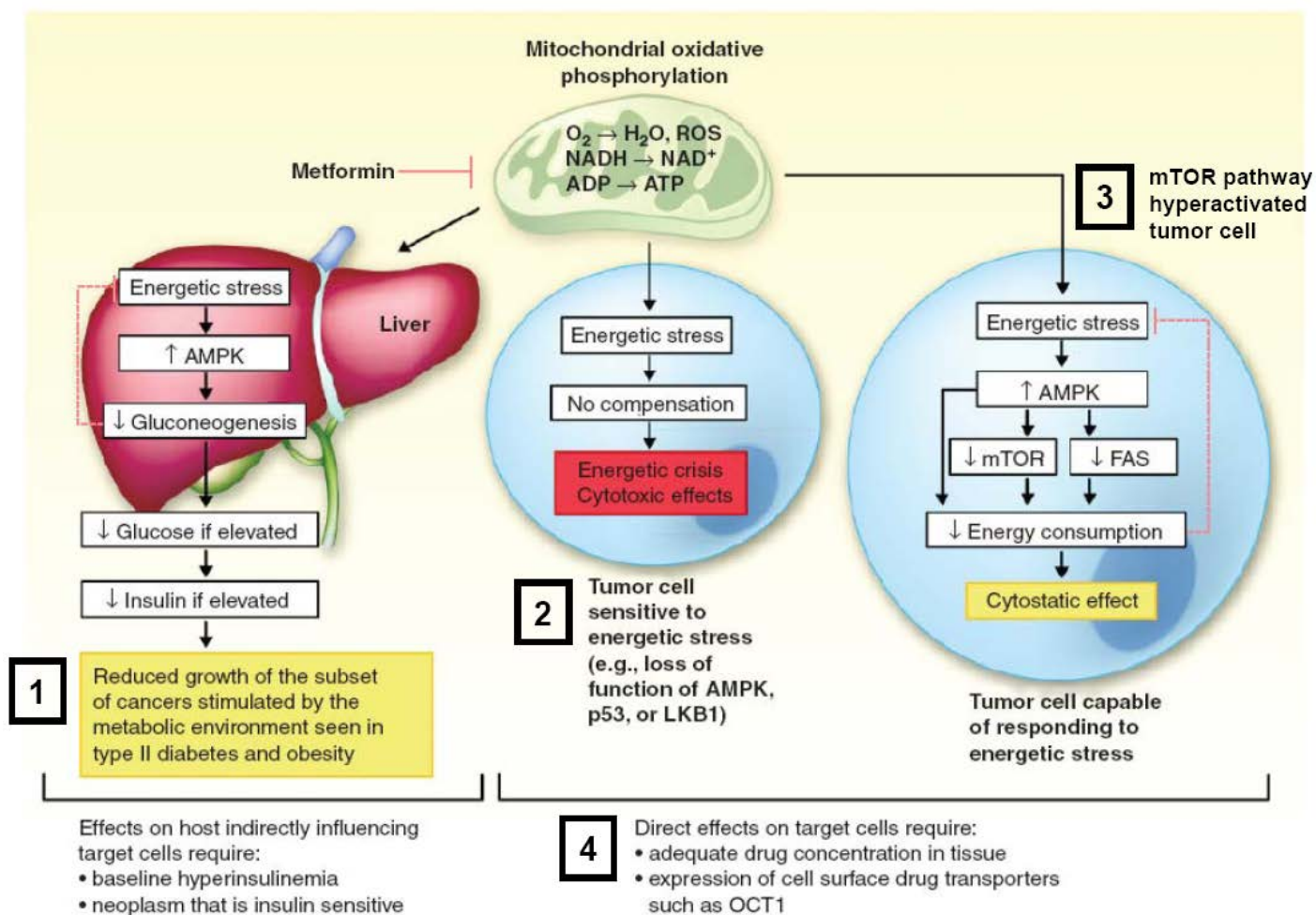


Figure 6. Schematic of the proposed mechanisms of the anti-tumorigenic effects of metformin.

As part of this proposed clinical trial, we will investigate potential biomarkers associated with critical underlying mechanisms of metformin's anti-tumorigenic actions, including (1) indirect effects on the metabolic environment seen in obese cancer patients, (2) genetic defects in tumor cells related to poor coping with energetic stress, (3) evidence of hyperactivation of the mTOR pathway in tumor cells and (4) expression of cation-selective transporter proteins responsible for the uptake of metformin into tumor cells. This figure was edited from Investigating Metformin for Cancer Prevention and Treatment: The End of the Beginning, *Cancer Discovery* (2012), 2:778-790.

One critical, unanswered question in regards to the action of metformin is the extent to which this drug accumulates in neoplastic tissues. Metformin is highly hydrophilic with a net positive charge at all physiologic pH values. Therefore, it requires cation-selective transport proteins that mediate its entry into cells. The cation-selective transporters organic cation transporter (OCT)1-3, plasma membrane monoamine transporter (PMAT) and multidrug and toxin extrusion transporters (MATE) 1-2 mediate metformin transport in the liver, intestine, and kidney⁸²⁻⁸⁸. Less is known about the expression of these transporter proteins in

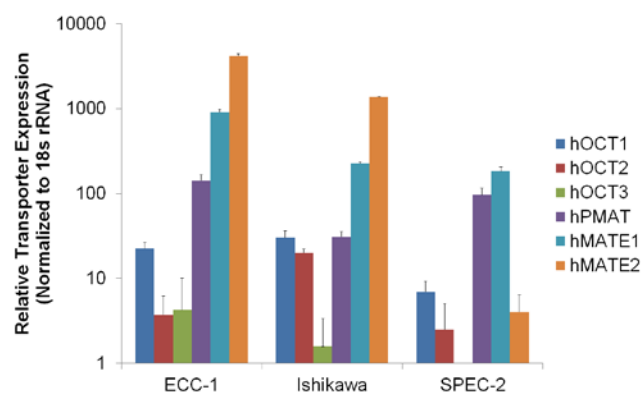
solid tumors. Studies in our laboratory show that metformin uptake into human breast cancer cell lines are dictated by the expression levels of these cation-selective transporters (unpublished data).

Therefore, it is reasonable to assume that metformin uptake into endometrial cancer cell lines and tissues must be mediated by specific cation-selective transporters.

Initial real-time polymerase chain reaction (RT-PCR) experiments to determine if cation-selective metformin transporters are expressed in endometrial cancer cell lines showed that all transporters of interest are present at varying levels (Figure 7) (unpublished data, submitted as an abstract to the Annual Meeting of the Society of Gynecologic Oncology). MATE1 and 2

were the most highly expressed transporters in endometrial cancer cell lines (Ishikawa and ECC-1) while OCT2 and 3 were the least expressed transporters. In the serous endometrial cancer cell line (SPEC-2), MATE1 and PMAT expression predominated, with much decreased expression of MATE2. We also assessed the expression of the metformin transporter proteins in fifteen human endometrial cancer specimens and adjacent benign tissues. MATE1 was found to be the predominant transporter in endometrial tumor and benign tissues; however, PMAT and OCT3 were also expressed in significant amounts (Figure 8). Thus, we anticipate that the highly expressed MATE1 will facilitate metformin intracellular uptake into endometrial tumors and be predictive of treatment response to this agent.

Figure 7. Relative Transporter Expression in Human Endometrial Cancer Cell Lines. All common cation-selective transporters were expressed in endometrial cancer cell lines, except OCT3 which was undetectable in SPEC-2 cells. MATE2 was the predominant transporter in ECC-1 and Ishikawa cell lines, followed by MATE1. In SPEC-2 cells, MATE1 showed highest expression followed by PMAT, whereas the expression of MATE2 was 1033- and 338-fold lower than that observed in ECC-1 and Ishikawa cells, respectively.



METFORMIN, OBESITY AND ENDOMETRIAL CANCER

Our laboratory has shown that metformin-mediated AMPK activation decreases endometrial cancer cell growth through inhibition of mTOR signaling¹⁷, and that metformin and paclitaxel have synergistic anti-proliferative effects¹⁸. Others have also shown that metformin blunts cell and tumor

growth *in vitro* and in xenograft models^{17, 31-33, 35-37}. In an animal mouse model for endometrial hyperplasia, metformin exhibited anti-proliferative effects on the endometrium that coincided with inhibition of downstream targets of the mTOR pathway. Some preclinical data suggest that the anti-tumorigenic efficacy of metformin is dependent on the obese and insulin resistant state^{38, 39}. The potential impact of obesity on response rates to metformin has not been explored in the setting of a clinical trial.

Recently, we have conducted a multi-institutional, retrospective cohort analysis of all endometrial cancer patients with type 2 diabetes treated from 2005-2010 (unpublished data, submitted as an abstract to the Annual Meeting of the Society of Gynecologic Oncology). 1561 endometrial cancer patients were identified, of which 377 were diabetic. Of these, 54% used metformin. The mean age was 63 yo (SD 11.6), and the mean was BMI 39.1 (SD 11.3). The majority of patients had tumors of endometrioid histology (297, 75%). Stage distribution included the following: 308 (78%) stage I, 16 (4%) stage II, 52 (13%) stage III and 18 (4%) stage IV. Median follow-up was 33 months (range of 19 to 87 months). Metformin use was significantly associated with improved progression free survival (HR 0.57, 95CI 0.391-0.852). Metformin users also had significantly improved overall survival with a hazard ratio of 0.05 (95CI 0.33-0.78, p<0.001). After adjusting for BMI, stage and adjuvant treatment, metformin use was associated with improved progression free survival (HR 0.56, 95CI 0.36-0.86) and overall survival (HR 0.47, 95CI 0.29-0.77). These results provide further support that metformin may have a role as adjuvant and maintenance therapy for

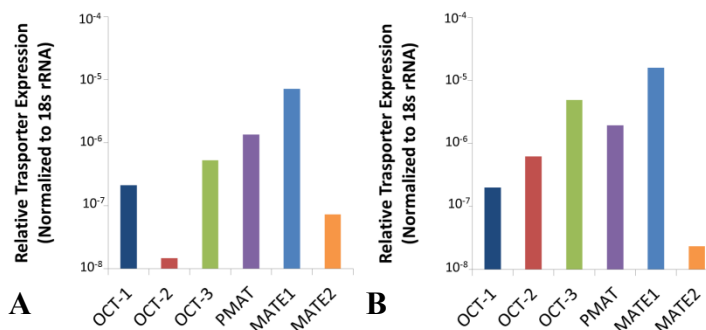


Figure 8. Relative Transporter Expression in Endometrial Tumor Tissues and Adjacent Non-malignant Tissues. In (A) 15 human endometrial tumor tissues and (B) 5 adjacent non-malignant tissues examined, MATE1 was the predominant transporter; however, PMAT and OCT3 were also expressed in significant amounts. The transporter expression pattern in tumor tissues and adjacent non-tumor tissues was similar.

endometrial cancer.

Thus, we aim to explore whether metformin in combination with paclitaxel and carboplatin is broadly useful for all women with endometrial cancer or more efficacious in the obese population. We hypothesize that the metabolic milieu of the host may play a critical role in oncogenesis, cancer proliferation, and transformation of the tumor into a unique pathology and prognosis dependent on the presence of obesity. As such, we propose that obese endometrial cancer patients would benefit most from treatment with metformin, which would inhibit or mitigate these obesity-driven tumor biological pathways. Through the comprehensive analysis of patients' metabolic factors as well as targets of metformin/mTOR signaling and expression of the metformin transporter proteins in their tumors, we strive to gain valuable insight into the relationship between the tumor, its host environment, and the critical role obesity plays in endometrial cancer treatment. As delineated in Figure 6, we will investigate potential biomarkers associated with critical underlying mechanisms of metformin's anti-tumorigenic actions, including (1) indirect effects on the metabolic environment seen in obese cancer patients, (2) genetic defects in tumor cells related to poor coping with energetic stress, (3) evidence of hyperactivation of the mTOR pathway in tumor cells and (4) expression of cation-selective transporter proteins responsible for the uptake of metformin into tumor cells. We speculate that hyperactivation of mTOR pathway-derived, metabolically-dependent, proliferative targets will be more characteristic of tumors derived from obese versus non-obese women with endometrial cancer and correspond to increased susceptibility to the metformin/paclitaxel/carboplatin regimen. Overall, obese women will be more responsive to metformin/paclitaxel/carboplatin treatment, and metabolic and molecular biomarkers will emerge that are predictive of sensitivity to this treatment regimen.

2.5 Patient-Reported Outcomes Research (12/05/16)

Rationale for assessing physical function, fatigue, and physical activity.

Many of the advanced or recurrent endometrial cancer patients suffer from obesity-driven comorbidities, including Type II diabetes, hypertension, heart disease, osteoarthritis, metabolic syndrome, and pulmonary disease. Their obesity affects not only comorbidity but also quality of life, including levels of fatigue and physical functioning.^{91, 106} As stated in 2.4, if obese women are more responsive to metformin/paclitaxel/carboplatin treatment, this responsiveness may be reflected in an improved self-reported health status, or in an advanced cancer patient population, a lower rate of decline than patients not treated with metformin.

This disease and its co-morbidities are linked to manifestations of fatigue⁹². In turn, cancer-related fatigue benefits from physical activity^{93, 94, 95}, including among endometrial cancer patients.⁹⁶ Fatigue has also been linked to shorter recurrence-free and overall survival.⁹⁷ Cancer-related fatigue (CRF) is a very

common and distressing symptom related to cancer and its treatment⁹⁸, is known to adversely affect quality of life, and may be a dose limiting toxicity for some agents. Because of its prevalence and impact, CRF has been the subject of much research [i.e., NCI Symptom Management and Quality of Life Steering Committee Cancer-related Fatigue CTPM Executive Summary, April 2010]. One recommendation from this CTPM was to “study host, disease, treatment, and environmental factors that result in different manifestations of CRF.” We suggest that these two PRO objectives provide a novel, and significant way in which to examine the mechanisms of CRF within an advanced cancer clinical trial, in which the disease, the host environment (e.g., obesity, level of physical activity), and the hypothetically beneficial exposure to Metformin could improve how CRF is conceptualized in this population.

This is not without precedent. For example, the NCIC is currently conducting a Phase III Randomized Trial of Metformin vs Placebo in Early Stage Breast cancer, in which health-related quality of life, physical activity, and diet are included as secondary outcome measures (www.clinicaltrials.gov/NCT01101438). Further, an NCI-funded BIQSFP supplement was awarded to NSABP to examine the association between markers of inflammation and symptoms of fatigue among patients with and without exposure to Metformin. Notably, one of the objectives of their supplement is to determine whether metformin is associated with reductions in inflammatory markers and corresponding decreases in fatigue. We are proposing to collect data in the phase II trial, so that the GOG moves to implement a phase III trial on metformin versus placebo, we will have the necessary data to estimate outcome variances and effect sizes for planning the patient reported outcome component of the trial. Because the effects of metformin on this population of advanced cancer patients is unknown (and thus estimates of effect size are unavailable), it is important for us to collect PRO data in phase II for appropriate planning of Phase III.

Additionally, if the study moves on to phase III, 300 patients will be recruited but the survival data will be analyzed together with the 240 patients from phase II, for a total sample size of 540. If PRO data are not collected during phase II the sample size for PRO data will be limited to only 300 patients, which may be inadequate for determining differences. In the phase II study we expect positive trends supporting these hypotheses. This would support inclusion of the outcomes in the phase III trial, which would have an adequate sample size for definitive testing of hypotheses examining between group differences.

Our proposed study differs from this trial in three fundamental ways: 1) This is an advanced cancer patient population, for which effective treatments and survival are paramount; 2) A sizeable proportion of this advanced cancer patient population will be obese, thereby creating hypothesized survival advantages if randomized to metformin. 3) Physical well-being, physical activity, and decreasing obesity are of critical importance to this patient advocacy population (per GCSC and NCI summary statement critique 11/12). Therefore, we include a

brief physical activity assessment (which collects information on vigorous and moderate activity, as well as walking and sitting) as part of the patient-reported outcome component of this trial for several reasons. First, physical activity provides a secondary measure of physical functioning. Measures of physical functioning reflect a patient's ability to do basic activities of daily living, but leisure time physical activity provides an indicator of functioning above and beyond the basic level that may be particularly useful among patients who have sufficient functioning to do basic daily activities and thus may have a ceiling effect on physical functioning measures. Second, leisure time physical activity has been associated with improved quality of life and outcomes in randomized trials of patients with a range of cancer types⁹⁹. Furthermore, higher physical activity levels after diagnosis have been associated with improved overall and disease specific survival in breast and colon cancer¹⁰⁰. The measure chosen also assesses time spent sitting. Sedentary behavior, independent of moderate to vigorous intensity physical activity, is increasingly being linked to negative health outcomes¹⁰⁷, including endometrial cancer¹⁰⁸. Time spent sitting is also likely to provide an indication of physical functioning. Data exists on physical activity in endometrial cancer survivors with early stage disease. These women report lower levels of moderate to vigorous activity than women their age who have not had cancer. In a survey of 120 survivors of early stage endometrial cancer, only 22% were meeting recommendations of 150 minutes of moderate to vigorous physical activity per week, compared with 46% of the general population¹⁰⁶. In this survey survivors who were sedentary had poorer physical functioning and more fatigue than those who were more active. However, data on physical activity in patients with advanced and recurrent endometrial cancer do not exist. Collecting data on physical activity and sedentary behavior in this trial would provide preliminary data which could aid in designing a physical activity intervention trial. Finally, physical activity targets some of the same mTOR related pathways as metformin (i.e., via AMPK pathway)¹⁰¹, and thus could be analyzed along with the translational endpoints of the trial. In addition, physical activity can be anti-inflammatory¹⁰² and reduce insulin resistance¹⁰³.

2.6 Inclusion of Women and Minorities

The Gynecologic Oncology Group and GOG participating institutions will not exclude potential subjects from participating in this or any study solely on the basis of ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire endometrial cancer population treated by participating institutions.

3.0 ELIGIBILITY CRITERIA

3.1 Eligible Patients

- 3.11 Patients must have measurable Stage III, measurable Stage IVA, Stage IVB (with or without measurable disease) or recurrent (with or without measurable disease) endometrial carcinoma.

Histologic confirmation of the original primary tumor is required. Patients with the following histologic epithelial cell types are eligible: Endometrioid adenocarcinoma, serous adenocarcinoma, undifferentiated carcinoma, clear cell adenocarcinoma, mixed epithelial carcinoma, adenocarcinoma not otherwise specified (N.O.S.).

- 3.12 Measurable disease is defined by RECIST (version 1.1). Measurable disease is defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded). Each lesion must be ≥ 10 mm when measured by CT, MRI or caliper measurement by clinical exam; or ≥ 20 mm when measured by chest x-ray. Lymph nodes must be ≥ 15 mm in short axis when measured by CT or MRI (See section 8).

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

- 3.14 Patients must have adequate:
NOTE: Institutional/laboratory upper limit of normal (ULN)
Institutional/laboratory lower limit of normal (LLN)

3.141 Bone marrow function:

- Absolute neutrophil count (ANC) greater than or equal to 1,500/mcl
- Platelets greater than or equal to 100,000/mcl.

3.142 Renal function:

- Creatinine less than 1.4 mg/dl.
(Per the manufacturer, metformin is contraindicated in the presence of renal dysfunction defined as a serum creatinine ≥ 1.4 mg/dL in females and in patients with abnormal clearance.) (05/19/14)

3.143 Hepatic function:

- Bilirubin less than or equal to 1.5 x ULN
- AST and ALT less than or equal to 3 x ULN
- Alkaline phosphatase less than or equal to 2.5 x ULN

- 3.15 Prior Therapy:
- 3.151 Patients must **NOT** have received prior chemotherapy or targeted therapy, including chemotherapy used for radiation sensitization for treatment of endometrial carcinoma.
 - 3.152 Patients may have received prior radiation therapy for treatment of endometrial carcinoma. Prior radiation therapy may have included pelvic radiation therapy, extended field pelvic/para-aortic radiation therapy, and/or intravaginal brachytherapy. All radiation therapy must be completed at least 4 weeks prior to the first date of study therapy.
 - 3.153 Patients may have received prior hormonal therapy for treatment of endometrial carcinoma. All hormonal therapy must be discontinued at least one week prior to the first date of study therapy.
- 3.16 Patients must be able to swallow and retain orally-administered medication.
- 3.17 Patients must have signed an approved informed consent and authorization permitting release of personal health information. Individuals with impaired decision-making capacity are not eligible to participate on the study.
- 3.18 Patients must meet eligibility criteria as specified in section 7.0.
- 3.19 Patients must be 18 years or older.

3.2 Ineligible Patients

- 3.21 Patients must NOT be taking metformin or have been on metformin in the past 6 months.
- 3.22 Patients with a history of other invasive malignancies, with the exception of non-melanoma skin cancer are excluded if there is any evidence of other malignancy being present within the last three years.
- 3.23 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

- 3.24 Patients who are pregnant or nursing. If patients are of reproductive age and have not undergone hysterectomy, they must use an effective contraceptive method for the duration of this study.
- 3.25 Any condition associated with increased risk of metformin-associated lactic acidosis. (e.g. congestive heart failure defined as New York Heart Association {NYHA} Class III or IV functional status, history of acidosis of any type; habitual intake of 3 or more alcoholic beverages per day)

4.0 STUDY MODALITIES

4.1 Paclitaxel (NSC #673089)

- 4.11 Formulation: Paclitaxel is supplied as a 6mg/mL non-aqueous solution in multi-dose vials containing 30mg/5mL, 100mg/16.7mL, or 300mg/50mL of paclitaxel. In addition to 6mg of paclitaxel, each mL of sterile non-pyrogenic solution contains 527mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.
- 4.12 Storage: Unopened vials of paclitaxel are stable to the date indicated on the package when stored between 20 to 25°C (68 to 77°F). Protect from light.
- 4.13 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 below, solutions of paclitaxel (0.3-1.2 mg/ml) are physically and chemically stable for 27 hours.
- 4.14 Preparation: Paclitaxel must be diluted prior to infusion. Paclitaxel should be diluted in 0.9% Sodium Chloride for Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2mg/mL. The solutions are physically and chemically stable for up to 27 hours at ambient temperature (approximately 25°C / 77°F) and room lighting conditions.

NOTE: In order to minimize patient exposure to the plasticizer DEHP, which may be leached from PVC infusion bags or sets, diluted paclitaxel solutions should be stored in bottles (glass, polypropylene) or plastic (polypropylene, polyolefin) bags and administered through polyethylene-lined administration sets.

Paclitaxel should be administered through an inline filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® or IVEX-HP®, which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP.

All patients should be premedicated with corticosteroids, diphenhydramine, and H2 antagonists prior to paclitaxel administration in order to prevent severe hypersensitivity reactions. Patients who experience severe hypersensitivity reactions to paclitaxel should not be re-challenged with the drug.

- 4.15 Adverse Effects: Consult the package insert for the most current and complete information.
- 4.16 Supplier/ How Supplied: Commercially available both from Bristol-Myers Squibb Oncology as well as generic manufacturers. Consult the American Hospital Formulary Service Drug Information guide, Facts and Comparisons, or the package insert for additional information.
- 4.2 Carboplatin (Paraplatin® - NSC #241240)
- 4.21 Formulation: Carboplatin is supplied as a sterile, pyrogen-free, 10mg/mL aqueous solution in multi-dose vials containing 50mg/5mL, 150mg/15mL, 450mg/45mL, or 600mg/60mL of carboplatin.
- 4.22 Storage: Unopened vials of carboplatin are stable to the date indicated on the package when stored at 25°C (77°F). Excursions from 15 to 30°C (59 to 86°F) are permitted. Protect from light. Carboplatin multi dose vials maintain microbial, chemical, and physical stability for up to 14 days at 25°C following multiple needle entries.
- 4.23 Preparation: Carboplatin aqueous solution can be further diluted to concentrations as low as 0.5mg/mL with 5% Dextrose in Water or 0.9% Sodium Chloride for Injection, USP. When prepared as directed, carboplatin aqueous solutions are stable for 8 hours at room temperature (25°C / 77°F). Since no antibacterial preservative is contained in the formulation, it is recommended that carboplatin solutions be discarded 8 hours after dilution.
- 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.
- 4.24 Adverse Effects: Consult the package insert for the most current and complete information.
- 4.25 Supplier: Commercially available both from Bristol-Myers Squibb Oncology as well as generic manufacturers. Consult the American Hospital Formulary Service Drug Information guide, Facts and Comparisons, or the package insert for additional information.
- 4.3 Metformin (NSC#91485)
- 4.31 Formulation: Metformin hydrochloride (N , N - dimethylimidodicarbonimidic diamide hydrochloride) is not chemically or

pharmacologically related to any other classes of oral antihyperglycemic agents. Metformin hydrochloride is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5 \cdot HCl$ and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of metformin is 12.4. The pH of a 1% aqueous solution of metformin hydrochloride is 6.68. Metformin tablets contain 850 mg, of metformin hydrochloride. Each tablet contains the inactive ingredients povidone and magnesium stearate. In addition, the coating for the tablets contains hypromellose.

- 4.32 Storage: Metformin/placebo will be stored at controlled temperatures of 20-25 degrees Celsius (68-77 degrees Fahrenheit). Metformin/placebo is dispensed in a light-resistant container
- 4.33 Preparation: Metformin is an oral agent and no preparation is needed.
- 4.34 Adverse Effects: Consult the package insert for the most current and complete information.
- 4.35 Supplier: Metformin and matched placebo will be supplied and distributed by Biologics. Metformin will be supplied in 850 mg capsules. Matched placebo will be supplied.
- 4.36 Drug Distribution: Following submission and approval of all required regulatory documents (as stated in Section 5.0), the Pharmacy Information Form will be forwarded to Biologics notifying them that an institution has been approved for patient entry.
- 4.361 Initial Supply: Upon notification of randomization, Biologics will ship an initial supply of study drug of the following quantities to institution:
- Metformin 850mg OR Placebo capsules - 126 count bottle to complete the initial 3 cycles.
- 4.362 Subsequent Supply: At approximately 7 weeks, Biologics will contact the institution to confirm if additional study drug is needed. Biologics will ship a subsequent supply of study drug of the following quantities to site:
- Metformin 850mg OR Placebo capsules - 126 count bottle to complete the next 3 cycles

All study drugs will be shipped with a patient specific label adhered to the bottle. Each bottle will be placed in a Ziploc bag with a study specific label adhered to the outside to avoid confusion at the sites.

Each shipment includes a patient specific label with the following information:

- The Study Number (i.e. GOG-0286B)
- Patient identification
- IND caution statement and/or local regulatory statements
- Expiration Information
- Dosing instructions (Take as Directed per Protocol)
- Storage instructions
- Emergency contact instructions

All drug orders are shipped via *FedEx Priority Overnight* delivery for shipments to US sites. Study Drug is shipped in a Biologics branded package with appropriate materials to maintain temperature stability.

4.363 Institution Instruction Upon Receipt of Study Drug: The designated site coordinator validates contents of package matches information provided on packing slip, signs off on the packing slip, and faxes completed form to Biologics to validate shipment has been received and is accurate.

4.37 Drug Accountability: All study drug must be accounted for during the course of this study. Sites must maintain a NCI drug accountability log.

4.38 Drug Destruction: At the conclusion of the study, remaining inventory is documented in the accountability records and unused drug is to be destroyed as per institution policy and record on the accountability record.

4.4 Emergency Unblinding

In the event of an emergency during normal business hours (Monday through Friday 9:00 am to 5:00 pm Eastern Time), contact the GOG Statistical and Data Center by phone at 1-800-523-2917. At all other times, call: 716-901-2853. If there is no answer, leave a message including a telephone number for a return call. A staff member from the GOG Statistical and Data Center will return your call. **Remember, this is only in the event of an emergency!** This procedure is to be used by the physician when the physician needs to know whether the patient is taking metformin or a placebo to manage an acute illness. Patients should be instructed that if they have any questions or symptoms they should contact the treating physician's office. The GOG Statistical and Data Center will require the protocol number (e.g., "GOG-0286B"), the patient ID number (e.g., "999-0286B-001"), and the patient initials (e.g., "FML") to unblind the patient.

5.0 TREATMENT PLAN AND ENTRY/RANDOMIZATION PROCEDURE

Before patient entries will be accepted, submit the following documents to the GOG Administrative Office via mail (**Attn: Regulatory Department, Protocol GOG-0286B**):

- IRB approval*
- IRB-approved informed consent
- IRB Membership list or IRB assurance number
- Study-specific signed original FDA Form 1572 for institution PI**
- Current CV (signed and dated) for institution PI and all sub-investigators listed on FDA Form 1572
- Medical License for institution PI and sub-investigators listed on FDA Form 1572
- Lab license, certificates, and Normal Lab Values (NLV) for labs listed on FDA Form 1572
- Signed Investigator Signature Page for protocol**
- Signed financial Disclosure Form for investigators listed on FDA Form 1572**
- Pharmacy Information Form**

The GOG Administrative Office will receive, review, and approve all regulatory documents. Please allow 7-10 days for review and approval of all documents prior to randomization of first patient.

* When submitting the IRB approval to the GOG, the CTSU IRB Certification Form must be used (form can be downloaded at www.ctsu.org). All initial, continuing and amendment reviews must be sent to the GOG Administrative Office.

** Please see GOG-0286B protocol documentation page to download forms by clicking on the “Regulatory Forms” link.

5.1 Patient Entry and Registration

When a suitable candidate has been obtained for protocol entry, the following steps should be taken:

OPEN (Oncology Patient Enrollment Network) Registration: All site staff will use OPEN to enroll patients to this study. OPEN can be accessed on the GOG web menu page by clicking on the OPEN link.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the group web site as a tool to verify eligibility.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for the CTSU members' web site.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the GOG or CTSU roster.
- To perform registrations you must have an equivalent 'Registrar' role on the Lead Group roster. Role assignments are handled through the Groups in which you are a member.

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the CTSU members' web site OPEN tab or within the OPEN URL. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctscontact@westat.com.

5.2 Treatment Plan (05/19/14) (08/18/14)(04/28/15)(12/05/16)

Randomization will be stratified within the following groups:

1. Performance status (0 or 1 versus 2)
2. Disease status (Stage III versus IV or recurrent)
3. Patient BMI (<30 or >=30)

Randomization:

Arm I:

Paclitaxel 175 mg/m² IV over 3 hours day 1

Carboplatin AUC = 5 IV day 1

Every 21 days x 6 cycles

Metformin 850 mg oral QD, beginning on day 1. If tolerated for 4 weeks, the dose will be increased to Metformin 850 mg BID.

Maintenance regimen (for patients in complete response, partial response or stable disease) – Metformin 850 mg oral BID

Patients continue to receive maintenance treatment until disease progression or until adverse events prohibit further therapy.

Arm II:

Paclitaxel 175 mg/m² IV over 3 hours day 1

Carboplatin AUC = 5 IV day 1

Every 21 days x 6 cycles

Placebo for metformin 850 mg oral QD, beginning on day 1. If tolerated for 4 weeks, the dose will be increased to placebo for metformin 850 mg BID.

Maintenance regimen – (for patients in complete response, partial response or stable disease) – Matched placebo oral BID
Patients continue to receive maintenance treatment until disease progression or until adverse events prohibit further therapy.

Metformin or placebo for metformin 850 mg will begin on cycle 1 day 1. Metformin or Placebo doses will be taken orally, once daily approximately 24 hours apart. If patients tolerate the once daily dosing of metformin/placebo for 4 weeks, the dose will be increased to twice daily, approximately 10-12 hours apart. Patients will be given a Patient Medication Calendar to complete (Appendix II). The Patient Medication Calendar should be reviewed prior to the start of each cycle.

Paclitaxel will be infused over approximately 3 hours.

Carboplatin will be infused over approximately 30 minutes, following paclitaxel.

For all cycles where paclitaxel is to be administered, it is recommended that a preparative regimen be employed, to reduce the risk associated with hypersensitivity reactions. This regimen should include dexamethasone (either IV or PO), an anti-histamine H1 (diphenhydramine 25-50 mg IV or orally, or an equivalent dose of an alternate H1 blocker such as loratadine or fexofenadine), and a standard dose of antihistamine H2 IV (Famotidine is preferred, but ranitidine can also be used. Cimetidine is not recommended as it may increase the serum concentration of metformin). The preparative regimen can be altered at the discretion of the treating physician.

NOTE: Patients with SD or PR who still have measurable disease at the completion of cycle 6 may continue to receive paclitaxel and carboplatin (with metformin or placebo) up to a total of 10 cycles (if deemed necessary by the treating investigator). Patients who continue with cycles 7-10 will continue with all study assessments as described for Prior to each cycle (cycles 1-6) (see [Section 7, Study Parameters](#)).

Dosing of Carboplatin: See Appendix IV for Carboplatin Dose Calculation Instructions.

Chemotherapy administration: See Appendix III for NRG Oncology General Therapy Guidelines.

5.3 Supportive Care Guidelines:

5.3.1 Nausea/Vomiting: It is anticipated that nausea and vomiting may be a significant side effect (due to carboplatin administration). It is recommended that ASCO, NCCN and/or your institutional anti-emetic guidelines be consulted. Antiemetic regimens can be altered at the

discretion of the treating physician. (05/19/14)

5.32 Routine supportive measures for cancer patients such as erythropoietin, analgesics, blood transfusions, antibiotics, bisphosphonates, and myeloid colony stimulating factors are permitted (see Section 6 for specific guidelines).

5.4 Criteria for removal from treatment

5.41 Inability to tolerate the lowest doses because of toxicity.

5.42 Patients may withdraw from the study at any time for any reason. Patients with evidence of disease progression or significant side effects will be removed from study therapy.

6.0 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. Any patient whose treatment is delayed must be evaluated on a weekly basis until adequate hematologic and non-hematologic parameters have been met. No dose escalation is planned for this study.

6.1 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
- Hematologic Nadirs, Table A
- Delayed Hematologic Recovery, Table B
- Non-Hematologic Toxicity, Table C

6.2 General Guidelines for Hematologic Toxicity

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

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

6.23 Lower Limits for ANC and Platelet Count

6.231 With Cytotoxic Chemotherapy - Subsequent courses of treatment which contain any cytotoxic chemotherapy (carboplatin, paclitaxel) will not begin (**day 1 of each cycle**) until the ANC is $\geq 1,500$ cells/ mcl and the platelet count is $\geq 100,000$ / mcl. Such treatment will 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 any protocol-directed therapy.

Exceptions:

Patients who received filgrastim or pegfilgrastim prior to the current cycle may begin (day 1 of cycle) with ANC ≥ 1000 cells/ mcl, if clinically appropriate, to allow for transient reductions in ANC after discontinuation of myeloid growth factors. Patients who are delayed more than 7 days may begin with ANC ≥ 1000

cells/ mcl, if clinically appropriate; and if they will receive filgrastim or pegfilgrastim with subsequent therapy.

- 6.232 During Maintenance – Metformin or Placebo is not expected to cause myelosuppression. Required blood tests during maintenance are detailed in Table 7.

6.24 Use of Hematopoietic Cytokines and Protective Agents

- 6.241 It is anticipated that myelosuppression may be a significant side effect of cytotoxic chemotherapy. Myeloid growth factors (either filgrastim or pegfilgrastim) can be used (it is recommended that ASCO, NCCN and/or your institutional guidelines be consulted). If myeloid growth factors are used, it is recommended that filgrastim (dose and schedule per institutional standard) or pegfilgrastim (dose and schedule per institutional standard) be administered.

- 6.242 Patients will NOT receive prophylactic thrombopoietic agents.

- 6.243 Patients may receive erythropoietin (EPO), iron supplements, and/or transfusions as clinically indicated for management of anemia. Treating physicians should be aware of prescribing information for the erythropoiesis stimulating agents (including Aranesp, Epogen and Procrit) which note that there is a potential risk of shortening the time to tumor progression or disease-free survival, and that these agents are administered only to avoid red blood cell transfusions. They do not alleviate fatigue or increase energy. They should NOT be used in patients with uncontrolled hypertension. They can cause an increased incidence of thrombotic events in cancer patients on chemotherapy. The updated package inserts should be consulted.

<http://www.fda.gov/Medwatch/safety/2007/safety07.htm>

6.3 Modifications for Hematologic Toxicity (Nadirs)

- 6.31 Initial occurrence of dose-limiting neutropenia (defined in 6.32) or dose limiting thrombocytopenia (defined in 6.33) will be handled according to Table A.

- 6.32 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 as a disorder characterized by an ANC $<1000/\text{mm}^3$ and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥ 38 degrees C

(100.4 degrees F) for more than one hour. Dose reductions will be handled according to Table A.

- 6.33 Dose-limiting thrombocytopenia (DLT-PLT) is defined by any occurrence of Grade 4 thrombocytopenia or bleeding associated with Grade 3 thrombocytopenia. There will be no modifications for uncomplicated Grade 3 thrombocytopenia. Dose reductions will be handled according to Table A.

DLT ANC	DLT PLT	First Occurrence	Second Occurrence	Third Occurrence
Yes [‡]	No	Reduce paclitaxel one dose level	Reduce carboplatin one AUC unit*	Discontinue protocol-directed cytotoxic chemotherapy
Yes [‡]	Yes	Reduce paclitaxel one dose level and carboplatin one AUC unit*	Reduce paclitaxel one dose level	Discontinue protocol-directed cytotoxic chemotherapy
No	Yes	Reduce carboplatin one AUC unit*	Reduce paclitaxel one dose level	Discontinue protocol-directed cytotoxic chemotherapy

*Minimum carboplatin dose = AUC 4.

[‡]Myeloid growth factors (either filgrastim or pegfilgrastim) can be used (it is recommended that ASCO, NCCN and/or your institutional guidelines be consulted). See section 6.241.

6.4 Modifications for Delayed Hematologic Recovery:

- 6.41 Delay on the basis of neutropenia (Delay-ANC) is defined if the ANC is less than 1,500 cells/mcl within 24 hours prior to scheduled day 1 therapy, or less than 1,000 cells/mcl, if the patient received filgrastim or pegfilgrastim during the previous cycle.
- 6.42 Delay on the basis of thrombocytopenia (Delay-PLT) is defined if the platelet count is less than **100,000**/mcl within 24 hours prior to scheduled day 1 therapy.
- 6.43 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.

Category	Delay (days)	Modification
Delay -ANC [‡]	1-7	No Change mandated
	8-21	Decrease paclitaxel one dose level
	>21	Discontinue Protocol Directed Cytotoxic Chemotherapy

Delay-PLT	1-7	No Change
	8-21	Decrease carboplatin one AUC unit*
	>21	Discontinue Protocol Directed Cytotoxic Chemotherapy

*Minimum carboplatin dose = AUC 4.

‡Myeloid growth factors (either filgrastim or pegfilgrastim) can be used (it is recommended that ASCO, NCCN and/or your institutional guidelines be consulted). See section 6.241.

6.5 Adjustments Specific for Metformin/Placebo (hypoglycemia, glucose intolerance and conditions predisposing to lactic acidosis)

6.51 If a patient develops a hypoglycemic condition (grade 3 or 4), the metformin/placebo will be discontinued until the condition resolves. If a patient develops a second episode of grade 3 or 4 hypoglycemia, the metformin/placebo will be discontinued, and unblinding will occur for appropriate continued management of the hypoglycemia. No dose adjustments will be made for hypoglycemia.

6.52 If a patient develops grade 2 or grade 3 glucose intolerance, any anti-diabetic medication can be initiated except for metformin, and the patient can remain on the trial with full follow-up. If grade 4 glucose intolerance develops, the metformin/placebo will be discontinued, and unblinding will occur for appropriate continued management of the glucose intolerance. 6.53 If a patient develops any condition predisposing to lactic acidosis such as renal dysfunction (serum creatinine \geq 1.4 mg/dl), CHF and dehydration, the metformin/placebo will be held until the condition resolves.

6.54 **NOTE: Temporarily discontinue metformin/placebo in patients undergoing radiologic studies in which intravascular iodinated contrast media are utilized.** It is generally recommended that metformin be should be temporarily discontinued prior to or at the time of intravascular administration of iodinated contrast media (potential for acute alteration in renal function). Metformin is recommended to be withheld for 48 hours after the radiologic study. Advise patients on temporarily discontinuing metformin/placebo for radiologic studies with intravascular iodinated contrast media according to your institutional policies.

6.6 Adjustments for Non-Hematologic Toxicity

Drug	Regimen -2 Level	Regimen -1 Level	Regimen Starting Dose
Paclitaxel	110 mg/m²	135 mg/m²	175 mg/m²

Carboplatin	Discontinue	AUC = 4	AUC = 5
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- 6.61 The development of Grade 2 (or greater) peripheral neuropathy requires reduction of one dose level in paclitaxel. If CTCAE Grade 3 or 4 peripheral neuropathy occurs then subsequent doses of paclitaxel will be delayed for a maximum of three weeks until recovered to CTCAE Grade ≤ 2 . If peripheral neuropathy fails to recover to Grade ≤ 2 by a maximum delay of three weeks from time therapy is due, then all paclitaxel should be withheld from all subsequent chemotherapy cycles.
- 6.62 Renal toxicity (associated with reduction in GFR) is not expected as a direct complication of chemotherapy in this chemotherapy naive patient population using the prescribed dose and schedule of each regimen. However, if the serum creatinine increases to ≥ 1.4 mg/dl, metformin or placebo must be held. Metformin is contraindicated in women with a serum creatinine ≥ 1.4 mg/dl. Metformin or placebo may be re-started once the serum creatinine improves to < 1.4 mg/dl. See Appendix IV (Carboplatin Dose Calculation Instructions). (05/19/14)
- 6.63 Hepatic toxicity is not expected as a direct complication of chemotherapy in this chemotherapy naive patient population using the prescribed dose and schedule for each regimen. However, the development of Grade 3 (or greater) elevations in AST, ALT, alkaline phosphatase or bilirubin requires reduction of one dose level in paclitaxel and delay in subsequent therapy for a maximum of three weeks until recovered to Grade 1.
- 6.64 There will be no dose modifications for alopecia or controllable nausea, vomiting, constipation, or diarrhea. It is recommended that routine medical measures be employed to manage nausea, vomiting, constipation, and diarrhea.
- 6.65 Potential modifications for uncontrolled nausea, vomiting, constipation or diarrhea or other non-hematologic toxicities Grade 2 (or greater) require discussion with the study chair except where noted below in Section 6.651.
- 6.651 Special Modifications Study Treatment 6.651 For any CTCAE Grade 3 non-hematologic adverse event (except controllable nausea/vomiting, constipation or diarrhea) considered to be at least possibly related to study treatment, protocol directed treatment should be held until symptoms resolve to \leq CTCAE Grade 1. Upon resumption of therapy, both paclitaxel and carboplatin should be reduced by one dose level. If a CTCAE Grade 3 adverse event persists for $>$ three weeks or recurs after resumption of

therapy, the patient may be taken off protocol directed treatment after consulting with the Study Chair.

6.65 For any CTCAE Grade 4 non-hematologic adverse event, the patient may be taken off protocol directed treatment therapy after consulting with the Study Chair.

7.0 STUDY PARAMETERS7.1 Observations and Tests (04/28/15)

The following observations and tests are to be performed and recorded on the appropriate forms.

Parameter	Pre-Therapy	Prior to Each Cycle (Cycles 1-6)	Prior to Cycle 3	Every 9 weeks	Every 3 Maintenance Cycles	Prior to each Cycle (Cycle 7-10) for patients continuing carboplatin /paclitaxel	Off All Study Therapy
History & Physical	1	X			X	X	
Vital Status							6
Review of Patient Medication Calendar		X			X	X	
Vital signs (Heart rate, blood pressure, and temperature)	1	X			X	X	
BMI (height and weight)*	1		X		X		
Hip to waist ratio	1, 7		X		X		
Performance status	1	X			X	X	
Toxicity Assessment	2	X			X	X	
CBC/Differential/Platelets	2	X (3)			X	X (3)	
Serum Pregnancy Test (for patients of childbearing potential)	2						
Fasting glucose	2		X				
Fasting insulin level	2		X				
Electrolytes, BUN, creatinine	2	X (3)			X	X (3)	
Bilirubin, AST, ALT, Alkaline Phosphatase, Albumin	2	X (3)			X	X (3)	
HgbA1C	1						
ECG	1						
Chest imaging (X-ray or CT scan of the chest)	1			4#	4#		4#
Radiographic tumor measurement (CT or MRI)	1			5#	5#		5#
Patient Reported Outcomes	X		X	X ⁸			

*Height only needs to be measured at baseline (pre-therapy). Weight should be measured and recorded at indicated intervals and BMI recalculated with current weight at the indicated intervals.

Until disease progression, death or initiation of a subsequent anti-cancer therapy.

Continue with the same frequency if the patient goes off of study therapy for reasons other than disease progression or death.

ONE CYCLE = 3 weeks

NOTE: Patients that discontinue metformin or placebo, but continue treatment with paclitaxel and/or carboplatin are considered on study therapy. All observations/tests and form completion must continue.

Notes:

1. Must be obtained within 28 days prior to initiating protocol therapy.
2. Must be obtained within 14 days prior to initiating protocol therapy.
3. Laboratory tests (including CBC/Differential/Platelets and creatinine) must be obtained within 4 days of re-treatment with cytotoxic therapy.
4. Repeat chest imaging every 9 weeks (+/- 7 days) and at any other time if clinically indicated based on symptoms or physical signs suggestive of progressive disease, if initially abnormal or if required to monitor tumor response. Imaging assessments as part of this protocol can be discontinued if disease progression is confirmed according to guidelines in section 8. **If a patient discontinues study treatment for any reason other than progression, imaging assessments should be performed every 9 weeks (+/- 7 days) until progression.** After 2 years of protocol therapy or follow-up (measured from approximately cycle 1, day 1), chest imaging interval will be conducted every 12 weeks (+/- 7 days). An Excel tool is provided to assist in determining imaging dates (see Appendix VI).
5. CT scan or MRI of the abdomen and pelvis every 9 weeks (+/- 7 days) and at any other time if clinically indicated based on symptoms or physical signs suggestive of progressive disease. Imaging assessments as part of this protocol can be discontinued if disease progression is confirmed according to guidelines in section 8. **If a patient discontinues study treatment for any reason other than progression, CT scan or MRI of the abdomen and pelvis should be performed every 9 weeks (+/- 7 days) until progression.** After 2 years of protocol therapy or follow-up (measured from approximately cycle 1, day 1), CT scan or MRI of the abdomen and pelvis interval will be conducted every 12 weeks (+/- 7 days). An Excel tool is provided to assist in determining imaging dates (see Appendix VI).
6. After completion all protocol treatment and radiologic assessments: Follow-up every 3 months for 2 years and then every 6 months for 3 years. Follow-up forms are collected for the 5 year follow-up period or until study termination.
7. Instructions on measuring hip to waist ratio (see section 7.5)
 - Measure hip circumference the maximum extension of the buttocks
 - Measure waist circumference at the belly button.
8. Patient reported outcomes are collected before cycle 1, 3, and 6, and at 26 weeks.

7.2 Stained Pathology Slide Requirements for Central Review to Confirm Protocol Eligibility

7.21 Eligibility Criteria: Endometrial cancer.

Patients with the following histologic epithelial cell types are eligible: Endometrioid adenocarcinoma, serous adenocarcinoma, undifferentiated carcinoma, clear cell adenocarcinoma, mixed epithelial carcinoma, and adenocarcinoma not otherwise specified (N.O.S.).

7.22 Primary Site: At least two H&E stained slides from the primary tumor must be submitted. These slides should document the range of histologic

features of the tumor (e.g. grade; clear cell, solid, serous or endometrioid features). More than 2 slides may be submitted. If the tumor is histologically homogeneous, two randomly selected slides should be submitted.

Most Advanced Stage: If histologically determined, at least one H&E stained slide documenting the most advanced stage should be submitted.

Recurrent Tumor: If histologically determined, at least one representative H&E stained slide documenting recurrent tumor should be submitted.

When submitting pathology material to the NRG Statistics and Data Monitoring Center individual slides must be labeled with NRG Patient ID, patient initials and the surgical / pathology accession number (e.g., S08-2355) and block identifier (e.g., A6). Do not label the slides with disease site (e.g., right ovary) or procedure date. Pack the labeled slides into plastic slide cassette(s). Tape plastic slide cassettes shut and wrap in bubble wrap or another type of padded material prior to shipping. Please include the NRG Patient ID, patient initials, and protocol number on all pages of the pathology report and black out the patient's name. Ship pathology slides, one copy of the official pathology report in your own shipping containing using postal mail at your own expense directly to the **Pathology Materials Coordinator at the NRG Statistics and Data Monitoring Center, Roswell Park Cancer Institute, Research Studies Center, Carlton and Elm Streets, Buffalo, New York, 14263**; phone (716) 845-5702 Please see section 10.2 for additional requirements and instructions. (04/28/15)

7.3 Translational Research (12/05/16)

7.31 Specimen Requirements (12/05/16)(XX/XX/XX)

If the patient gives permission for her specimens to be collected and used for this optional translational research component, then participating institutions are required to submit the patient's specimens as outlined below (unless otherwise specified).

Required Specimen (Specimen Code)	Collection Time Point	Sites Ship Specimens To
FFPE Primary Tumor (FP01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)	Prior to all treatment	GOG Tissue Bank within 8 weeks of registration ¹
FFPE Metastatic Tumor (FM01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)		
FFPE Recurrent Primary Tumor	Prior to study treatment	

(FRP01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)		
FFPE Recurrent Metastatic Tumor (FRM01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)		
Pre-Treatment Whole Blood (WB01) 7-10mL drawn into purple top (EDTA) tube(s)	Prior to study treatment	GOG Tissue Bank the day the specimen is collected ¹

- * A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the GOG Tissue Bank
1 GOG Tissue Bank / Protocol GOG-0286B, Nationwide Children's Hospital, 700 Children's Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2865, FAX: (614) 722-2897, Email: BPCBank@nationwidechildrens.org

7.32 Laboratory Testing (12/05/16)

Assay details are included in [Appendix VII](#).

7.321 Analysis of PI3K/AKT/MTOR Pathway Mutations and Amplifications (Integrated Biomarker)

DNA extracted from FFPE and whole blood will be used for targeted sequencing using a hybrid capture approach in conjunction with next generation sequencing. PIK3CA mutations/amplifications and PIK3R1/PIK3R2 mutations will specifically be assessed.

7.322 Immunohistochemical Analysis of Metformin Transporter Proteins (Integrated Biomarker)

FFPE will be used for metformin transporter proteins (OCT 3, MATE 1/2, and PMAT) immunohistochemistry.

7.323 Immunohistochemical Analysis of Key Targets of the Insulin/IGF-1/mTOR Pathway (Exploratory Biomarker)

FFPE will be used for p-IGF1R, p-S6 and p-4E-BP-1 immunohistochemistry.

7.324 Analysis of the Genetic Variants of the Metformin Transporters (Exploratory Biomarker)

DNA extracted from FFPE and whole blood will be used for targeted sequencing using a hybrid capture approach in conjunction with next generation sequencing. Genetic variants of the metformin transporters will specifically be assessed (see Table 2 of [Appendix VII](#)).

7.33 Future Research

Details regarding the banking and use of specimens for future research can be found in [Appendix V](#).

7.4 Patient-reported Outcomes Measures (05/19/14)

Physical Function The physical functioning (PF) subscale of the SF-36 has been used previously to study physical functioning and ability to do activities of daily living in endometrial cancer survivors [Basen-Engquist et al, 2009; Nout et al 2011]. Basen-Engquist et al found that endometrial cancer survivors' scores on the physical functioning scale were 8.1 points lower, on average, than age-matched norms. In this sample, the SF-36 physical functioning scale was negatively correlated with ratings of pain, fatigue, and age, as expected (Basen-Engquist, personal communication). The scale was used in a study of long term outcomes of endometrial cancer patients randomized to surgery plus radiation versus surgery alone; patients in the radiation treatment group reported poorer physical functioning 15 years after treatment than those who received surgery alone [Nout et al 2011]. The scale is also sensitive to differences in BMI in endometrial cancer survivors^{91, 96}, and physical activity level⁹⁶. The PF subscale consists of 10 items designed to assess the ability to conduct activities of daily living requiring large motor skills; a high score indicates better PF and a difference of 7 points on the scale is suggested as a minimally important difference.¹⁰⁹ The internal consistency of the subscale is excellent (Cronbach alpha, 0.89-0.92) [Ware et al, 1997] and it correlates with other PF measures and distinguishes between serious and mild medical conditions [McHorney et al, 1993].

Fatigue The Functional Assessment of Chronic Illness Therapy-Fatigue Scale (FACIT-F Scale) is a 13-item questionnaire that assesses self-reported fatigue and its impact upon daily activities and function. The 13-item FACIT-F Scale is formatted for self-administration on one-page, and uses a 5-point Likert-type scale (0 = Not at all; 1 = A little bit; 2 = Somewhat; 3 = Quite a bit; and 4 = Very Much). The FACIT-F Scale has been found to be reliable and valid (Yellen et al, 1997; Cella et al, 2002). Since its initial validation report published in 1997, the FACIT-F Scale has been used on well over 20,000 people with cancer, and represents the multistep process for validation [Smith et al, PM&R, 2010]. It is currently being used in GOG 249 for validation of the PROMIS Fatigue-SF1. A difference of 4.1 scale points is the best estimate of the minimal clinically important difference (MCID) when comparing the FACIT-Fatigue subscale between treatment groups.

Physical Activity

To measure physical activity patients will complete short form of the International Physical Activity Questionnaire (IPAQ). The IPAQ includes 7 questions asking patients to report the frequency and duration of their vigorous, moderate, and

walking (at least 10 minutes) activity in the past 7 days, and the amount of time they spend sitting per day. The IPAQ has very good test-retest reliability for the overall score; in a 12 country study of the IPAQ's reliability and validity¹¹⁰, 75% of the test-retest estimates were greater than 0.65, and the pooled estimate was 0.76. The test retest reliability of the sitting question alone is also very good, with 2/3 of the estimates greater than 0.70. In comparisons with accelerometer data, the short IPAQ was moderately correlated with total activity (2/3 of the correlations coefficients $r > 0.30$) and sitting (over half of the correlation coefficients $r > 0.30$). When the data are categorized based on meeting the criteria of >150 minutes per week, agreement coefficients between accelerometer and the short IPAQ ranged from .46 to .93, with 80% of the coefficients greater than 0.70. The question responses can be used to estimate MET minutes/week for walking, moderate intensity activity, and vigorous intensity activity, as well as total physical activity MET minutes/week.

PRO Measurement Intervals

Patients will complete the Questionnaires at 4 times:

- 1) Prior to beginning therapy
- 2) 6 weeks after initiating therapy (approximately prior to cycle 3)
- 3) 15 weeks after initiation of treatment (prior to cycle 6)
- 4) 26 weeks following initiation of therapy

The timing of the first three assessments is scheduled to coincide with regular treatment follow-up visits and to take account of expected changes in this patient population during active treatment, which might include improvements in fatigue and decreased sedentary behavior in the experimental arm. The final or 4th assessment at 26 weeks/approx 6 months post baseline will measure quality of life after patients have completed chemotherapy on this protocol, but will provide an opportunity to detect potential early differences in treatment arms responses between those on or off maintenance therapy. QOL assessments should be administered at assessment times, regardless of whether the patient progresses or is removed from study for any reason. It is worth noting the GOG experience in two recent advanced endometrial cancer studies (GOG-0122, GOG-0209) indicates that compliance is substantial (>94%) initially, and continues to be impressive even at later assessment points (e.g., GOG-0209 @ 81%), with minimal missing data. Data monitoring and institution training for compliance and data quality is ongoing.

7.5 Anthropometrics (05/19/14)

Height, weight, and waist circumference will be measured at the clinic site by site staff for each participant enrolled in the trial. The physical examination form site will be entered electronically via Medidata Rave. Specific instructions for measurement are detailed below.

Height

Each participant will have her height in indoor clothes and without shoes measured at baseline and yearly at the GOG clinic managing her regular oncology clinical exams. The participant should be standing evenly on a flat surface at a right angle to the movable vertical board or cap that is pulled down to lightly touch the most superior point of the head. Hair may need to be compressed to ensure correct measurement of the participant's height. The height should be recorded on the physical exam form to the nearest 1.0 centimeter.

Weight

Each participant will have her weight measured at baseline and every three months (to be in line with follow-up physical exams) at the GOG clinic managing her regular oncology clinical exams. Participants will be weighed using a calibrated standard beam scale that was calibrated to zero just prior to use. The scale should be located on a level area and the participant in lightweight, indoor clothing and shoeless should step onto the scale so that her body weight is centered on the platform and distributed evenly between both feet. The weight should be recorded on the physical exam form to the nearest 0.5 kilogram.

Waist Circumference

At baseline and every 3 cycles (9 weeks) (along with weight and physical examination), the participant will have her waist circumference measured by local GOG oncology care clinic staff. Clothing should not be covering the waist area at the time of the waist-circumference measurement. The participant should be standing relaxed, with her hands hanging at her sides. The measurement should be taken by facing the participant and placing the measurement tape horizontally around the participant at the umbilicus. The tape measure should not be stretched nor the skin compressed when the waist circumference is being measured. Special care should be taken to ensure that the tape measure is straight and not up on one side or the other. Clinics may find it helpful to have another staff member or mirror present to confirm correct alignment of the tape measure. The waist circumference should be recorded to the nearest 0.1 centimeters.

Buttocks (Hip) Circumference

The patient stands erect with feet together and weight evenly distributed on both feet. The examiner squats on the right side of the SP and places the measuring tape around the buttocks. The tape is placed at the maximum extension of the buttocks. Ensure that the tape is horizontal and parallel to the floor. The tape is held snug but not tight. The examiner takes the measurement from the right side and calls it to the recorder. The hip circumference should be recorded to the nearest 0.1 centimeters.

8.0 EVALUATION CRITERIA

8.1 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

8.11 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment on study.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

8.12 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable unless progression is documented or a biopsy is obtained to confirm persistence at least 90 days following completion of radiation therapy.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by

CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

8.13 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans), but NOT lung.

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for

use with RECIST measurements. PET-CT scans are not always done with oral and IV contrast. In addition, the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed. **For these reasons, the GOG will not allow PET-CT use for RECIST 1.1 response criteria.**

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: *While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:*

- a. *Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.*
- b. *No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will*

be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

- c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

8.14 Response Criteria

8.141 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to

qualify for PD

8.142 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Non-CR/Non-PD: Persistence of one or more non-target lesion(s)

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

8.143 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	

PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥ 4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
<p>* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** Only for non-randomized trials with response as primary endpoint. *** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.</p> <p><u>Note:</u> Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “<i>symptomatic deterioration</i>.” Every effort should be made to document the objective progression even after discontinuation of treatment.</p>				

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
<p>* ‘Non-CR/non-PD’ is preferred over ‘stable disease’ for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised</p>		

8.15 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from study entry until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

8.16 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from date of study entry to time of progression or death, whichever occurs first.

8.17 Survival

Survival is defined as the duration of time from date of study entry to time of death or the date of last contact.

9.0 DURATION OF STUDY

- 9.1 Patients will receive therapy until disease progression or intolerable toxicity intervenes. The patient can refuse the study treatment at any time.
- 9.11 **NOTE:** Patients with SD or PR who still have measurable disease at the completion of cycle 6 may continue to receive paclitaxel and carboplatin (with metformin or placebo) up to a total of 10 cycles (if deemed necessary by the treating investigator). Patients who continue with cycles 7-10 will continue with all study assessments as described for Prior to each cycle (cycles 1-6) (see [Section 7, Study Parameters](#)). (04/28/15)
- 9.2 All patients will be treated (with completion of all required case report forms) until disease progression or study withdrawal. Patients will then be followed every three months for the first two years and then every six months for the next three years. Patients will be monitored for delayed toxicity and survival for this 5-year period with Follow-Up Forms submitted to the GOG Statistical and Data Center, unless consent is withdrawn. Follow-Up Forms will no longer be required if the study is terminated prior to the completion of the 5-year follow-up period.
- 9.3 A patient is considered off study therapy when the patient has progressed or died, a non-protocol drug or therapy (directed at the disease) is initiated or **all** study therapy is totally discontinued. Report all treatment received on Cycle Drug Information Forms and adverse events on Adverse Event Forms up until the patient qualifies as being off study therapy.

10.0 STUDY MONITORING AND REPORTING PROCEDURES

10.1 Adverse Event Reporting for a Commercial Agent

10.11 Definition of Adverse Events (AE)

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that occurs in a patient administered a medical treatment, whether the event is considered related or unrelated to the medical treatment.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (<http://ctep.cancer.gov>). The CTCAE v4.0 Manual is also available on the GOG member web site (<http://www.gog.org> under MANUALS).

10.12 Reporting Expedited Adverse Events (05/19/14)

Depending on the phase of the study, use of investigational or commercial agents, and role of the pharmaceutical sponsor, an AE report may need to reach multiple destinations. For patients participating on a GOG trial, all expedited AE reports should be submitted by using the CTEP automated system for expedited reporting (CTEP-AERS). All CTEP-AERS submissions are reviewed by GOG before final submission to CTEP-AERS. Submitting a report through CTEP-AERS serves as notification to GOG, and satisfies the GOG requirements for expedited AE reporting. All adverse reactions will be immediately directed to the Study Chair for further action.

The requirement for timely reporting of AEs to the study sponsor is specified in the Statement of Investigator, Form FDA-1572. In signing the FDA-1572, the investigator assumes the responsibility for reporting AEs via CTEP-AERS. In compliance with FDA regulations, as contained in 21 CFR 312.64, AEs should be reported by the investigator.

10.13 Phase 2 and 3 Trials Utilizing a Commercial Agent: CTEP-AERS Expedited Reporting Requirements for Adverse Events That Occur Within 30 Days of the Last Dose of Any Commercial Study Agent (05/19/14)

Reporting Requirements for Adverse Events that occur within 30 Days¹ of the Last Dose of the Commercial Agent on Phase 2 and 3 Trials

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5 ²	Grades 4 & 5 ²
	Unexpected and Expected	Unexpected	Expected	Unexpected With Hospitalization	Without Hospitalization	Expected With Hospitalization	Without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	7 Calendar Days	Not Required	7 Calendar Days	Not Required	7 Calendar Days	7 Calendar Days
Possible Probable Definite	Not Required	7 Calendar Days	Not Required	7 Calendar Days	7 Calendar Days	7 Calendar Days	Not Required	24-Hrs; 3 Calendar Days	7 Calendar Days

¹ Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with a commercial agent require reporting as follows:

CTEP-AERS 24-hour notification followed by complete report within 3 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 7 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

² Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

Please see exceptions below under the section entitled, “Additional Instructions or Exceptions to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing a Commercial Agent.” **March 2005**

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
 - “24 hours; 3 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 3 calendar days of the initial 24-hour report.
 - “7 calendar days” – A complete CTEP-AERS report on the AE must be submitted within 7 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported to GOG via CTEP-AERS if the event occurs following treatment with a commercial agent.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial

registration on all reports.

Additional Instructions or Exceptions to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing a Commercial Agent:

The following SAEs will be exempted from expedited reporting through CTEP-AERS

- All Grade 2, 3 and 4 myelosuppression (including leukopenia, neutropenia, anemia, and thrombocytopenia) is exempt from expedited reporting.
- G3-4 Neutropenia/febrile neutropenia, regardless of hospitalization.
- G3-4 Diarrhea, Nausea, Vomiting, or Dehydration, regardless of hospitalization.

10.14 Procedures for Expedited Adverse Event Reporting: (05/19/14)

10.141 CTEP-AERS Expedited Reports: Expedited reports are to be submitted using CTEP-AERS available at <http://ctep.cancer.gov>. The CTEP, NCI Guidelines: Adverse Event Reporting Requirements for expedited adverse event reporting requirements are also available at this site.

Secondary Malignancy:

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

Second Malignancy:

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting via CDUS unless otherwise specified.*

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to GOG by telephone at: 215-854-0770. An electronic report MUST be submitted immediately upon re-establishment of internet connection. Please note that all paper

CTEP-AERS forms have been removed from the CTEP website and will NO LONGER be accepted.

10.2 GOG Data Management Forms (04/28/15)(XX/XX/XX)

The following forms must be completed for all patients registered and submitted to the GOG Statistical and Data Center (SDC) according to the schedule below. GOG electronic case report forms must be submitted through the Medidata Rave Electronic Data Entry System (www.imedidata.com). All amendments to forms must also be submitted through Medidata Rave. Pathology reports can be sent to the GOG Statistical and Data Center via postal mail or uploaded in Medidata Rave. The upload option is an alternative method for submitting paper reports. Patient Reported Outcomes (PRO) questionnaires are to be completed on the original Scantron forms and submitted via postal mail.

Form	Comments
Baseline Folder <i>(Forms due within 2 weeks of registration)</i>	
Baseline/History Forms: <ul style="list-style-type: none"> - Visit Information – Baseline Form - Registration Form - History Information Form - Primary Surgery Form - Pre-Treatment Summary Form - Specimen Consent Solid Tumor Evaluation Forms: <ul style="list-style-type: none"> - Target Lesions Form - Non-Target Lesions Form Non-Measurable Evaluation: <ul style="list-style-type: none"> - No Target Lesions Form - Non-Target Lesions Form Concomitant Medications Form Diabetes and Cardiovascular History Form	The appropriate forms will load in the Baseline Folder based on the answers reported on the corresponding Baseline Visit Information form.
Visit Folder <i>(Forms due within 2 weeks of the completion of each cycle)</i>	
Cycle Information and Treatment Forms: <ul style="list-style-type: none"> - Visit Information Form - Cycle Patient Information Form - Cycle Drug Information Form - Labs and Chemistries Form - Blood Pressure Assessment Form Toxicity Forms: <ul style="list-style-type: none"> - Section 1 Form - NADIRS Form - Adverse Event Form - Adverse Event Grades Solid Tumor Evaluation Forms: <ul style="list-style-type: none"> - Target Lesions Form - Non-Target Form 	The appropriate forms will load in the Visit Folder based on the answers reported on the corresponding Visit Information forms.

<ul style="list-style-type: none"> - New Lesion Form - Status and Response Form <p>Non-Measurable Evaluation:</p> <ul style="list-style-type: none"> - No Target Lesions Form - Non-Target Lesions Form - New Lesion Form - Status and Response Form 	
Pathology Folder <i>(Reports and slides due within 6 weeks of registration)</i>	
<p>Primary disease: Pathology Report Stained Slides</p> <p>Recurrent or Persistent Disease: Pathology Report Stained Slides</p>	<p>Submit stained slides with two copies of the pathology report to SDC via postal mail or upload the pathology report online via RAVE.</p> <p>Stained pathology slides are required for central review by the GOG Pathology Committee. See Section 7.2 for mailing instructions for Pathology eligibility. All stained slides MUST be submitted via postal mail.</p>
Translational Research Folder	
<p>TR Form:</p> <ul style="list-style-type: none"> - FFPE Primary Tumor (FP01) - FFPE Metastatic Tumor (FM01) - FFPE Recurrent Primary Tumor (FRP01) - FFPE Recurrent Metastatic Tumor (FRM01) - Pre-Treatment Whole Blood (WB01) 	<p>A completed copy of Form TR must accompany each specimen shipped to the GOG Tissue Bank (or alternate laboratory). Handwritten forms will not be accepted.</p> <p>FP01, FM01, FRP01, and FRM01 are due 8 weeks from registration. WB01 is due 2 weeks from registration.</p>
Patient-Reported Outcomes Measures (PRO) Folder	
<p>PRO Scantron Form</p>	<p>Patients will complete the Questionnaires at 4 times:</p> <ol style="list-style-type: none"> 1) Prior to beginning therapy 2) 6 weeks after initiating therapy (approx. prior to cycle 3) 3) 15 weeks after initiation of treatment (prior to cycle 6) 4) 26 weeks following initiation of therapy
Treatment Completion Folder <i>(Forms due within 2 weeks of treatment completion)</i>	
<p>Treatment Completion Form</p>	
Follow-up Visit Folder <i>(Forms due within 2 weeks of follow-up visits, disease progression or death)</i>	
<p>Visit Information Follow-Up Form</p> <p>Follow-Up Form</p> <p>Follow-Up Period Adverse Event:</p>	<p>Follow-up visits should be scheduled quarterly for 2 years, semi-annually for 3 more years, and annually thereafter.</p> <p>The appropriate forms will in the Follow-up Visit Folder based on the answers reported on the corresponding Follow-up Visit Information forms.</p>

<ul style="list-style-type: none">- Reporting Form – Part 1- Reporting Form – Part 2 <p>Solid Tumor Evaluation:</p> <ul style="list-style-type: none">- Target Lesions Form- Non-Target Form- New Lesion Form- Status and Response Form <p>Non-Measurable Evaluation:</p> <ul style="list-style-type: none">- No Target Lesions Form- Non-Target Lesions Form- New Lesion Form- Status and Response Form	
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This study will be monitored by the Complete Clinical Data System (CDUS) Version 3.0.
CDUS data will be submitted quarterly to CTEP by electronic means.

11.0 STATISTICAL CONSIDERATIONS

11.1 **Study Design Summary:**

This is a phase II/III, placebo controlled study. The phase II study is a randomized clinical trial designed to assess the impact of metformin in combination with carboplatin and paclitaxel (arm 1) against the reference regimen of placebo with carboplatin and paclitaxel (arm 2) through PFS. The study will accrue 240 patients and test the equivalence of PFS between the regimens after 60 PFS events are observed in the reference regimen at a 20% level of significance. If the true HR of arm 1 to arm 2 is 2/3, then the study has 90% power. The 60th event time may occur during study accrual, or up to four months after the study finishes accrual. If the phase II study is positive, accrual will resume for a 300 patient phase III trial (i.e. targeting a total of 540) and use OS as a primary endpoint. An interim futility analysis using OS will be conducted in both the phase II and phase III cohorts after 52 deaths are observed on the reference arm, which should yield at least 90 deaths (on average) in the entire study. If the estimated risk of death is not greater on the experimental regimen than on the reference regimen, then the study will recommend continued accrual. On the other hand, if the risk is greater, consideration will be given to closing the phase III portion of study for futility. Assuming the phase III study completes accrual, follow-up will continue until 180 deaths are observed in the reference arm of the phase II and phase III studies. A log-rank test of equivalent OS will be conducted at the 5% level of significance (one sided). Simulations indicate that a test utilizing both phases will reduce the false positive rate (i.e. alpha) to less than 5% while increasing overall power (i.e. the joint probability of having both a positive phase II study and a positive phase III study) when OS and PFS HR=2/3 to about 88%.

This study will use an intent-to-treat principle. A dynamic allocation procedure will be used that tends to allocate each treatment arm in the ratio 1:1 within the stratification factors defined below: performance status (0 or 1 versus 2), disease status (Stage III versus Stage IV or recurrent), and patient body mass index (BMI < 30 versus >=30). (04/28/15)

11.2 **Principal parameters:**

Parameters used to determine the relative benefit of each treatment are listed below:

11.21 Primary Endpoints

11.211 Progression-free survival (PFS) for the Phase II study only.

Overall survival (OS) if the Phase III study is opened. In this case, OS for the patients enrolled onto the phase II portion will also become a primary endpoint for the Phase II/III study.

11.22 Secondary Endpoints

11.221 Among patients with measurable disease, the proportion of patients

responding to therapy by treatment.

11.222 The duration of response by treatment.

11.223 OS if the study closes before opening the phase III study (i.e. for the phase II study only). If the study continues with a phase III clinical trial, then PFS will be a secondary endpoint.

11.224 The frequency and severity of adverse events as assessed by CTCAE v4 within each arm.

11.225 Proportion responding, PFS, OS, and toxicity by treatment and level of obesity.

11.23 Translational Research Endpoints (12/05/16)

Specific Integrated Biomarker Endpoints

11.231 Whether or not a patient has PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations with PFS.

11.232 Expression of MATE 2 (dichotomized into two groups as high expression and low expression) with PFS.

11.233 BMI, hip-to-waist ratio, diabetes status, HgBA1C, fasting insulin and glucose levels, HOMA scores by treatment and tumor response, PFS and OS.

Other Exploratory Endpoints

11.232 Expression levels of key targets of the metformin/mTOR signaling pathway (p-IGF1R, p-S6 and p-4EBP-1) by treatment and tumor response, PFS and OS.

11.3 **Study Duration and Accrual Rate:**

The anticipated rate of accrual onto this study is 13 patients per month (156 patients per year). The period of active accrual to the phase II portion of the study is expected to be 18.5 months. The study will suspend for the observation of 60 PFS events in the reference arm. The 60th event is anticipated to occur about 17 months after the trial activates (likely range from 15 to 20 months, making the period of trial suspension small to non-existent). The period of active accrual to the phase III study is expected to be 23 months (at least 42 months for the entire study). The time of final data maturation is expected to occur around 70 months after the phase II trial opens (5 to 6 years).

11.4 Hypothesis and Sample Size (12/05/16)

The population under examination has a survival function for both PFS and OS that can be modeled quite well with a gompertz model:

$$S(t) = \exp\{(\alpha/\beta)*(1-\exp(\beta*t))\}$$

For PFS, alpha (α) has been estimated at 0.0909 and beta (β) has been estimated at 0.0431. For OS, alpha was estimated at 0.0326 and beta at 0.0126. A plot of the survival functions is provided below:

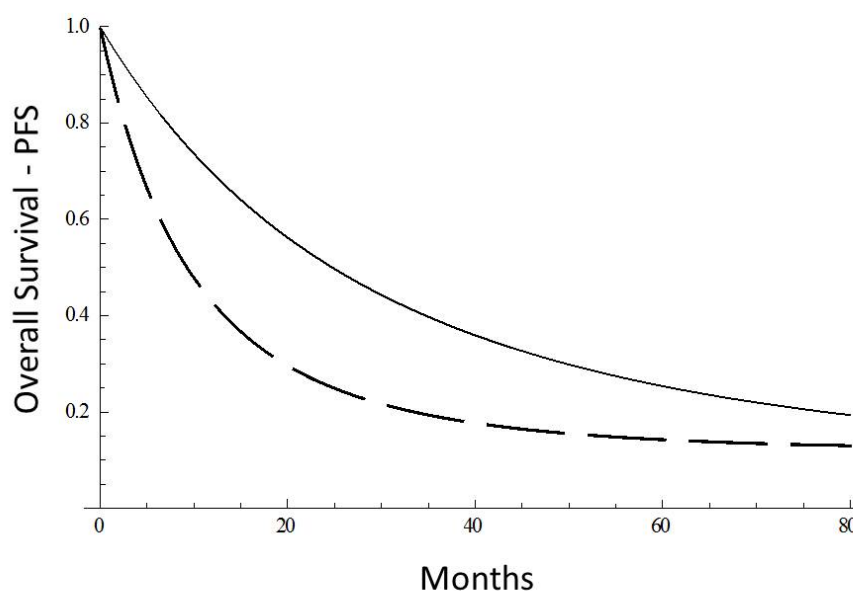


Figure 11.1: Plots of survival functions for OS (solid line) and PFS (dashed line).

Because the survival functions indicate a proportion of patients who are cured as well as having decreasing hazards of death or progressing, it is necessary to accrue larger sample sizes than typically seen in phase II studies.

Phase II

The null hypothesis is that the PFS hazard ratio (HR) of the experimental regimen to the reference regimen is 1.0. That is, $H_0: HR \geq 1.0$. Ordinarily, phase II studies restrict the level of significance to 10%. However, the current study is exceptional in several respects. Most importantly, metformin is not as toxic as many experimental agents examined in clinical trials. Therefore the harm is not as great if the drug was incorrectly taken into a phase III study. Furthermore, there is a desire to keep the accrual momentum high, which can be more easily

achieved if the study does not suspend for long periods of time (if at all).

When testing the equivalence of hazard rates with a stratified log-rank test at $\alpha = 20\%$ (1-sided) and $\beta = 10\%$, Schoenfeld's equation indicates that we need to observe 110 PFS events in total when clinically significant hazard ratios of 2/3 or less are deemed interesting (i.e. $H_a: HR \leq 2/3$). Requiring the observation of 60 events in the reference arm under the alternative will give us a design with these characteristics (simulation). The expected time to observe 60 events with an accrual rate of 13 patients per month and a maximum sample size of 240 is about 17 months (simulation). Follow-up for OS will continue as a secondary endpoint (if the study is negative) or as a primary endpoint for combining with a subsequent phase III study. In the case that a phase III study commences, data for the phase II and phase III patients will be frozen when 52 deaths are observed in the reference arm. The purpose of the data freeze when 52 deaths are seen is to conduct an interim analysis of OS for guidance in carrying out the rest of the study. If this analysis indicates a greater risk of death in the experimental regimen, the phase III study will be considered for early termination for futility. The timing of this analysis is expected to occur approximately 26 months after the phase II study opens (simulation) if there is no delay in opening the phase III trial.

Phase II/III Trial

The null hypothesis for the Phase III and Phase II/III study is that the overall survival hazard ratio is 1.0 ($H_0: OS\ HR \geq 1.0$). For the purposes of this study, reductions in the hazard by 1/3 or more are deemed clinically significant (i.e. $H_a: OS\ HR \leq 2/3$). A case can be made for smaller reductions being considered clinically significant (e.g. $HR \leq 0.80$), but such designs are not practically feasible.

This study will target an accrual of 300 patients to the phase III portion (540 in total). The primary endpoint will be OS. The null hypothesis of equivalent hazards will be assessed at the final analysis with a stratified log-rank test statistic at an overall 1-sided 5% level of significance when 180 deaths are observed in the reference arm (phase II and phase III patients). This should guarantee the observation of at least 300 deaths in the entire study under the alternative hypothesis ($OS\ HR = 2/3$ and $PFI\ HR = 2/3$) and provide approximately 88% power (probability of both phase II and phase II/III trials being positive).

An interim analysis will be conducted after the observation of 52 deaths in the reference arm (phase II and phase III patients as stated above). The purpose of this analysis is primarily for futility. Assuming no interruption of accrual, this analysis will be conducted approximately when 60% of the targeted accrual is attained. If the futility analysis indicates a higher hazard of death on the experimental arm (as assessed with a stratified log-rank statistic before squaring being greater than zero) using the method of Wieand⁹⁰, then the trial could close at that time.

There will also be a test for substantial activity in the regimen that administers metformin using a Lan-DeMets alpha spending function:

$$\alpha(t) = \alpha * t^3.$$

This spending function mimics an O'Brien-Fleming procedure when the total alpha is 0.05 (if alpha was 0.025, then t^4 may be more suitable). If the interim analysis is conducted at 28% information time, then 0.001 alpha will be spent at that time. The estimated probability (through simulation) of observing a sufficient result for early stopping when the PFI and PFS HR are both equal to 0.67 and the OS HR is equal to 0.67 is 33%.

The time of final data maturation on the phase II/III study is considerably longer than the phase II study. It is anticipated that it will take about 70 months after initiating the phase II study (about 5 to 6 years) before 180 deaths are observed in the reference arm (phase II and phase III patients).

Table 11.1 of Operating Characteristics

Event	P(Event) ¹ HR _{PFI} = 1.0 ² HR _{PFS} = 1.0 HR _{OS} =1.0	P(Event) ¹ HR _{PFI} = 0.667 HR _{PFS} = 0.77 HR _{OS} =1.0	P(Event) ¹ HR _{PFI} = 0.80 HR _{PFS} = 0.80 HR _{OS} =0.80	P(Event) ¹ HR _{PFI} =0.667 HR _{PFS} = 0.71 HR _{OS} =0.80	P(Event) ¹ HR _{PFI} = 0.667 HR _{PFS} = 0.667 HR _{OS} =0.667
Start Phase II/III from Phase II PFS (Starting at Study Initiation)	20.01	73.24	64.52	85.23	90.92
Complete Phase II/III Study After OS IA ³ at 52 Deaths (Starting at Study Initiation)	13.67	42.09	59.39	76.52	89.52
Reject H ₀ at End Phase II/III Study (Starting at Study Initiation)	1.46	3.96	43.99	55.70	88.16
Reject H ₀ at End Phase II/III Study (Beginning at Phase III After a Positive Phase II Study) ³	7.30	5.40	68.18	65.35	96.96

¹ Probabilities are provided in percentages (%).

² HR_{PFS} was estimated through simulation

² IA = Interim Analysis

³ The higher probabilities than the design's advertised level of significance in the first two

columns is not a serious concern since Phase II/III trials are designs to examine the properties from study initiation.

The null hypothesis in the table is that the experimental regimen does not reduce the risk of death (i.e. H_0 : OS HR=1.0).

The above table provides a sampling of the operating characteristics of the design at interesting points in the parameter space using simulation (n=10000) based on the Gompertz model provided above.

The first column shows the characteristics when the metformin has no effect on PFI, PFS, or OS. Only 20% of the phase II trials lead to opening a phase III trial, and only 14% of all studies continue on to complete the phase III study after the phase II/III interim analysis utilizing OS. The probability of a phase II/III study (that combines data from both phases) declaring metformin active is 1.46% of all studies that are initiated. Conditional on a phase II study being positive, the probability of the phase II/III study being positive is about 7.3%.

The second column gives an example where metformin has an effect on PFI and PFS but not on OS. In this case, the majority of phase II trials open to phase III studies, however, when the data are analyzed by OS, the Phase II/III study reach the correct conclusion within the required probability (<5%).

The third column provides an example of modest activity on both PFI and OS, and the 5th column provides the characteristics of the design when the drug is fully active by both PFS and OS.

11.5 Secondary Endpoints

With about 25% of patients being non-measurable, we could expect 200 measurable patients in each arm at the end of a phase II/III study. Testing a one-sided alternative hypothesis at $\alpha = 5\%$, the study should have 90% power of detecting a difference when the $P(\text{response} \mid \text{arm 1}) = 65\%$ and $P(\text{response} \mid \text{arm 2}) = 50\%$. The 95% CI for the probability of response should be no greater than 14% per treatment arm (actual length depending on the number of measurable patients). The duration of response will be assessed from the date of response to disease progression, death, or date last seen. These durations will be characterized by treatment using Kaplan-Meier curves and quartile estimates.

Toxicities will be assessed according to CTCAE v4 by organ or organ system. For each category of toxicity, each patient will be evaluated by the worst grade experienced during the course of therapy. These data will then be summarized by their frequency and severity according to the regimen administered. Comparisons between regimens will be examined through exact Chi-Square methods by breaking the severity of the worst toxicities experienced by each patient into

severe versus not severe (or into groups of none to mild, moderate, and severe or worse).

Obesity will be quantitatively assessed by body mass index (BMI) and will be assessed for its predictive and prognostic significance.

The interaction between BMI and metformin treatment will be examined with an interaction term in a Cox proportional hazards model. Historical data indicate that approximately 50% of people are obese ($BMI \geq 30$). For the purposes of examining the relationship, we will classify patients into two levels (high versus low) at the median BMI, which will increase the likelihood of detecting an interaction between metformin treatment and obesity. The power to detect an association between metformin treatment and obesity in this manner is approximated in the graph below where $R = \Delta_1 / \Delta_2$ and Δ_1 is the hazard ratio of control to experimental therapy for obese people and Δ_2 is the hazard ratio of control to experimental therapy for people who are not obese:

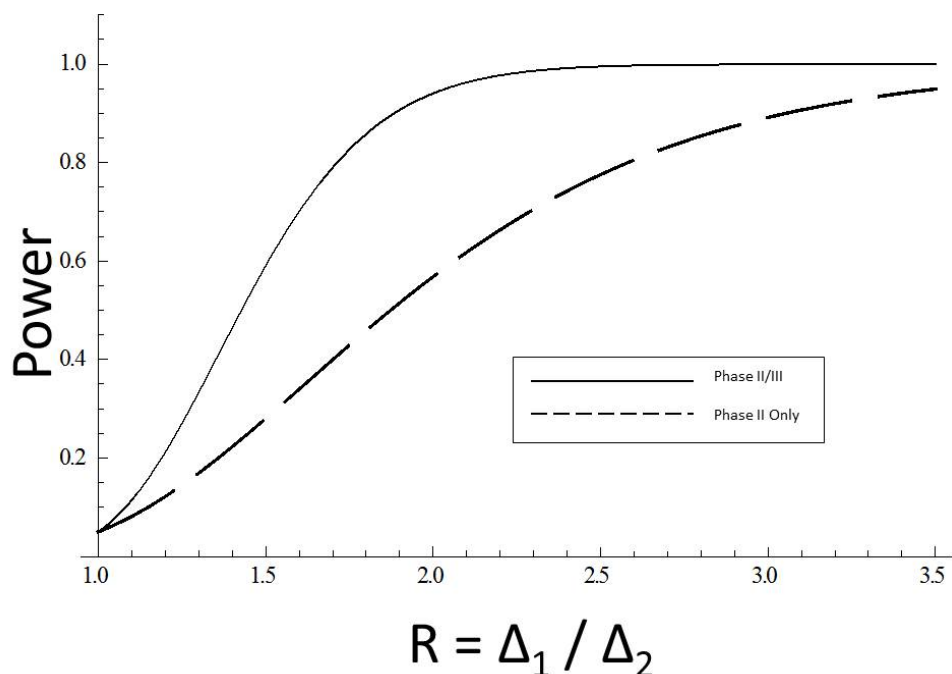


Figure 11.2: Power to detect a significant association between weight and metformin therapy as a function of R when testing a one-sided alternative hypothesis at the 5% level of significance. The dashed line gives power after observing 110 events (phase II data only); the solid line gives power after observing 342 events (phase II and III data combined).

The power to detect associations using interaction terms is unfortunately not high for clinically significant values of R (e.g. in the range of 1.5). In addition to this rigorous test, we will examine the impact of obesity with subset analyses as well

as inspect the impact of BMI (as a continuous variable) in a multivariate Cox PH model or logistic regression (depending on the endpoint). The latter analyses will be reported if the model diagnostics indicate that they are adequate. The operating characteristics for a log-rank test of equivalent PFS or OS by regimen within obese patients as a function of HR (control to experimental) are provided in the figure and paragraph below:

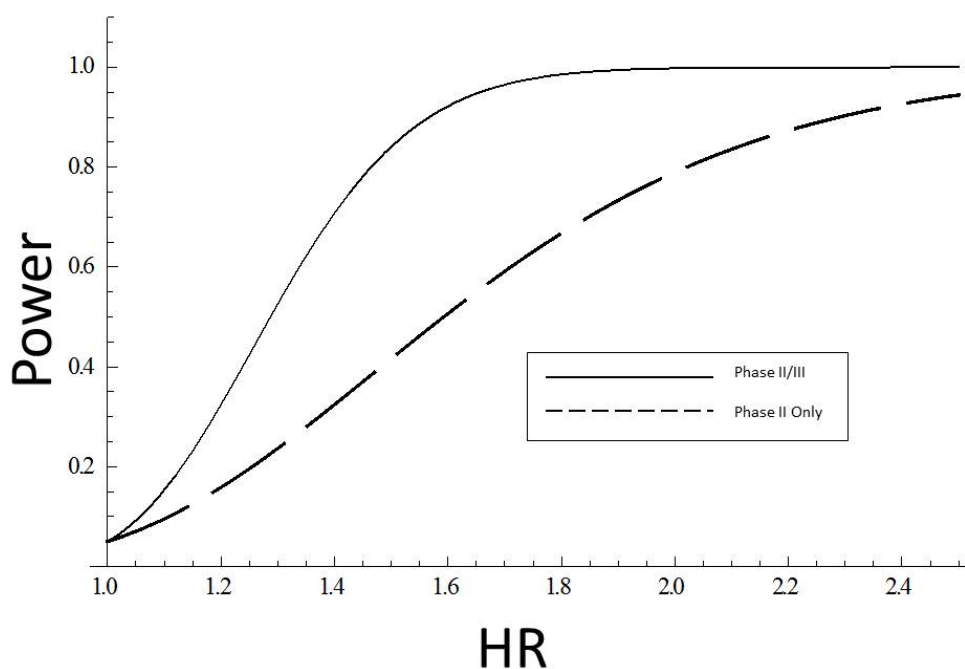


Figure 11.3: Power to detect the activity of metformin in a subset analysis as a function of the hazard ratio (HR) within obese patients assuming that approximately 50% of the population are obese in (A) the phase II study only [long dashed line] with 50 PFS events within this group, and (B) the combination of both phase II and III studies [solid line] with 170 events in this group.

The power to detect metformin activity within the phase II study is 20% and 42% when the HR is 1.25 and 1.5, respectively. The power to detect metformin activity with the studies combined is 42% and 84% when the HR is 1.25 and 1.5, respectively. If metformin is more active in obese patients than non-obese patients, then the power of the subset analysis may be less than indicated here because fewer patients will event in the experimental arm of this subgroup at the time when the analysis is triggered.

11.6 Exploratory and Translational Research (12/05/16)

Integrated Biomarker Analysis (Hypothesis Testing)

There will be two hypotheses tested within the metformin treatment arm (i.e. these will be subset analyses). The first hypothesis will examine whether patients with PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations have a lower hazard of progression or death (PFS endpoint). Therefore patients will be split into two groups: those with mutations and those without, and the clinical outcome will be PFS. The null hypothesis will be that the hazard ratio (HR) of the PFS event for those with mutations to without is 1.0, i.e. $H_0: HR = 1.0$. The alternative will be 1-sided indicating that patients with mutations have a lower risk of the PFS event compared to those without mutation, i.e. $H_a: HR < 1.0$. The null hypothesis will be tested at the 2.5% level of significance.

The number of events at the time of data maturation for the phase II study will be relatively small. By design, we anticipate about 110 PFS events in the entire study, and we expect there to be about 50 to 55 PFS events within the experimental regimen, depending on the relative level of activity of the regimen compared to the reference arm. If the number of patients with any mutation is approximately 50%, then the power of the study will be at a maximum. If patients with mutations have a hazard of 50% for the PFS event compared to those without mutations, then power will be 69%. On the other hand, if 2/3 of patients have mutations, the power of the study will be 63%. A graph of the study's power as a function of the proportion with any mutation is provided below.

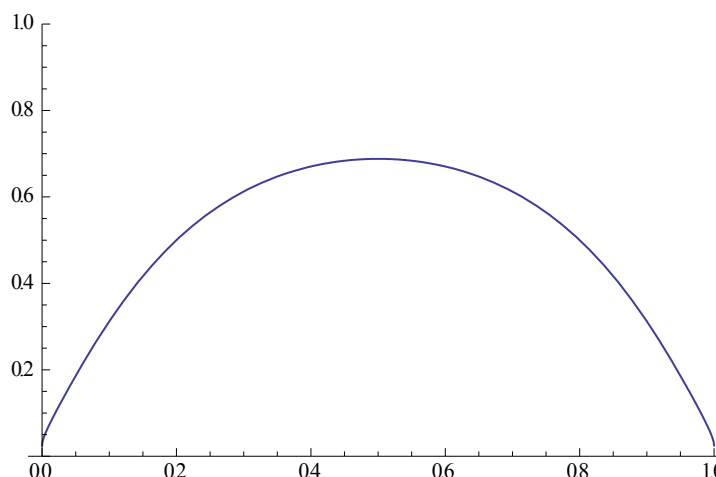


Figure: The asymptotic approximation of the study's power as a function of the proportion of patients who have the mutation of interest when the HR is 50% with 50 events.

If the number of PFS events in one group is less than 10, then a permutation test will be conducted instead of an asymptotic test to assess the null hypothesis.

As can be seen from the graph, the power of the subset analysis will not be very high for the phase II study. If the study stops after the phase II study, it is possible that a subsequent analysis could be done at a later time as part of a long-term

follow-up study.

If the study continues on to the phase III study, then we expect to see many more PFS events. We anticipate on seeing at least 120 PFS events and possibly up to 216 within the experimental regimen. The variability is greater because the primary endpoint will be overall survival for the second study. It is reasonable to assume we will see 170 PFS events within the metformin arm at the conclusion of the data maturation. The power of the study will be 99% in this case, regardless of whether the proportion is 50% or 67%. A graph is provided below to give the reader a better understanding of the relationship in this setting:

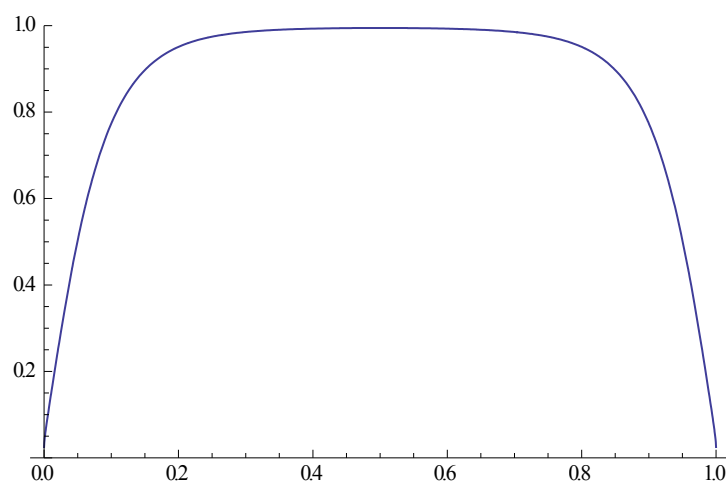


Figure: The asymptotic approximation of the study's power as a function of the proportion of patients who have the mutation of interest when 170 PFS events are observed in the metformin arm when the HR is 50%.

If 67% of the patients have the mutation, then the power of the study will be 2.5%, 28%, 59%, and 88% when the HR is 1.0, 0.80, 0.70, and 0.60, respectively. The graph as a function of the hazard ratio is also provided below:

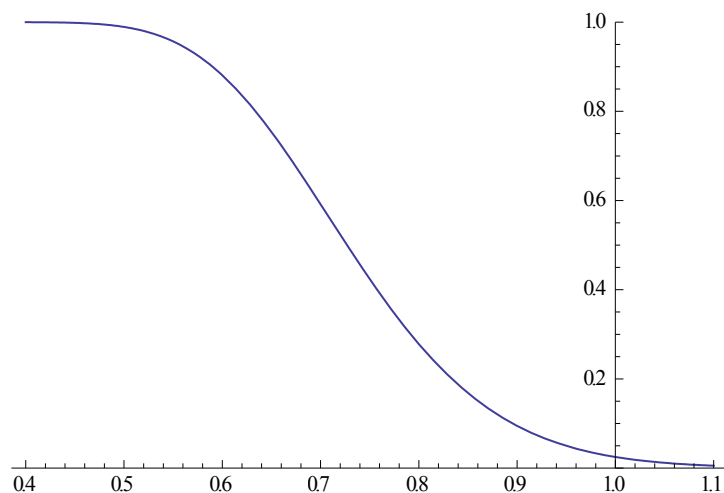
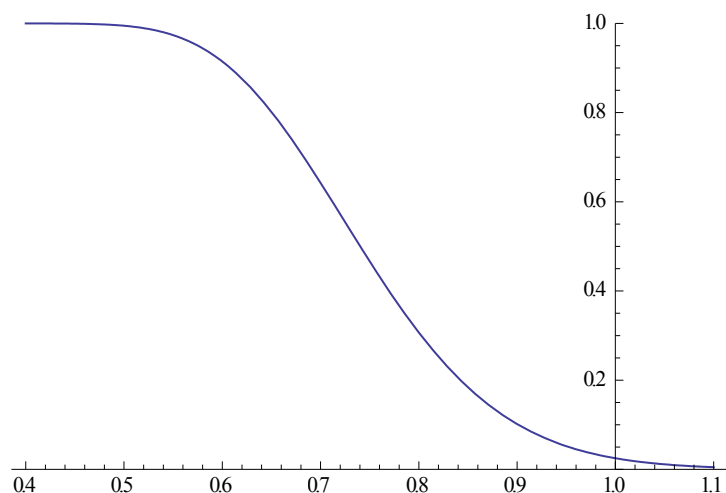


Figure: The asymptotic approximation of the study's power as a function of the HR for patients who have the mutation of interest to those who do not when 170

PFS events are observed in the metformin arm with 67% of patients carrying the mutation.

The second integrated biomarker to be analyzed will be MATE 2 expression. Expression will be examined by IHC with intensity of staining and the percentage of cells staining positive. From these statistics, we will calculate an H-score which is the (intensity score) x (% staining). The distribution of the H-score is expected to be fairly continuous, so we will further dichotomize expression as high expression and low expression at the median to maximize the power of the study. If the median is equal to zero, then the dichotomized variable will be broken into those patients whose scores are 0 against those whose scores are greater than 0. The null hypothesis will be that the HR for those who have high expression to those with low expression is 1.0, i.e. $H_0: HR = 1.0$. The alternative hypothesis will be that the HR for those who have high expression to those with low expression is less than 1.0, i.e. $H_a: HR < 1.0$. The null hypothesis will be tested at the 2.5% level of significance.

The operating characteristics for the testing of this biomarker will be identical to the one listed above. However, since the distribution of the H-score is expected to be fairly continuous, we have a higher chance of seeing 50% with high expression. Below provides a graph of the power when the groups are split 50:50:



If 50% of the patients have high expression, then the power of the study at the end of the phase III part will be 2.5%, 31%, 64%, and 91% when the HR is 1.0, 0.80, 0.70, and 0.60, respectively.

Although not explicitly included as part of the integrated analysis to be a part of the official hypothesis testing procedure, a test for an interaction between treatment regimen and biomarker level (e.g. mutant or high value versus wild type or low value) will be conducted as part of the exploratory data analysis. The relationship between power and the parameter R where $R = \Delta_1 / \Delta_2$ and Δ_1 is the hazard ratio of experimental to control therapy for biomarker level 1 people and Δ_2 is the hazard ratio of experimental to control therapy for biomarker level 2

people, p = the proportion of people who have biomarker level 1, d = the total number of events in the study, and Z_{α} is the standardized critical value for rejecting the null hypothesis that $R = 1$, and α = the probability of a type I error, is given by:

$$1 - \beta = \Phi \left[\pm 0.5 \times \sqrt{p(1-p)d} \ln\{R\} - Z_{\alpha} \right]$$

The operating characteristics will be the same as the test involving the hazard ratios for obese and non-obese subjects when the proportion within each group is 50%. If the proportion is different, then the power will not be as high. For example, if 67% of the patients have biomarker level 1 and 33% have biomarker level 2, then power for the test of interaction will drop from 44% (at $p=0.50$) to 40% (at $p=0.33$ or 0.67) when 110 patients event and $R = 0.50$. The following graph provides the operating characteristics for the phase II and phase II/III studies.

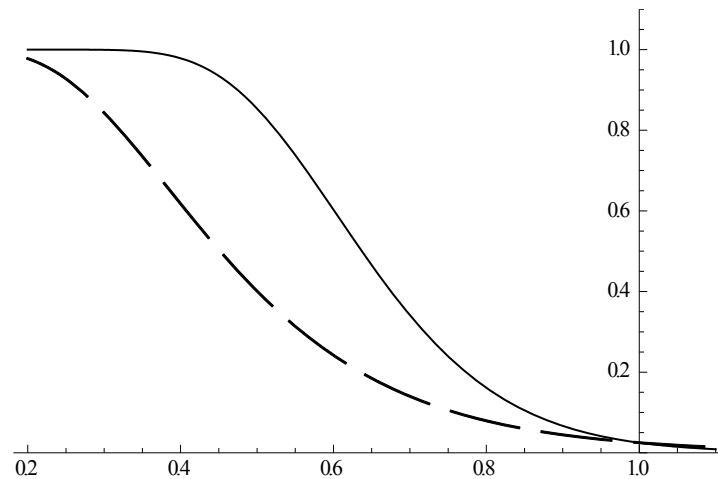


Figure: Power to detect a significant interaction association between biomarker level and metformin therapy as a function of R when testing a one-sided alternative hypothesis at the 2.5% level of significance. The dashed line gives power after observing 110 events (phase II data only); the solid line gives power after observing 342 events (phase II and III data combined) when the proportion with biomarker level 1 is 67%.

Analyses similar to those involving BMI will also be done on related measures of obesity and diabetes. Measurements to be examined include hip-to-waist ratios, HgBA1C values, fasting insulin and glucose levels, and HOMA scores.

Continuous measures will be dichotomized as it is proposed for BMI, so the power of these studies is expected to be similar. In addition, these variables will be analyzed as continuous covariates (or as appropriate with transformations such as the logarithm) with Cox models or logistic regression. If systematic differences exist by institution, consideration will be given for including them as covariates or using random effects models. If serial measurements are available, the data may be analyzed for associations with risks of progression or death as

time dependent covariates in Cox models for PFS or OS.

The field of translational research is one that is rapidly developing technologically and quickly changing as our knowledge of the relationships between various biomarkers and cancer development evolves. Therefore, it is difficult to predict which hypotheses will be of primary interest at this time. In spite of these problems, a general outline of the analytical procedures used to uncover important associations and interactions can still be made.

Two important goals of the study will be to determine whether the biomarkers under consideration have prognostic value or predictive value. A variable is prognostic if varying levels of the factor influences the hazard of death (for example), irrespective of treatment. Performance status and stage of disease are common examples. A variable is predictive of response to treatment if varying levels of the factors differentially influences the hazard of death by the treatment administered. A biomarker would be predictive if, for example, high levels of protein expression were associated with a substantial reduction in the risk of progression within the arm that administered metformin but not in the arm that administered chemotherapy only. Such factors can be identified with analyses that look at biomarker-treatment interactions. The identification of these factors would help guide physicians to the appropriate therapy.

Two mutually exclusive sets of patients will be used to assess the value of the biomarkers as prognostic factors or predictive factors. The first set of patients will serve as a training dataset, which will be used to screen through possibly many biomarkers in order to develop a model (or a small number of models) that is believed to relate the biomarker data to clinical outcomes (such as survival, PFS, and response) in a meaningful way. Commonly used model building techniques involve data examination (with the possible exclusion of observations believed to be outliers resulting from laboratory errors), subjective judgment by the statistician about whether to include certain variables, and collaboration with knowledgeable investigators who can comment on the possible meaning of the results. Although the method is useful for arriving at models that functionally relate biomarker data to clinical outcomes, given the relatively large number of relationships examined, it would be difficult to determine the reliability of the final set of models strictly from this process because the usual limits on type I and type II errors are no longer applicable. Fortunately, there will be enough patients in the trial to test the reliability of these models with the use of validation datasets. Variables that demonstrate statistically significant associations in the validation dataset should increase our level of confidence that relationship detected in the model building phase is real.

Associations between biomarkers and overall survival or progression-free survival will be examined in a Cox proportional hazards model that includes significant prognostic variables based on prior research such as performance status and stage of disease. Since the translational research analysis will not occur before the

reporting of clinical outcomes, the number of events for survival analysis should be adequate to screen biomarkers in the model building phase. For example, an independent, normally distributed biomarker that has a prognostic relationship to the hazard of survival of 1.5 for one standard deviation increase in value is almost certainly going to be detected (assuming 170 events with $\alpha = 0.05$ while testing against a two-sided alternative; power is nearly 1 in this case). If the coefficient of determination with other biomarkers is relatively high, for example equal to 50%, then the marginal probability of detecting the relationship is still greater than 95%. However, if the biomarker is dichotomous with roughly equal proportions of each value, the relationship between the hazard of death and the biomarker must be stronger to assure a good chance of detection. For example, if the hazard ratio associated with the biomarker is only 1.5, then the marginal probability of detection is about 75%. On the other hand, if the hazard ratio is 2, the marginal probability of detection is nearly certain with this event size.

Unfortunately, when many biomarkers are being screened for associations, the chances of erroneously including at least one biomarker into the model is quite high. Assuming independence between biomarkers (not likely in reality but assumed in order to give a flavor of the likelihood of these errors), the probability of including at least one biomarker erroneously into the model is 40% with 10 variables, 64% with 20 variables, and 92% with 50 variables. Compounding the problem are multiple analyses (e.g. analyses of response in addition to survival and PFS), making use of the validation dataset a valuable part of the analysis.

The detection of significant predictive biomarkers is going to be more difficult because it involves an analysis of interactions. Following Peterson and George's definition for interaction, it is possible to look at the probability of detection of a dichotomous biomarker. For example, if the hazard ratio for treatment on metformin to no metformin is 0.5 for patients with biomarker level L_1 , and the hazard ratio is equal to 1.0 for those patients who have biomarker level L_2 , the probability of detecting the interaction is approximately 59% (assuming about 160 events in the training dataset). In order to detect the interaction with 80% power, the hazard ratio in L_1 needs to be 0.41 or 2.43. To make such a detection with 90% power, the hazard ratio in L_1 needs to be 0.36 or 2.79.

The analysis of biomarkers may be conducted as categorical data types, ordinal categorical data, or continuous data. Mutations are most naturally thought of as categorical data for statistical analysis. The IHC intensity scores are inherently ordinal but can be analyzed as categorical. The IHC percent staining positive are continuous, and the histoscore data may be thought of as being continuous. For survival analysis, we intend to carry out the analyses of biomarker data as either categorical data or continuous data. The operating characteristics of the analyses using dichotomized data are expected to behave similarly to the analysis of dichotomized BMI data since we will attempt to split into two levels at the median. The analysis of continuous biomarker data requires more care because it is susceptible to influential cases having an undue effect on the results. This can

be risky with some data if they are skewed or have outlying cases. However, these analyses tend to have more statistical power. See Table 11.2 which demonstrates the difference in power when analyzing a biomarker as a numerical covariate versus a dichotomized covariate (when the covariate is unlikely to yield influential cases). As can be seen from the Table 11.2, the gain in power from analyzing the covariate as a raw numerical score can be anywhere from 6% to about 14% when the parameter is significantly within the alternative parameter space.

Table 11.2. Statistical power of test for association between a categorized or numerical covariate and the hazard of eventing (death, progression, etc.) when 240 patients are enrolled with approximately 20% of patients censored.

Hazard Ratio ¹	Power (categorized) ²	Power (numerical) ²
1.01	5.50	5.26
1.13	28.67	36.86
1.21	52.88	66.65
1.30	78.09	89.08
1.41	92.33	97.89

¹ Hazard ratio is defined by the numerical covariate as the hazard at 1 standard deviation above the median covariate value to the hazard rate at the median covariate value.

² Power estimated with 10,000 simulations. The covariate is either categorized or analyzed as a numerical value.

Table 11.3. Statistical power of test for association between a categorized or numerical covariate and the hazard of eventing (death, progression, etc.) when 120 patients (phase II study) are examined within the subset of interest with approximately 20% of patients censored. This table should approximate the power of a study after the first data freeze for clinical analysis (about 100 events).

Hazard Ratio ¹	Power (categorized) ²	Power (numerical) ²
1.01	5.05	5.03
1.13	16.32	19.71
1.21	29.86	38.69
1.30	48.28	61.19
1.41	65.73	79.68
1.53	81.20	92.74

¹ Hazard ratio is defined by the numerical covariate as the hazard at 1 standard deviation above the median covariate value to the hazard rate at the median covariate value.

² Power estimated with 10,000 simulations. The covariate is either categorized or analyzed as a numerical value.

Table 11.3B. Statistical power of test for association between a categorized or numerical covariate and the hazard of eventing (death, progression, etc.) when 270 patients (phase III study) are examined within the subset of interest with approximately 20% of patients censored. This table should approximate the power of a study after the last data freeze for clinical analysis.

Hazard Ratio ¹	Power (categorized) ²	Power (numerical) ²
1.00	5.28	5.48
1.13	30.45	38.93
1.21	57.85	71.30
1.30	82.55	92.66
1.41	94.89	98.90

¹ Hazard ratio is defined by the numerical covariate as the hazard at 1 standard deviation above the median covariate value to the hazard rate at the median covariate value.

² Power estimated with 10,000 simulations. The covariate is either categorized or analyzed as a numerical value.

A reason why this analysis is not carried out universally has to do, in part, with the

problem of influential cases. Cases with potentially undue influence will need to be examined with deviance or martingale residual diagnostics, changes in the estimated effects (e.g. diff betas), and likelihood displacement measures to assure that particular cases (or set of cases) are not overwhelming the results. If a problem exists, subsequent analyses will be conducted with these cases removed as part of an exploratory analysis (these cases will be explicitly stated as being dropped from the analysis). Furthermore, martingale or deviance residuals can help uncover non-linear relationships between the hazard function and various numeric covariates which can help create transformed covariates that are more sensitive prognostic factors.

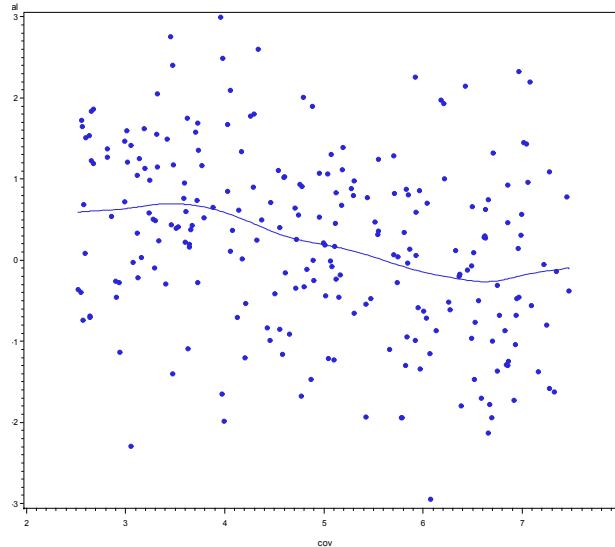


Figure 11.4: Example of a deviance residual plot where the covariate is truly linearly related to the hazard of death (higher values have lower risk).

It is possible to expect patients on the extremes of a biomarker scale to be at a higher or lower risk of death than those who have more normal levels. Analyzing a relationship such as this with either a dichotomized transformed variable or as raw values in a linear model will not likely reveal an association between the covariate and the hazard. In cases like these, plotting the martingale or deviance residual can help investigators uncover more complex relationships. For example as shown in **Fig 11.5** is a theoretical relationship between the hazard function and a biomarker which can be discerned in a sample of 240 patients who are about 20% censored (**Fig 11.6**).

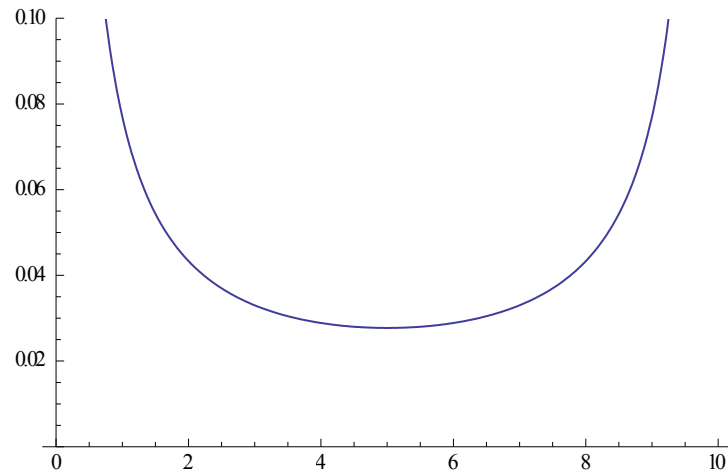


Fig 11.5: Theoretical Hazard Rate as a Function of a Covariate

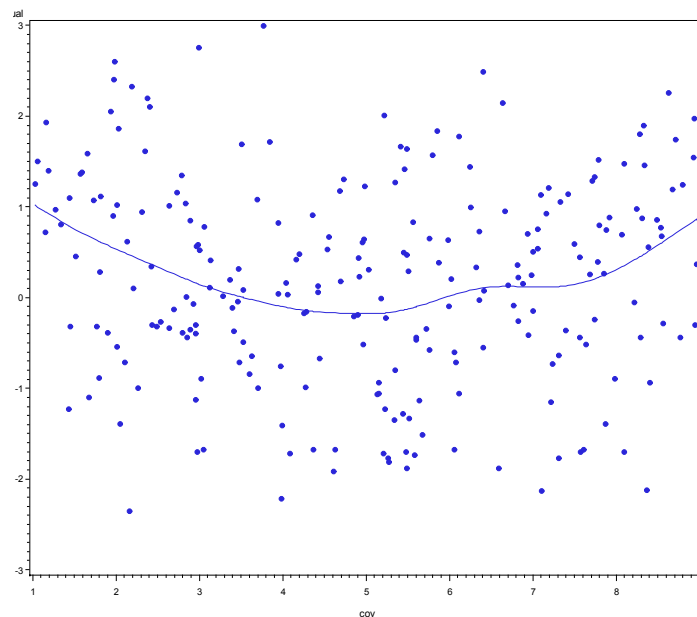


Fig 11.6: Example Deviance Residual Plot with a Smoothed Spline Function
Corresponding to the Theoretical Hazard Function Plotted Above
(240 patients)

If such a relationship was suspected with real data, a preliminary analysis could be conducted with an indicator variable that designated a patient into a higher risk group if the covariate value was less than 3 or greater than 7. This variable could be included into a Cox PH model and would yield a greater likelihood of detecting a true relationship. On the other hand, since the analysis would be exploratory, a follow-up analysis with independent data would be necessary to validate such findings. Assuming the study goes to a phase III trial, a natural point for data splitting would be at the interim between the phase II and phase III components. Alternatively, all of the data could be randomly split at the end of

the study, which has several advantages.

Logistic regression will be used to help assess the value of biomarkers in predicting response to a particular treatment or determine associations with response. Covariates determined by previous research to be likely associated with response will be included in all models. If the biomarker is normally distributed and associated with response such that when it increases by one standard deviation above the mean, the odds of responding increases by 50% (i.e. the odds ratio for those at $\mu + \sigma$ to those at μ is 1.5; $P_0 = 0.30$; $P_a = 0.39$), then there is a marginal probability of detecting this association in 225 evaluable patients is about 80% (with the assumption of a 30% baseline response rate). If the coefficient of determination with other covariates is 50%, the probability of detection is 50%. However, if the odds ratio is 2 ($P_0 = 0.30$; $P_a = 0.46$), the marginal probability of detection is about 92% (even with a 50% coefficient of determination). If the biomarker is dichotomous, the ability to detect an association becomes more difficult. In the case of an independent biomarker where the odds ratio is 2, the probability of detection is approximately 71%. On the other hand, the probability of detection is approximately 92% when the odds ratio is 2.5 ($P_0 = 0.30$; $P_a = 0.52$). If the coefficient of determination is 50%, power drops to 65%. Some additional examples follow:

Table 11.4: Statistical power of test for association between a categorized or numerical covariate and the odds ratio of responding at a high value to a lower value for various sample sizes within the subset of interest using logistic regression or Fisher's Exact Test (FET).

Odds Ratio (categorized) ¹	Odds Ratio (numerical) ²	Power (categorized) ³	Power (numerical) ³	Power (FET) ⁴	Sample Size
1.02	1.01	5.18	5.09	3.51	60
1.78	1.40	18.99	22.39	14.91	60
2.71	1.80	47.46	58.00	40.96	60
3.73	2.20	70.32	81.33	64.29	60
4.81	2.60	83.85	92.35	79.35	60
5.92	3.00	90.96	96.98	88.14	60
1.02	1.01	5.67	4.71	4.79	120
1.78	1.40	35.43	42.56	32.09	120
2.71	1.80	76.72	87.34	73.84	120
3.73	2.20	94.12	98.27	92.79	120
4.81	2.60	98.73	99.84	98.38	120
1.02	1.01	5.98	6.31	5.90	270
1.18	1.10	57.60	69.99	57.21	270
1.27	1.15	88.51	95.37	88.39	270
1.37	1.20	98.28	99.73	98.25	270
1.78	1.40	100.00	100.00	100.00	270

¹ The parametric value of the odds ratio of response for values of a biomarker dichotomized above the median to those below assuming the underlining biomarker is distributed as a uniform with a marginal probability of response equal to 50%.

² Odds ratio is defined by the numerical covariate as the odds at 1 standard deviation above the median covariate value to the odds at the median covariate value where the covariate is uniformly distributed.

³ Power estimated with 10,000 simulations using logistic regression. The covariate is either categorized (dichotomized) or analyzed as a numerical value. The analysis depends on asymptotic results to make inferences.

⁴ Power using Fisher's Exact Test (estimated). This estimate differs from standard power calculations because the simulation used a parametric median to classify biomarkers as high or low values. This procedure does not depend on asymptotic results but has smaller statistical power.

11.7 Study Monitoring (04/28/15)

Safety monitoring: The Safety Review Committee (SRC) reviews accumulating summaries of adverse event reports and all serious adverse event (SAE) reports submitted during the interval between group meetings. This committee also reviews deaths in which treatment may have contributed to the cause. Based upon these reviews, the SRC may recommend study amendments to the DMC pertaining to patient safety.

11.8 Patient-Reported Outcomes (PROs) Research

The PRO research questionnaire consists of three patient-reported outcomes measures:

- The physical functional subscale of the SF-36,
- International physical activity questionnaire (IPAQ) short form,
- FACIT-F subscale.

PRO Measurement time points

Patients will complete the Questionnaires at 4 times:

- 1) Prior to beginning therapy
- 2) 6 weeks after initiating therapy (approximately prior to cycle 3)
- 3) 15 weeks after initiation of treatment (prior to cycle 6)
- 4) 26 weeks following initiation of therapy

Scoring of PRO measures and Minimal Important Differences (MID)

- The physical functioning subscale of the SF-36 is a 10-item scale of the Medical Outcome Study-Short Form. Each item is rated on a 3-point scale of limitation of activity due to the patient's health: 1=limited a lot, 2=limited a little, 3=not limited at all. If at least 50% of items provide valid answers, the physical functioning score will be calculated as follows:
 - 1) First averaging the item responses across all non-missing items,
 - 2) Transform scale to 0-100 using following formula:

$$\frac{\text{individual mean subscale score} - \text{minimum possible score}}{\text{maximum possible score} - \text{minimum possible score}} \times 100$$

A higher score indicates a better physical functioning.

A difference of 7 points is suggested as the minimally important difference (MID) for physical functioning subscale of the SF-36 (Jayadevappa R, Malkowicz SB, Wittink M, Wein AJ, Chhatre S. Comparison of distribution- and anchor-based approaches to infer changes in health-related quality of life

of prostate cancer survivors. *Health Services Research*, 47:5, 1902-1924, (2012)). The GOG has applied physical functioning subscale of the SF-36 to a recently closed randomized phase III trial (GOG #213) which evaluates the effect of the addition of bevacizumab to second-line and maintenance phases of treatment on overall survival in patients with recurrent platinum sensitive ovarian cancer. A total of 522 patients have participated in QOL component of this study. The estimated standard deviation of physical functioning subscale scores at baseline is about 26. Assuming the same standard deviation, the MID of 7 points is corresponding to an effect size (ES) of 0.27 by Cohen's criteria.

- International physical activity questionnaire (IPAQ) short form consists of 4 generic items regarding the types (e.g. vigorous-intensity, moderate-intensity, walk, and sitting) of physical activities and the time people spend per week as part of their everyday lives. The volume of physical activity is summarized with MET-minute, which weights each type of activity with its energy requirements defined in Metabolic Equivalent of Task (MET). The following formula is used to calculate the MET-minute per week:

$$\text{MET-minutes/week} = \text{MET level} \times \text{minutes of activity/day} \times \text{days per week}$$

The MET levels used in IPAQ short form are: Walking= 3.3 METs, Moderate intensity=4.0 METs, and Vigorous intensity = 8.0 METs. The total physical activity MET-minutes/week is calculated as the sum of walking MET-minutes/week, Moderate MET-minutes/week, and Vigorous MET-minutes/week. A larger number of MET-minutes/week indicates larger amount of physical activity per week. Since no MCID has been established for IPAQ scale, a medium effect size of 0.5 would be an interest to detect in this study. We will also analyze the single item measuring minutes of sitting time.

- The FACIT-F Scale is a 13-item questionnaire that assesses self-reported fatigue and its impact upon daily activities and function. It uses a 5-point Likert-type scale (0 = Not at all; 1 = A little bit; 2 = Somewhat; 3 = Quite a bit; and 4 = Very Much). A subscale score will be computed if more than 50% of subscale items were answered. A subscale score S_i with N_i items was calculated as:

$$S_i = N_i \times \frac{\sum (\delta_{ij} \times s_{ij})}{\sum \delta_{ij}}$$

where δ_{ij} is equal to 1 when the j th item has a valid response; otherwise it is equal to 0 and s_{ij} is the score of the j th item. A higher score indicates worse fatigue. A difference of 4 scale points is considered the best estimate of the minimal clinically important difference (MCID) when comparing the FACIT-Fatigue subscale between treatment groups (Yost KJ, Eton DT. Combining distribution- and anchor-based approaches to determine minimally important difference, the

FACIT experience. Evaluation & the health professions, Vol. 28 No. 2, June 2005, 172-191). The FACIT-Fatigue subscale has been applied to a recently closed randomized phase III study (GOG 249) which evaluates the effect of vaginal cuff brachytherapy followed by three cycles of chemotherapy on recurrence or death rate. A total of 546 patients completed baseline QOL assessment. The estimated standard deviation of the FACIT-Fatigue subscale is approximately 11 points. Therefore this MCID of 4 points is corresponding an effect size of 0.36 assuming a standard deviation of 11.

Statistical power and analysis on patient-reported outcomes research

The primary objective on patient-reported outcomes in this study is to estimate treatment differences in physical functioning, physical activity, and fatigue between treatment arms.

Since this study is randomized phase II/III study for primary clinical endpoints, the hypothesis testing for PROs will be conducted only if the phase III is resumed. The overall type I error for testing entire PRO outcomes is set as 5% (two-sided) for phase III study. In order to control the PROs-wide error for three PROs, a type one error of 1.67% will be allocated to each of the PROs.

Based on the GOG experiences on advanced endometrial cancer studies (e.g. GOG 0209), at least 86% eligible patients are evaluable for PRO assessments. A patient is evaluable for PRO assessment if she completes baseline and at least one follow-up assessment. Assuming the same evaluable percentage for this study, we expect at least 232 patients in each group will be evaluable for PRO in phase III trial of this study.

Since the PRO outcomes are measured repeatedly over time, the repeated outcomes are most likely correlated each other. The following table displays the statistical powers to detect MCIDs or ESs if the correlations (e.g. compound symmetry) among the repeated measures are ranged 0.6 to 0.7. The effect size is defined as the ratio of treatment difference to the standard deviation in the control group at baseline. If the phase II trial moves forward to a phase III study, the standard deviations of the physical functioning and FACIT Fatigue scales will be re-estimated using the data collected from phase II trial and statistical power will be adjusted correspondingly.

	MCID	STD	Effect size	correlations	Statistical Power
Physical Functioning	7	26	0.27	0.6 ~0.7	80.5% ~ 84.2%
FACIT-Fatigue	4.0	11	0.36	0.6~0.7	97.4%~98.4%
Physical activity			0.50	0.6~0.7	99.9%~99.9%

A linear mixed model will be applied to assess the treatment difference of each PRO scores and its interaction with assessment points. The baseline PRO score will be included in the model as a covariate along with the patient's age, and performance status at baseline. Patients will be categorized by their randomized treatment group rather than the treatment received. Patients who complete

baseline and at least one follow-up PRO assessment will be included in the PRO analysis. The interaction between treatment assignment and assessment time will be assessed first. If there is statistically significant evidence that the relative treatment effects vary over assessment time, then treatment comparisons will be performed for each assessment time points.

Missing information

Patient death, noncompliance, missed appointments, and patient illiteracy, can cause missing information. One or more of the QoL assessments may be missing for an individual on any occasion. Missing information is troublesome particularly in studies involving repeated patient assessments. Data management procedures will be used to reduce missing data. To this end, a calendar of events which lists the dates for the required QoL assessments for each patient will be made available to the patient's health care provider as soon as the patient has been registered onto this study. Also, the clinic staff will use the GOG web-based forms tracking system to obtain reminders of the upcoming QoL assessments.

At semi-annual group meetings the data managers and nurses will be given presentations, which describe the goals of this study and stress the importance of obtaining complete assessments. A study contact person will be designated to answer questions that arise throughout the study.

Women, who are unable to read or have difficulty reading, will not be required to participate in the QoL part of this study. Also, any woman, who does not wish to participate in the QoL portion of this study, can refuse and remain eligible for the therapeutic portion of the study.

11.9 Planned gender, minority and ethnic inclusion:

The following are the race and ethnicity distributions anticipated for this trial based upon historical data. All patients in this study will be female by definition of disease.

Ethnic Category	Number of patients anticipated
Hispanic or Latino	22 (4.1%)
Not Hispanic or Latino	518 (95.9%)
Total	540 (100.0%)
Racial Category	Number of patients anticipated
American Indian/Alaskan Native	4 (0.7%)
Asian	10 (1.9%)

Native Hawaiian or Other Pacific Islander	0 (0.0%)
Black or African American	67 (12.4%)
White	459 (85.0%)
Total	540 (100.0%)

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APPENDIX I - Clinical Staging (FIGO)

CARCINOMA OF THE ENDOMETRIUM
FIGO CLASSIFICATION
2009

Stage I*	Tumor confined to the corpus uteri.
IA*	No or less than half myometrial invasion
IB*	Invasion equal to or more than half of the myometrium
Stage II*	Tumor invades cervical stroma, but does not extend beyond the uterus**
Stage III*	Local and/or regional spread of the tumor
IIIA*	Tumor invades the serosa of the corpus uteri and/or adnexae [#]
IIIB*	Vaginal and/or parametrial involvement [#]
IIIC*	Metastases to pelvic and/or para-aortic lymph nodes [#]
IIIC1*	Positive pelvic nodes
IIIC2*	Positive para-aortic lymph nodes with or without positive pelvic lymph nodes
Stage IV*	Tumor invades bladder and/or bowel mucosa, and/or distant metastases
IVA*	Tumor invasion of bladder and/or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

*Either G1, G2, or G3.

**Endocervical glandular involvement only should be considered as Stage I and no longer as Stage II.

[#]Positive cytology has to be reported separately without changing the stage.

APPENDIX II - Patient Medication Calendar (05/19/14)(04/28/15)

PILL DIARY

INSTRUCTIONS FOR THE PATIENT:

This is a calendar on which you are to record the total number of pills (study medication) you take each day and the time of day you take your pills (study medication). You will take metformin (850 mg) or placebo once a day for 4 weeks and then twice a day for the remainder of the study.

Bring the bottle(s) with any unused pills and your calendar with you each time you have an appointment.

If you have any questions, please contact: _____

Telephone: _____

Your next appointment is: _____

#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>	#of pills taken _____ Time: a.m. <input type="checkbox"/> Time p. m. <input type="checkbox"/>
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This section is to be completed by the physician, nurse or staff:

Reporting period (mm/dd/yy): Start _____ Stop _____ Total # of pills taken: _____ # pills left in bottle: _____

Patient's Signature: _____ Date: _____

APPENDIX III - NRG Oncology General Therapy Guidelines (12/05/16)

- For 21 or 28 day cycles, a patient will be permitted to have a new cycle of therapy delayed up to 7 days (without this being considered to be a protocol violation) for major life events (e.g., serious illness in a family member, major holiday, vacation which is unable to be re-scheduled). Documentation to justify this decision should be provided.
- It will be acceptable for individual doses to be delivered within a “24-hour window before and after the protocol-defined date” for “Day 1” treatment of 21 or 28 day cycles. If the treatment due date is a Friday, and the patient cannot be treated on that Friday, then the window for treatment would include the Thursday (1 day earlier than due) through the Monday (day 3 past due).
- For weekly regimens, it will be acceptable for individual doses to be delivered within a “24-hour window,” for example; “Day 8 therapy” can be delivered on Day 7, Day 8, or Day 9 and “Day 15 therapy” can be given on Day 14, Day 15, or Day 16.
- Doses can be “rounded” according to institutional standards without being considered a protocol violation (most institutions use a rule of approximately +/- 5% of the calculated dose).
- Doses are required to be recalculated if the patient has a weight change of greater than or equal to 10%. Patients are permitted to have chemotherapy doses recalculated for less than 10% weight changes.

APPENDIX IV - CARBOPLATIN DOSE CALCULATION INSTRUCTIONS

- 1) The Cockcroft-Gault formula will be used in GOG trials.
- 2) Conversion of IDMS creatinine levels to “non-IDMS” values will not be permitted.
- 3) The carboplatin calculation tool is available on the GOG website (Web Menu, Tools).

Dosing of Carboplatin:

- 1) The carboplatin dose will be calculated to reach a target area under the curve (AUC) according to the Calvert formula using an estimated glomerular filtration rate (GFR) from the Cockcroft-Gault formula.
- 2) The initial dose of carboplatin must be calculated using GFR. In the absence of renal toxicity greater than or equal to CTCAE Grade 2 (serum creatinine >1.5 x ULN) or toxicity requiring dose modification, the dose of carboplatin **will not** need to be recalculated for subsequent cycles, but will be subject to dose modification for toxicity as noted in the protocol.
- 3) Carboplatin doses are required to be recalculated if the patient has a weight change of greater than or equal to 10%. Patients are permitted to have chemotherapy doses recalculated for < 10% weight changes.
- 4) At the time of dose modification, if the patient’s age had changed (the patient has had a birthday), the site can use the current age.
- 5) In patients with an abnormally low serum creatinine (less than 0.7 mg/dl), the creatinine clearance should be estimated using **a minimum value of 0.7 mg/dl**. For trials where patients enter and are treated within less than or equal to 12 weeks of surgery: If a more appropriate (higher) baseline creatinine value is available from the pre-operative period (within 4 weeks of surgery date), that value may also be used for the initial estimation of GFR.

CALVERT FORMULA:

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

NOTE: the GFR used in the Calvert formula should not exceed 125 ml/min.

Maximum carboplatin dose (mg) = target AUC (mg/ml x min) x 150 ml/min.

The maximum allowed doses of carboplatin are:

AUC 6 = 900 mg

AUC 5 = 750 mg

AUC 4 = 600 mg

For the purposes of this protocol, the GFR is considered to be equivalent to the estimated creatinine clearance. The estimated creatinine clearance (ml/min) is calculated by the method of Cockcroft-Gault using the following formula:

$$\text{Creatinine Clearance (mL/min)} = \frac{[140 - \text{Age (years)}] \times \text{Weight (kg)} \times 0.85}{72 \times \text{serum creatinine (mg/dl)}}$$

Notes:

- 1) Weight in kilograms (kg):
 - a. Body Mass Index (BMI) should be calculated for each patient. A BMI calculator is available at the following link: <http://www.nhlbisupport.com/bmi/>
 - b. Actual weight should be used for estimation of GFR for patients with BMI of less than 25.
 - c. **Adjusted** weight should be used for estimation of GFR for patients with **BMI of greater than or equal to 25**.
 - d. Adjusted weight calculation:
 Ideal weight (kg) = (((Height (cm)/2.54) – 60) x 2.3) + 45.5
Adjusted weight (kg) = ((Actual weight – Ideal weight) x 0.40) + Ideal weight
- 2) The Cockcroft-Gault formula above is specifically for women (it includes the 0.85 factor).

At the time of a dose modification for toxicity: If the creatinine at the time of a dose modification is lower than the creatinine used to calculate the previous dose, use the previous (higher) creatinine; if the creatinine at the time of a dose modification is higher than the creatinine used to calculate the previous dose, use the current (higher) creatinine. This will ensure that the patient is actually receiving a dose reduction.

APPENDIX V – Translational Research Specimen Procedures (12/05/16)(XX/XX/XX)

I. Summary of Specimen Requirements

If the patient gives permission for her specimens to be collected and used for this optional translational research component, then participating institutions are required to submit the patient's specimens as outlined below (unless otherwise specified).

Required Specimen (Specimen Code)	Collection Time Point	Sites Ship Specimens To
FFPE Primary Tumor (FP01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)	Prior to all treatment	GOG Tissue Bank within 8 weeks of registration ¹
FFPE Metastatic Tumor (FM01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)		
FFPE Recurrent Primary Tumor (FRP01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)	Prior to study treatment	
FFPE Recurrent Metastatic Tumor (FRM01)* 1 st Choice: block 2 nd Choice: 25 unstained slides (15 charged, 5µm & 10 uncharged, 10µm)		
Pre-Treatment Whole Blood (WB01) 7-10mL drawn into purple top (EDTA) tube(s)	Prior to study treatment	

* A copy of the corresponding pathology report must be shipped with all tissue specimens sent to the GOG Tissue Bank

1 GOG Tissue Bank / Protocol GOG-0286B, Nationwide Children's Hospital, 700 Children's Drive, WA1340, Columbus, OH 43205, Phone: (614) 722-2865, FAX: (614) 722-2897, Email: BPCBank@nationwidechildrens.org

II. Obtaining a GOG Bank ID for Translational Research Specimens

Only one GOG Bank ID (#### - ## - G ###) is assigned per patient. All translational research specimens and accompanying paperwork must be labeled with this coded patient number.

A Bank ID is automatically assigned once the Specimen Consent is completed and indicates that a patient has agreed to participate in the translational science component. If a patient has previously been assigned a Bank ID, please ensure the Bank ID appearing in Rave is the same as the previously assigned Bank ID.

Please contact GOG User Support if you need assistance or have assigned more than one Bank ID to a patient (Email: support@gogstats.org; Phone: 716-845-7767).

III. Requesting Translational Research Specimen Kits

Kits are not provided for this protocol.

IV. Labeling Translational Research Specimens

A waterproof permanent marker or printed label should be used to label each translational research specimen with:

GOG Bank ID (#### - ## - G ###)
GOG protocol number (GOG- ####)
specimen code (see section I)
collection date (mm/dd/yyyy)
surgical pathology accession number (tissue specimens only)
block number (tissue specimens only)

Note: If labeling slides, only label on the top, front portion of the slide. Do not place a label on the back of the slide or over the tissue. The label must fit on the slide and should not be wrapped around the slide or hang over the edge.

V. Submitting Formalin-Fixed, Paraffin-Embedded Tissue

Formalin-fixed, paraffin embedded (FFPE) tissue should be the most representative of the tumor type (primary, metastatic, recurrent).

Every attempt should be made to provide a FFPE block; however, if a block cannot be provided on a permanent basis, then 25 unstained consecutive slides (15 charged, 5 μ m + 10 uncharged, 10 μ m) should be submitted. All tissue sections should be cut sequentially from one same block.

Note: If stained slides (required to confirm patient eligibility by central pathology review) will be cut from the same block that will be submitted for translational research, your pathology department should cut the slides for staining prior to submitting the block for translational research.

The type of specimen (block or slides) should be specified on Form TR. If submitting slides, the slide type, thickness, and count should also be specified.

All FFPE tissue should be submitted with the corresponding pathology report.

VI. Submitting Whole Blood

1. Label the lavender/purple top (EDTA) collection tube(s) as described above. Multiple tubes may be used to collect the required amount.
2. Draw 7-10mL of blood into the labeled lavender/purple top tube(s). A minimum of 3mL is needed for processing.
3. Immediately after collection, gently invert the tube 5-10 times to mix the blood and EDTA.

4. Whole blood specimens should be refrigerated (4°C) until the specimens can be shipped. Ship whole blood to the GOG Tissue Bank the day the specimen is collected (i.e., Monday through Friday, but not the day before a holiday). If the whole blood absolutely cannot be shipped the day it is collected, the tube(s) should be refrigerated (4°C) until the specimen can be shipped.

VII. Submitting Form TR

A specimen transmittal form (i.e., Form TR) for each biospecimen will be available in the **Translational Research Folder in Rave**, once the Specimen Consent (located in the Baseline Folder) has been completed.

An electronically (i.e., Rave) completed copy of Form TR must accompany each specimen shipped to the GOG Tissue Bank. Handwritten forms will not be accepted.

Note: A copy does not need to be sent to the GOG Tissue Bank if specimens are not collected.

Form TR should be printed from the Translational Research Form screen in Rave using the **“PDF File” link at the top of the form**. Do not use the “Printable Version” or “View PDF” links at the bottom of the form or any other method to print the form, as these formats will not be accepted.

Retain a printout of the completed form for your records.

Please contact User Support if you need assistance (Email: support@gogstats.org; Phone: 716-845-7767).

VIII. Shipping Translational Research Specimens

A completed copy of Form TR must be included for each translational research specimen.

A. FFPE Tissue

FFPE tissue and a copy of the corresponding pathology report should be shipped using your own container at your own expense to:

GOG Tissue Bank / Protocol GOG-0286B
Nationwide Children’s Hospital
700 Children’s Dr, WA1340
Columbus, OH 43205
Phone: (614) 722-2865
FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

Do not ship FFPE tissue for Saturday delivery.

B. Whole Blood

All whole blood specimens should be shipped to the GOG Tissue Bank (address above).

Whole blood specimens can be shipped to the GOG Tissue Bank Monday through Friday for Tuesday through Saturday delivery. Please do not ship whole blood the day before a holiday. Use your own shipping container to ship specimens via FedEx priority overnight.

When shipping whole blood specimens, **please be aware that your institution must comply with IATA standards** (www.iata.org). If you have questions regarding your shipment, contact the GOG Tissue Bank at BPCBank@nationwidechildrens.org or by phoning 866-GOG-BANC (866-464-2262).

To ship whole blood specimens you will need (1) a sturdy shipping container (e.g., a cardboard or styrofoam box), (2) a leak proof biohazard envelope with absorbent material*, (3) a puncture and pressure resistant envelope (e.g. Tyvek envelope), (4) an Exempt Human Specimen sticker, and (5) a pre-paid FedEx air bill.

**If you will be shipping whole blood specimens from more than one patient, please put each specimen in a separate plastic zip-lock bag before placing the specimens in the shipping bag. You may include up to four different blood specimens in one biohazard envelope.*

If you do not have these materials available at your institution, you may order them from any supplier (e.g., Saf-T-Pak; Phone: 800-814-7484; Website: www.saftpak.com).

Instructions for Shipping Whole Blood Using Your Own Shipping Container

1. Place the whole blood specimen in a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the bag.
2. Wrap the biohazard envelope in bubble wrap or another padded material.
3. Place the padded tube(s) into a Tyvek envelope. Expel as much air as possible before sealing the envelope.
4. Place the Tyvek envelope in a sturdy shipping container (e.g., cardboard FedEx box).
5. Insert a copy of the TR Form(s) into the box.
6. Attach an Exempt Human Specimen sticker to the outside of the shipping container.

7. Print a pre-paid FedEx air bill using the Kit Management system (<https://ricapps.nationwidechildrens.org/KitManagement/>). Attach the air bill.
8. Make arrangements for FedEx pick-up through your usual institutional procedure or by calling 800-238-5355.

IX. Distributing Translational Research Specimens

The GOG Statistical and Data Center and Tissue Bank will coordinate the distribution of translational research specimens to approved investigators.

Investigators will not be given access to any personal identifiers.

Investigators will be responsible for the direct supervision and oversight of translational research and for keeping accurate records.

Investigators will ensure the results are linked to the appropriate translational research specimen-specific identifiers and are responsible for transferring relevant laboratory data to the GOG Statistical and Data Center.

At the discretion of the Chair of the Committee on Experimental Medicine and the Director of the GOG Tissue Bank, investigators may be required to ship any specimens (or by-products) remaining after the completion of the translational research to the GOG Tissue Bank.

A. FFPE

FFPE will be batch shipped to:

Dr. David Neil Hayes
 UNC Lineberger Comprehensive Cancer Center
 School of Medicine CB#7295
 University of North Carolina at Chapel Hill
 450 West Drive
 Chapel Hill, NC 27599-7295
 Phone: 919-966-3786
 Fax: 919-966-1587
 Email: hayes@med.unc.edu

B. Whole Blood

The GOG Tissue Bank will extract DNA from whole blood. DNA will be batch shipped to Dr. David Neil Hayes (address above).

X. Banking Translational Research Specimens for Future Research

Specimens will remain banked in the GOG Tissue Bank and made available for approved research projects if the patient has provided permission for the use of her specimens for future health research. The patient's choices will be recorded on the signed informed consent document and electronically via the Specimen Consent. At the time of specimen selection for project distribution, the most recent consent information will be used.

Institutions can amend a patient's choices regarding the future use of her specimens at any time if the patient changes her mind.

If the patient revokes permission to use her specimens, the GOG Tissue Bank will destroy or return any remaining specimens. The patient's specimens will not be used for any further research; however, any specimens distributed for research prior to revoking consent cannot be returned or destroyed. In addition, the patient cannot be removed from any research that has been done with her specimens distributed prior to revoking consent.

Note: If return of specimens is requested, shipping will be at the institution's expense.

APPENDIX VI – CT Scan Calculator

Patient Number:	
Initials:	
Date of First Dose:	
Week 9	
Week 18	
Week 27	
Week 36	
Week 45	
Week 54	
Week 63	
Week 72	
Week 81	
Week 90	
Week 99	
Week 111	
Week 123	
Week 135	
Week 147	
Week 159	
Week 171	

Instructions:

Enter Patient Number in Cell B1

Enter Patient Initials in Cell B2

Enter Date of Cycle 1, Day 1 (first dose) in Cell B3

Projected CT Scan dates will appear in cells B4 through B20. Please use these dates to schedule CT scans for this patient. You may print this sheet for your reference.

APPENDIX VII – Translational Research Laboratory Testing Procedures (12/05/16)

A. OVERVIEW: Endometrial cancer (EC) is the most common cancer of the female reproductive organs. More than 50,000 new cases are diagnosed annually in the USA, and more than 8,000 women die in the same period. Despite available chemotherapy, overall 5-year survival for advanced EC remains poor at 21-56%. Thus, there is a great need for novel agents to improve EC outcomes.

Obesity, diabetes and insulin resistance are associated with increased risk and worse outcomes for EC¹⁻³. Metformin is a widely used first line treatment for type 2 diabetes. Epidemiological evidence⁴⁻⁶ and our own retrospective studies in EC patients⁷ suggest that metformin use lowers cancer risk and reduces cancer deaths among diabetic patients. Although controversial, metformin is thought to exert anti-tumorigenic activity through indirect effects on the metabolic milieu (\downarrow insulin, \downarrow glucose) and direct effects on the tumor *through* inhibition of mitochondrial complex 1 and subsequent AMPK activation/mTOR inhibition *via* cation-selective transporters (**Fig 1**)⁸⁻¹¹.

We found differential patterns of gene expression in endometrioid ECs arising in obese women compared with non-obese women in The Cancer Genome Atlas (TCGA) database, including upregulation of modulators of the insulin/insulin growth factor-1 (IGF-1) pathway, suggesting that ECs arising in obesity may have distinct metabolic targets for treatment. In addition, we completed a phase 0 clinical trial in obese EC patients and found a favorable response to short-term metformin treatment¹². Treatment with metformin resulted in decreased expression of the IGF-1 receptor and targets of the mTOR pathway. Metabolomic profiling revealed that responders to metformin treatment had higher pre-treatment serum levels of several fatty acids and glycolipids, suggesting that these biomarkers could predict increased benefit to metformin therapy. Lastly, in the LKB1^{fl/fl}p53^{fl/fl} genetically engineered mouse model of endometrioid EC, diet-induced obesity (DIO) resulted in a doubling of tumor size, and metformin had increased efficacy against EC in obese *versus* lean mice.

Based on this preliminary work, we will assess the contribution of indirect effects (via downregulation of insulin/IGF-1 signaling) and direct effects (via transporter-dependent cell entry and modulation of mitochondrial complex 1/AMPK/mTOR signaling) of metformin to its overall anti-cancer efficacy in GOG-0286B, an ongoing two arm, randomized phase 2/3 clinical trial of metformin *versus* placebo, in combination with paclitaxel/carboplatin, for the treatment of EC [through the NRG Oncology Group]. Since obesity is linked to upregulation of insulin/IGF-1 signaling, and metformin is believed to elicit its anti-cancer effects, in part, by lowering plasma insulin/glucose, *we hypothesize that metformin (in combination with paclitaxel/carboplatin) will be more efficacious in obese women versus their non-obese counterparts. In addition to obesity, expression of transporters and hyperactivation of insulin/IGF-1/mTOR signaling in the tumor tissue will predict response to metformin.*

SIGNIFICANCE AND PRELIMINARY DATA: EC is the fourth most common cancer

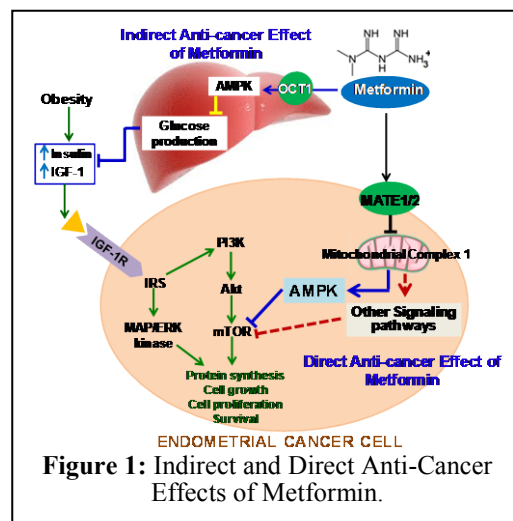


Figure 1: Indirect and Direct Anti-Cancer Effects of Metformin.

among women in the United States. EC frequency has been increasing, secondary to an aging female population and changes in dietary and hormonal factors, with obesity as a major culprit. In 2015, approximately 54,870 new cases will be diagnosed, and 10,170 women will succumb to this disease¹³. Obesity, diabetes and insulin resistance are well-known factors associated with both increased risk of developing EC¹⁴⁻¹⁶ and increased risk of death^{1-3,17}. *Obese women with EC have a 6.25-fold increased risk of death from this disease, as compared to their non-obese counterparts*².

It has been postulated that hyperglycemia and hyperinsulinemia in obese patients provide abundant nutrients and growth factors to cancer cells, resulting in an ideal environment for tumor signaling cascades, such as the insulin/insulin-like growth factor-1 (IGF-1) and PI3K/Akt/mTOR pathways¹⁸. Hyperinsulinemia, IGF-1 and IGF-1 receptor (IGF1R) levels are important in EC development and progression^{19,20}. Signaling through IGF-1R leads to activation of the PI3K/Akt/mTOR pathway, and components of this pathway are often mutated, amplified or aberrantly expressed in EC²¹⁻²⁴. Activation of the PI3K/Akt/mTOR pathway, through PIK3CA amplifications, PIK3CA/PIK3R1/PIK3R2 mutations and phosphatase and tensin homolog (PTEN) mutations/loss of function, is common in EC and has been linked to more aggressive tumor behavior²²⁻²⁵.

Taken together, obesity is a high-energy, pro-inflammatory condition that culminates in increased growth factor signaling *via* the insulin/IGF-1 axis, as well as a nutrient-saturated environment *via* increased glucose (and other nutrients), ultimately resulting in excessive stimulation of the PI3K/Akt/mTOR pathway²⁶⁻²⁹. In experimental animal models, diet-induced obesity leads to activation of Akt and mTOR in epithelial tissues^{30,31}. Conversely, calorie restriction represses signaling through this pathway^{30,31}. *Therefore, obesity may create a unique environment that can be exploited by a therapeutic approach as a strategy to improve EC outcomes.*

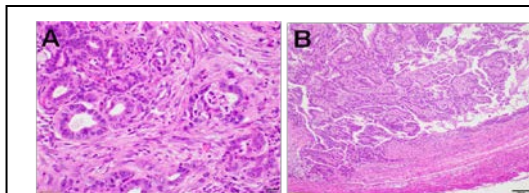


Figure 2. (A) Endometrioid ECs develop in the $LKB1^{fl/fl}p53^{fl/fl}$ mouse model, 12 weeks after AdeCre injection, as evidenced by anisocytosis, anisokaryosis, stromal invasion/fusion of glands, cellular atypia and prominent nucleoli. (B) Invasion of endometrial tumor into the myometrium.

Obesity drives significant EC progression in $LKB1^{fl/fl}p53^{fl/fl}$ mice. We have assessed the impact of obesity on EC development in a unique endometrioid EC mouse model ($LKB1^{fl/fl}p53^{fl/fl}$). The $LKB1^{fl/fl}p53^{fl/fl}$ mouse has specific and somatic deletions of the tumor suppressor genes, *LKB1* and *TP53*, in adult endometrial epithelial cells. Knockout of *LKB1* and *p53* are conditional and only activated *via* injection of an adenoviral vector expressing Cre (AdCre) into the uterine horn of adult female mice. Within 12 weeks (wks) post induction, tumors develop in the affected uterine horn, while the un-injected uterine horn remains normal (**Fig 2**).

$LKB1^{fl/fl}p53^{fl/fl}$ mice were fed a control low fat diet (LFD; 10% calories from fat) *versus* a high fat diet (HFD; 60% calories derived from fat) to mimic diet-induced obesity, starting at 3 wks of age. AdCre was injected at 6 wks to induce invasive EC. There was a significant difference in body weight between the two groups (obese mice: 32.8 g \pm 8.6, lean

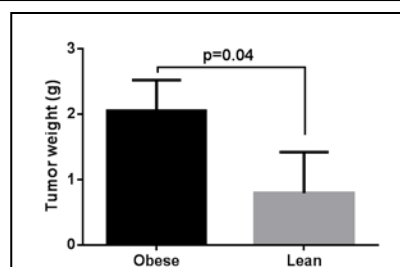


Figure 3. Obesity increases endometrial tumor size in $LKB1^{fl/fl}p53^{fl/fl}$ mice. Mice were fed low fat or high fat diets to induce obesity for 15 wks during tumorigenesis (N=10 mice/group). Comparison of tumor size from lean and obese mice (*p=0.040).

mice: $24.0 \text{ g} \pm 2.8$; $p=0.033$). After 15 wks of exposure to HFD/LFD, tumors in HFD-fed (obese-ECs) mice were more than double the size of those in LFD-fed (lean-ECs) mice (mean tumor size 2.1 cm^2 versus 0.79 cm^2 , $p=0.040$) (**Fig 2**). Thus, the obese state promotes tumor aggressiveness in the $LKB1^{fl/fl}p53^{fl/fl}$ mouse model of endometrioid EC.

Metabolically relevant alterations in gene expression are associated with BMI in human endometrioid ECs.

To assess genomic differences between human endometrioid ECs and BMI, we took advantage of the publically available gene expression analysis from The Cancer Genome Atlas (TCGA) database. 181 genes were found to be significantly up- or down-regulated with BMI status ($\text{BMI} < 30$ versus $\text{BMI} \geq 30$) among the endometrioid ECs ($q\text{-value} < 0.1$), including metabolically relevant genes related to lipid metabolism and the insulin/IGF-1 signaling pathway (**Fig 4**), including up-regulation of lipoprotein lipase, insulin receptor substrate-1 (IRS-1), insulin-like growth factor binding protein 7 (IGFBP7) and insulin-like growth factor binding protein 4 (IGFBP4). This suggests that the metabolic consequences of obesity may be crucial in

EC pathogenesis, resulting in biologically distinct cancers than those that arise in a non-obese environment.

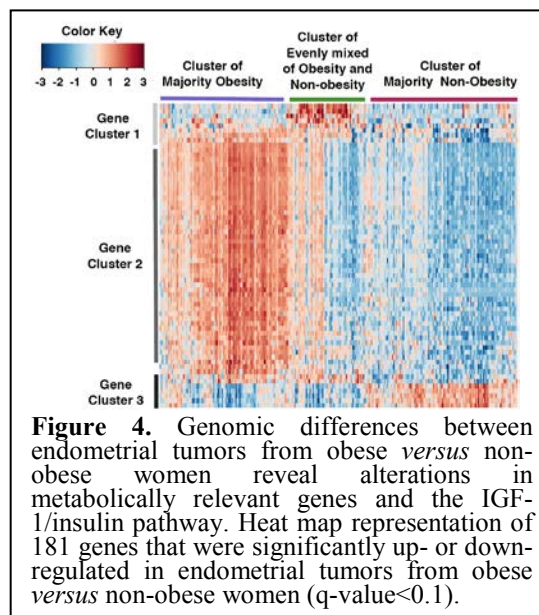


Figure 4. Genomic differences between endometrial tumors from obese versus non-obese women reveal alterations in metabolically relevant genes and the IGF-1/insulin pathway. Heat map representation of 181 genes that were significantly up- or down-regulated in endometrial tumors from obese versus non-obese women ($q\text{-value} < 0.1$).

Proposed Mechanisms of the Anti-tumorigenic Effects of Metformin.

Approved in the US in 1994, metformin is an effective, well-tolerated and inexpensive medication for normalizing hyperglycemia in the treatment of type 2 diabetes³². Metformin is also used to treat menstrual dysfunction and infertility in women with polycystic ovarian syndrome³³, and prevents the development of diabetes in patients with obesity and metabolic syndrome^{34,35}. Epidemiological evidence, including our own retrospective analysis of metformin and EC outcomes⁷, suggests that metformin lowers cancer risk and reduces cancer incidence and deaths among diabetic patients⁴⁻⁶. This has led to the hypothesis that metformin has a role in cancer treatment and prevention.

Metformin may have both indirect and direct effects on tumor growth (**Fig 1**)⁹. Its indirect effects are postulated to be due to a reduction in circulating glucose and insulin levels in the host *via* inhibition of gluconeogenesis in the liver, and subsequent decreased growth factor stimulation in tumor cells. On the cellular - or direct - level, metformin inhibits mitochondrial respiratory

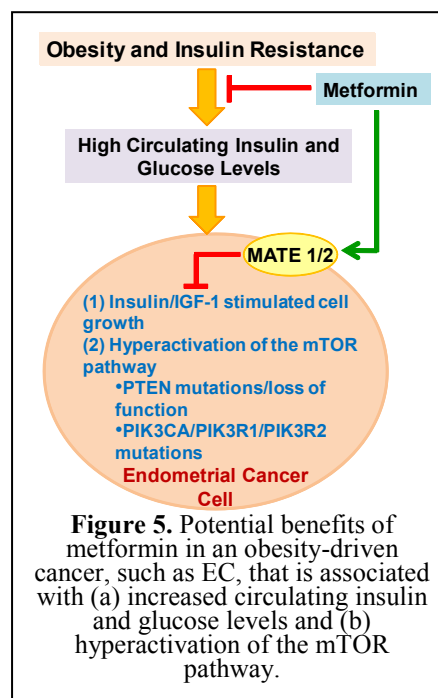


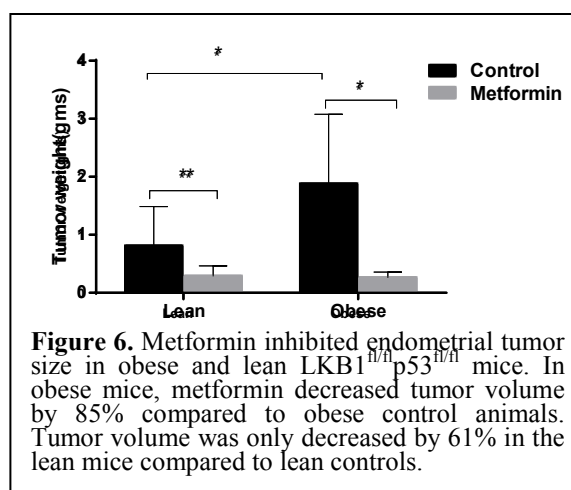
Figure 5. Potential benefits of metformin in an obesity-driven cancer, such as EC, that is associated with (a) increased circulating insulin and glucose levels and (b) hyperactivation of the mTOR pathway.

complex 1, leading to suppression of tricarboxylic acid (TCA) cycle flux, interrupted oxidative phosphorylation and decreased mitochondrial ATP production⁸⁻¹¹. Tumors engineered to express a surrogate for complex 1 that is refractory to metformin were found to be resistant to metformin *in vivo*¹¹, supporting that metformin's effects on mitochondrial metabolism are critical to direct inhibition of tumor growth. The resulting cellular energetic stress from inhibition of complex 1 raises the AMP/ATP ratio, resulting in increased AMPK signaling and stimulated glycolysis and fatty acid oxidation. AMPK is a central regulator of multiple signaling pathways that control cellular proliferation and metabolism, including inhibition of the mTOR pathway (i.e. specifically mTORC1 inhibition)⁹. In addition, metformin has also been found to inhibit the mTOR pathway *via* AMPK-independent mechanisms, potentially through its effects on the Ragulator complex (Rag GTPase) and REDD1 upregulation or *via* enhanced PRAS40 binding to RAPTOR^{9,36-39}. Thus, it is logical that a drug such as metformin, that indirectly decreases circulating glucose and insulin levels and specifically disrupts the insulin/IGF-1 and PI3K/Akt/mTOR pathways, may break the link between obesity and cancer, and be particularly useful in obesity-driven cancers, such as EC (**Fig 5**).

Metformin has anti-EC effects *in vitro* and *in vivo*:

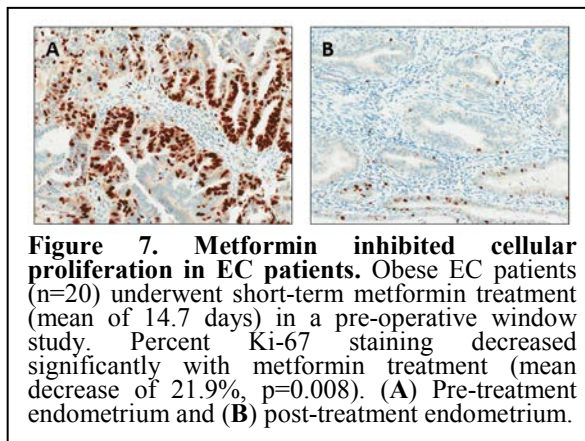
Our laboratory has shown that metformin-mediated AMPK activation decreases EC cell growth *via* inhibition of mTOR signaling⁴⁰, and that metformin and paclitaxel have synergistic anti-proliferative effects⁴¹. Others have shown that metformin blunts cell and tumor growth *in vitro* and in xenograft models^{40,42-47}. In a mouse model of endometrial hyperplasia, metformin induced anti-proliferative effects on the endometrium that coincided with inhibition of downstream targets of the mTOR pathway⁴⁸.

Importantly, metformin has increased anti-tumorigenic efficacy in our obese LKB1^{fl/fl}p53^{fl/fl} mouse model. LKB1^{fl/fl}p53^{fl/fl} mice were subjected to a HFD or LFD diet starting at 3 wks of age, and AdCre injection was performed at 6 wks of age. Once tumor growth was confirmed by palpation of a 1 cm tumor, obese and lean mice were treated with vehicle or metformin orally (200 mg/kg/day) for 4 wks (N=10 mice/group). Metformin inhibited tumor volume growth in both the HFD-fed and LFD-fed mice after 4 wks of treatment (**Fig 6**). However, metformin-induced decreases in tumor volume in HFD-fed animals were significantly greater than in LFD-fed animals (85% versus 62%, respectively, p=0.033). Thus, our pre-clinical studies suggest that metformin's anti-tumorigenic efficacy may be dependent on obese and insulin resistant states, which has also been shown in breast and lung cancer mouse models as well as our own work in ovarian cancer⁴⁹⁻⁵².



Metformin inhibits endometrial cell proliferation in EC Patients: Based on our preliminary *in vitro* work^{40,41}, we conducted a phase 0 clinical trial of metformin in EC patients at UNC-CH¹². Twenty obese women, who were to undergo surgical staging for EC, received short-term metformin treatment until the day before their surgery. Metformin significantly reduced Ki-67 staining, a marker of cell proliferation, in the endometrial tumors when comparing post-treatment

hysterectomy specimens to pre-treatment endometrial biopsies ($p=0.008$)¹² (**Fig 7**). Overall, 65% of patients (13/20) responded to metformin treatment, with a mean decrease in Ki-67 staining of 21.9% among responders to metformin¹². Responders to metformin treatment were defined as those patients with an absolute decrease in %Ki-67 staining (decrease range of 7-50%). Non-responders were defined as those who had no decrease in %Ki-67 staining. Glucose levels decreased in both metformin responders and non-responders, but were only statistically significant in responders ($p=0.007$)¹². Metformin also decreased expression of downstream targets of the insulin/IGF-1 and mTOR pathway in the endometrial tumors, including phosphorylated (p)-IGF1R (24%, $p=0.035$), p-S6 (51.2%, $p=0.0002$) and p-4E-BP-1 ($p=0.001$), as demonstrated by immunohistochemistry¹².



Metabolic Pathway	Metabolic Sub-Pathway	Biochemical Name	Responders/ Non-responders*
Amino Acid	Glycine, Serine and Threonine Metabolism	N-acetylthreonine	1.47
	Alanine and Aspartate Metabolism	aspartate	1.81
	Histidine Metabolism	trans-urocanate	1.8
	Creatine Metabolism	guanidinoacetate	1.94
Peptide	Dipeptide	phenylalanylphenylalanine	2.78
		phenylalanyltryptophan	2
Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	lactate	1.23
	Pentose Metabolism	xylulose	0.67
Lipid	Polyunsaturated Fatty Acid (n3 and n6)	arachidonate (20:4n6)	1.73
	Fatty Acid, Monohydroxy Lysolipid	2-hydroxypalmitate	1.64
		1-stearoylglycerophosphoinositol	2.94
		1-arachidonoylglycerophosphoinositol	1.67
		1-arachidonoylglycerophosphate	3.35
	Secondary Bile Acid Metabolism	glycodeoxycholate	0.36
Nucleotide		xanthine	1.86

Table 1. Baseline Metabolic Differences in Serum between Responders and Non-responders to Metformin Treatment (* $p<0.05$).

For the EC patients enrolled on the phase 0 clinical trial, metabolomic profiling was performed on serum pre- and post-metformin treatment. When comparing pre- and post-treatment serum, metformin significantly altered the concentration of 173 metabolites (37 up and 136 down) in obese EC patients¹². Comparison of global biochemical serum profiles revealed several key metabolic differences between metformin responders and non-responders. Metformin-driven metabolic alterations in the responders were primarily related to elevated lipolysis, more efficient amino acid metabolism and altered gut microbiome-associated metabolites.

Metabolomic profiling revealed that responders had higher pre-metformin treatment

serum levels of amino acids, dipeptides, glycolytic intermediates, arachidonic acid, monohydroxy fatty acids and lysolipids when compared to non-responders ($p < 0.05$) (**Table 1**). These metabolites could serve as potential biomarkers predictive of response to metformin treatment. *Higher pre-treatment serum levels of several fatty acids and glycolipids may be indicative of increased insulin resistance underlying increased benefit to metformin therapy.*

Cation-selective transporters mediate efficacy of metformin.

Metformin is one of the most hydrophilic drugs known (logD of -6.13 at pH 7.4) with a net positive charge at all physiologic pH values, necessitating transporters to mediate its entry into cells. Metformin transport in the intestine, liver and kidney is mediated by organic cation transporters (OCT)1-3, and/or the plasma membrane monoamine transporter (PMAT), and/or MATE1/2⁵³⁻⁵⁹. Studies in our laboratory show that metformin uptake into human breast cancer cell lines is dictated by the expression levels of these cation-selective transporters⁶⁰.

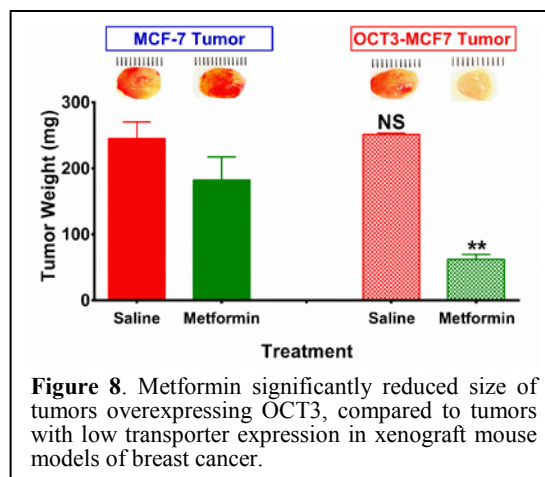


Figure 8. Metformin significantly reduced size of tumors overexpressing OCT3, compared to tumors with low transporter expression in xenograft mouse models of breast cancer.

In addition, we have found that metformin response in breast cancer requires metformin transport capability⁶¹. Xenograft mouse models of breast cancer were generated using MCF7 cells (low OCT3 transporter-expressing control group) and OCT3 overexpressing MCF7 cells (OCT3-MCF7 group). Cells (2×10^6) were injected subcutaneously into athymic nude mice. When tumor volumes reached 100 mm^3 , mice were treated with intraperitoneal saline or metformin (50 mg/kg, daily). After a 20-day treatment, metformin-induced decrease in tumor size was greater in OCT3-MCF7 tumors compared to MCF7 tumors (74.5% versus 24.7%, respectively) (**Fig 8**). *Thus, overexpression of a transporter increased response to metformin, suggesting variation in expression of transporters may contribute to differential susceptibility of tumors that arise in lean and obese animals.*

Metformin transporters are expressed in EC cell lines and tissues: Initial quantitative real-time PCR (qRT-PCR) experiments demonstrated the expression of cation-selective metformin transporters at varying levels in EC cell lines (**Fig 9**). MATE1 and 2 were the most highly-expressed transporters in two EC cell lines, namely Ishikawa and ECC-1, while OCT2 and 3 were the least expressed. In the serous EC cell line SPEC-2, MATE1 and PMAT expression predominated, with low expression of MATE2. Metformin transporter expression in 15 pairs of human EC specimens and adjacent benign tissues showed that MATE1 and 2 were expressed in significant amounts as well as PMAT (**Fig 10**). In our pre-operative window study of metformin in EC patients, MATE2 (26%, $p = 0.0017$) expression decreased with metformin treatment, and approached significance in predicting response to metformin ($p = 0.0625$). *Thus, we anticipate that the highly-expressed MATE2 may facilitate metformin*

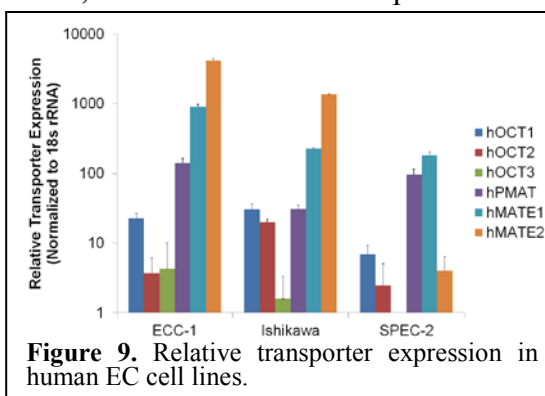


Figure 9. Relative transporter expression in human EC cell lines.

intracellular uptake into endometrial tumors and be predictive of treatment response to this agent.

It is also reasonable to postulate that genetic diversity in cation-selective transporters could affect outcomes of metformin treatment in cancer, as genetic variations in transporters have been shown to influence metformin's pharmacokinetics and glucose-lowering effects in humans⁶²⁻⁶⁶. Single nucleotide polymorphisms (SNPs) are the most common genetic variations in the human genome; therefore, SNPs in transporters can cause variability in response to drug treatment. *The effect of genetic variations in transporters has yet to be explored in clinical trials of metformin for cancer treatment, and will be assessed for the first time as part of the proposed work.*

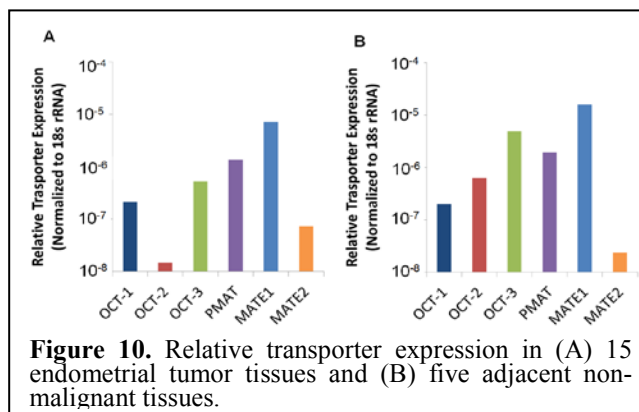


Figure 10. Relative transporter expression in (A) 15 endometrial tumor tissues and (B) five adjacent non-malignant tissues.

SUMMARY: Controversy surrounds whether metformin's anti-tumorigenic effects are indirect, *via* decreasing circulating insulin and glucose levels, or direct, through inhibition of mitochondrial complex 1, AMPK activation and insulin/IGF-1/mTOR pathway inhibition. Furthermore, it is unknown if metformin will be more effective in obese and insulin resistant patients, given its favorable impact on the metabolic syndrome and/or disruption of obesity-driven, intra-tumoral metabolic changes. Since obesity drives EC formation through alterations in metabolic pathways and hyperactivation of insulin/IGF-1/mTOR signaling, it is highly likely that an agent such as metformin, in combination with other chemotherapeutic agents, will be more efficacious in obese patients through its ability to lower plasma insulin/glucose, disrupt mitochondrial function and inhibit insulin/IGF-1/mTOR activation. Metformin enters cells *via* cation-selective transporters (OCT1-3, MATE1/2, and PMAT). However, the expression/action of these transporters in solid tumors is poorly understood. Our preliminary work suggests that metformin transporter expression patterns differ among breast, ovarian and ECs. If metformin is to be taken up by tumor cells *in vivo*, the expression of these transporters will be critical to its efficacy among cancer types and individual patients, as demonstrated by our preliminary studies in xenograft mouse models of breast cancer. *This knowledge would ultimately aid the development of improved metformin-based drugs that specifically target individual transporters.* The proposed studies will yield important insights into the relationship between the direct and indirect anti-cancer effects of metformin in EC. If our hypothesis is proven, we may need to consider obesity status and transporter expression in the individualization of metformin therapy in EC. *Most importantly, for the first time in a clinical trial, this proposal will explore the inter-relationship between both tumor and metabolic environment as predictors of drug efficacy.*

If metformin is found to be efficacious in this phase 2/3 clinical trial, the metabolic and molecular factors investigated in this study will be critical in identifying the best candidates for this unique targeted therapy and guiding further studies and management strategies for EC. Alternatively, if metformin is not found to be overall efficacious in this phase 2/3 trial, we may be able to identify subgroups of patient who did derive benefit from this therapy.

I. Insulin/IGF-1/mTOR Pathway

Integrated Biomarker Research Objective

(1) To test whether PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations are associated with a lower hazard of progression or death (PFS endpoint) among those EC patients treated with metformin. Targeted DNA sequencing (PIK3CA mutations/amplifications, PTEN mutations and PIK3R1/PIK3R2 mutations) analysis will be performed of the EC tumor specimens from patients enrolled on both arms of this trial. Hazard of progression or death will be compared between patients with and without PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations in their tumors on both arms of this trial, although we hypothesize that these mutations/amplifications will only be predictive of a lower hazard of progression or death in the metformin arm.

Other Exploratory Research Objectives

(1) To test whether genomic profiles (i.e. PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations) differ between the tumors of obese and non-obese EC patients. Targeted DNA sequencing (PIK3CA mutations/amplifications, PTEN mutations and PIK3R1/PIK3R2 mutations) analysis will be performed of the EC tumor specimens from patients on both arms of this trial, and genomic profiles compared between the endometrial tumors of obese and non-obese women.

(2) To correlate expression of key targets of the insulin/IGF-1/mTOR signaling pathway (p-IGF1R, p-S6 and p-4EBP-1) with treatment response to metformin/paclitaxel/carboplatin, PFS, OS and obesity status. Immunohistochemical (IHC) analyses will be performed of the EC tumor specimens from patients on both arms of this trial to determine expression of targets of the insulin/IGF-1/mTOR pathway, including p-IGF1R, p-S6 and p-4EBP-1. Hazard of progression or death will be compared between patients with and without high expression of p-IGF1R, p-S6 and p-4EBP-1 in their tumors, although we hypothesize that these alterations will only be predictive of a lower hazard of progression or death in the metformin arm. In addition, expression of p-IGF1R, p-S6 and p-4EBP-1 will also be compared between the endometrial tumors of obese and non-obese women.

Expected Findings: Among those EC patients treated with metformin (plus paclitaxel/carboplatin), we hypothesize that patients with PIK3CA mutations/amplifications, PTEN mutations or PIK3R1/PIK3R2 mutations in their tumors will have a lower hazard of progression or death (PFS endpoint) than those without these genomic alterations. As seen in our TCGA data on BMI and EC (Fig 3), we anticipate that the tumors from obese *versus* non-obese women will have evidence of hyperactivation of the insulin/IGF-1/mTOR pathway, as will be demonstrated by (1) increased expression of p-IGF1R (2) PTEN mutations, (3) PIK3CA mutations/amplifications and PIK3R1/PIK3R2 mutations, and (4) increased expression of downstream targets of the mTOR pathway, including p-S6 and p-4E-BP1. In addition, these alterations in insulin/IGF-1/mTOR signaling will be predictive of responsiveness to metformin (plus paclitaxel/carboplatin) and improvements in PFS and OS. If we unexpectedly find that hyperactivation of the insulin/IGF-1/mTOR signaling pathway is not associated with heightened

efficacy of metformin, it may be metformin's indirect effects on improving the metabolic milieu are much more critical in beneficial response to this agent, as will be explored by following the metabolic characteristics of patients enrolled on this trial (i.e. BMI, fasting insulin glucose levels, HOMA scores and hip-to-waist ratio).

II. Metformin Transporters

Integrated Biomarker Research Objective

(1) To test whether higher expression of MATE2 is associated with a lower hazard of progression or death (PFS endpoint) among EC patients who are treated with metformin. Using pre-treatment EC specimens from patients enrolled on both arms of this trial, IHC analyses of the metformin transporters (OCT3, MATE1/2, PMAT) will be performed. Hazard of progression or death will be compared between patients with and without high expression of these transporters in their tumors, although we hypothesize that high MATE2 expression alone will be predictive of a lower hazard of progression or death in only the metformin arm.

Other Exploratory Research Objectives

(1) To determine if the genetic variants of the metformin transporters correspond to metformin responsiveness, PFS and OS, next generation sequencing of the EC tumor specimens from the patients treated with metformin (plus paclitaxel/carboplatin) will be performed targeted to the regions coding the SNPs associated with increased or decreased uptake of metformin or related to efficacy of this agent (**Table 2**)⁶⁴. We anticipate that the racial distribution of our patients will be largely Caucasian (83%) and African American (10%); and thus, we will primarily focus on SNPs related to these two ethnic groups. Hazard of progression or death will be compared between patients with and without these SNPs of interest.

Expected Findings: As per our preliminary data in EC cell lines and tumors (**Fig 9 and 10**), we expect that the MATE2 transporter will be expressed in the EC tumors of women enrolled on GOG286B. We hypothesize that higher expression of this transporter will correspond with a lower hazard of progression or death (PFS endpoint) among patients who are treated with metformin. The MATE transporters are commonly thought to mediate metformin secretion in the kidney⁸, although some evidence suggests that MATE1 is a bi-directional transporter⁶⁷. If expression of MATE2 does not correspond with a lower hazard of progression or death (PFS), this could be because the MATE2 transporter is primarily related to metformin efflux in the endometrium and not uptake of this agent. In this case, we would assess expression of the other cation-selective transporters, including MATE1, OCT1, OCT3 and PMAT, and determine if these were alternatively predictive of response to metformin.

Although the phase 2/3 clinical trial is a combination of metformin with paclitaxel and carboplatin, it should not interfere with our ability to assess the relationship between transporter expression and response to metformin. Others have shown that the organic anion transporting polypeptide 1B3 and copper transporter 1 are essential for the uptake of paclitaxel and carboplatin, respectively in human EC, and not OCT1-3, PMAT or MATE1/2⁶⁸.

Genetic variants of transporters associated with decreased uptake of metformin will correlate with decreased efficacy of this drug, which could be related to either its indirect effects (uptake by the liver) or direct effects (uptake by the endometrial tumors). Conversely, genetic variants of transporters associated with increased uptake or enhanced efficacy will correlate with improved response to metformin.

Table 2. Transporter Coding SNPs Associated with Response to Metformin

Transporter	dsSNP ID	Amino Acid	Allele Frequencies	Metformin Uptake
OCT1	rs34447885	Ser14Phe	AA: 3.1%	↓
	rs12208357	Arg61Cys	C: 9.1-9.7%	↓
	rs34104736	Ser189Leu	C: 0.5%	↓
	rs361003319	Gly220Val	AA: 0.5%	↓
	rs34130495	Gly401Ser	C: 3.2%	↓
	rs628031	Met408Val	AA: 73.5% C: 59.7%	Positive predictor of efficacy
	rs202220802	Met420del	C: 15.7%	↓
	rs34059508	Gly465Arg	C: 1.5%	↓
OCT3	rs8187715	Thr44Met	AA: 0.6% C: 0.6%	↑
	rs8187725	Thr400Ile Val423Phe	C: 0.5%	↓
MATE1	rs35790011	Val338Ile	C: 0.4%	↓
	rs35395280	Cys497Phe/Ser	AA: 2.4%	↓

AA=African American, C=Caucasian

III. Metabolic Factors

(1) To correlate biomarkers of obesity and insulin resistance with response to metformin treatment (plus paclitaxel/carboplatin), PFS and OS, BMI, waist-to-hip ratio, HgBA1C and diabetes status will be documented throughout treatment. Fasting insulin and glucose levels will be obtained prior to cycles 1 and 3. Homeostasis Model Assessment Score (HOMA) (fasting insulin (microunits.ml) X fasting glucose (mmol/22.5)), a measurement of insulin resistance, will be calculated prior to cycles 1 and 3.

Expected findings: For the phase 2/3 clinical trial, we postulate that increased baseline BMI, waist-to-hip ratio, and fasting insulin and glucose levels will predict response to treatment with metformin/paclitaxel/carboplatin, with a greater response seen in the obese patients. We expect that BMI and fasting insulin and glucose levels will decrease with metformin/paclitaxel/carboplatin treatment as compared to placebo/paclitaxel/carboplatin. If metabolic factors are not predictive of response to metformin treatment, it would suggest that metformin's direct effects may be most important for its anti-tumorigenic benefits. In this case, metformin may be universally effective for cancer treatment and not limited to patients who are obese and insulin resistant; other biomarkers of response to metformin treatment could be elucidated from this work such as the transporters and alterations in the insulin/IGF-1/mTOR pathway as being explored above.

B. Laboratory Testing Procedures

Insert detailed procedures including assay specifics and quality control elements.

IMMUNOHISTOCHEMICAL ANALYSIS

Chromogenic Immunohistochemistry (IHC): IHC will be performed with the assistance of the UNC-CH Anatomic Pathology Translational Core Lab as previously done for our phase 0 study

of metformin in obese EC patients¹². All rabbit monoclonal and polyclonal antibodies used in this study are listed in the **Table 3**. All IHC stains will be carried out in the Bond fully-automated slide staining system (Leica Biosystems). Briefly, slides will be dewaxed in Bond Dewax solution (AR9222) and hydrated in Bond Wash solution (AR9590). Heat induced antigen retrieval for each antibody will be performed for 30 min at 100°C in Bond-Epitope Retrieval solution 1 pH-6.0 (AR9961) or solution 2 pH-9.0 (AR9640) as shown in **Table 3**. The antigen retrieval will be followed with 5 min Bond peroxide (Bond Polymer Refine kit DS9800) and 10-30 min protein blocking (DAKO, DS9800) steps. After pretreatment, slides will be incubated with the appropriately diluted primary antibody followed by chromogenic detection and hematoxylin counterstaining using Bond Polymer Refine Detection Kit (DS9800). Stained slides will be dehydrated and coverslipped. Positive and negative controls (no primary antibody) will be included *for* each run.

Antibody	Host M=mono- cl P=poly- cl	Vendor	Catalog #	Clone or Phos- phory- lated Site	Antigen Retrieval	Peroxidase Block (Bond Polymer Refine Kit DS9800). Min	Protein Block (DAKO X0909). min	Primary Ab dilution	Primary Ab Incubation	Bond Polymer Refine Kit (DS9800)	Analysis Software
MATE1: SLC47A1	Rabbit P	Thermo Scientifi c	PA5- 25272		pH-6.0, 30 min (AR9961)	5	10	1:400	1 h	yes	Aperio membrane algorithm
MATE2: SLC47A2	Rabbit P	Sigma- Aldrich	HPA044 412		pH-6.0, 30 min (AR9961)	5	10	1:200	1 h	yes	Aperio Nuclear
Oct3: SLC22A3	Rabbit M	OriGene	TA3108 52	EPR6 630	pH-6.0, 30 min (AR9961)	5	10	1:600	30 min	yes	Definiens Tissue Studio. H score and % of positive cells in epithelial enriched ROI (not stroma)
Phospho- 4E-BP1	Rabbit M	Cell Signalin g Technol ogy	2855	Thr37/ 46, 236B4	pH-6.0, 30 min (AR9961)	5	N/A	1:400	1 h	yes	Aperio cytoplasmic & color deconvolution algorithms
Phospho- IGF1R	Rabbit P IgG	Santa Cruz Biotechn ology	sc- 101703	Tyr 1161	N/A	5	10	1:200	1 h	yes	Aperio cytoplasmic algorithm
Phospho- S6 Ribosoma l Protein	Rabbit M	Cell Signalin g Technol ogy	4858	D57.2. 2E	pH-6.0, 30 min (AR9961)	5	10	1:200	2 h	yes	Aperio cytoplasmic & color deconvolution algorithms
PMAT	Rabbit P	Thermo Scientifi c	PA5- 11228		pH-6.0, 30 min (AR9961)	5	10	1:200	1 h	yes	Aperio cytoplasmic algorithm

PTEN	Rabbit M	Cell Signaling Technology	9559S	138G6	pH-9.0, 30 min (AR9640)	5	10	1:400	30 min	yes	Definiens Tissue Studio. H score and % of positive cells in the stroma and epithelial enriched ROIs
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Table 3: Rabbit Monoclonal and Polyclonal Antibodies and Protocols

Digital imaging and image analysis: Stained whole tissue sections (WTS) slides will be digitally imaged using 20X objective in the Aperio ScanScope XT (Leica Biosystems). Digital images will be stored within the Aperio eSlide Manger Database.

Phosphorylated (p)-4E-BP1, p-AKT, PMAT, p-IGF1R and p-S6 biomarkers will be quantified using Aperio v9 color deconvolution (area analysis) and Aperio Cytoplasmic v2 (cell quantification) algorithms. Aperio membrane v9 and nuclear v9 cell quantification algorithms will be used for MATE1 and MATE2, respectively. Definiens Tissue Studio image analysis software (Architect XD v 2.0.4, Tissue Studio v 3.5, Munich, Germany) will be used to quantify PTEN and OCT3 positive cells within the stroma and epithelial rich regions defined by the algorithm.

Protocol for Optimization of Automated Analysis of Digital Images from IHC Stained Slides:

Control slides will be constructed for each antibody with endometrial tissues known to be representative of both positive and negative biomarker status, in addition to being representative of a range of staining intensities. These control slides will be used to confirm that staining conditions remain consistent between staining runs. Additionally, sections of control slides will be cut at the same time as patient sample slides and all will be stored together in nitrogen desiccator chambers. In addition, we will minimize the number of batches run and ensure the same lot number is used for reagents, including that of the antibodies used.

Background IHC staining: Background staining levels for each IHC marker shall be defined as the average DAB staining intensity on negative control tissues (tissues that are negative for each marker). Average background staining intensities will be measured on digital images using the Color Deconvolution algorithm (v9, Aperio). The positive intensity threshold for each IHC marker will then be set above the average DAB background staining intensity.

Determination of % Positive cells: The algorithms that will be in this study are commercially available from Aperio and Definiens. Our Core facility has had extensive experience analyzing a wide range of normal and tumor tissues using both of these analysis platforms. We have worked with pathologists in the past to optimize algorithms, and our automated analysis results correlated well with both manual review of TPL stains and in comparison to original clinical diagnostics from multiple hospital sites (please see TPL references Allott et al., 2015 and Taylor et al., 2015 for our published work with the Carolina Breast Cancer Study and the AMBER consortium; manuscripts evaluating additional markers are currently under review or in preparation).

To ensure that automated algorithms are accurately quantifying DAB positive cells in endometrial tissues, for each marker a pathologist (Dr. S. O'Connor, pathologist involved with Dr. Bae-Jump's other IHC studies conducted at UNC-CH) will manually review a control TMA with tissue from multiple patients. We will then optimize automated algorithms to match these manual evaluations. Typically algorithms are adjusted for cell shape and size, stain and counterstain optical densities, and background staining levels. Our goal is to reach at least 90% agreement with manual reviews.

Intensity scores: For some markers such as phosphorylated H2AX (p-H2AX), there is a direct relationship between amounts of DNA damage and intensity levels of protein as measured by both Western blot and IHC staining (please see TPL reference Nikolaishvilli-Feinberg et al., 2014; automated analysis of p-H2AX using both AQUA and Tissue Studio was compared to Western blot data and radiation dosage). For other markers such as the use of CK5/6 or EGFR for basal-like subtyping in breast cancer, staining intensity is not considered.

The relevance of the staining intensity for the IHC markers that will be used in this study is unknown at this time but can still be evaluated (this information may be useful as the study continues). Intensity scores above background (1+, 2+, 3+) will be set for each IHC marker based on pathologist (Dr. S. O'Connor) manual review of control tissues with a range of staining intensities (e.g. a TMA slide with tissue cores from multiple patients). We will measure the average DAB intensity for each of these manually determined intensity score categories using the Color Deconvolution algorithm. These values will be utilized to set 1+, 2+ and 3+ thresholds in Cellular Analysis algorithms (average intensities will represent the center of each intensity score bin). Our goal is to reach at least 90% agreement with manual reviews.

Determining positive vs negative cutoffs for tissues: IHC markers that are clinically approved use a variety of factors to determine cut-offs for tissue positivity, including treatment efficacy and molecular subtyping. The markers that will be used for this study are not currently used clinically so no such guidelines exist. Based on our (TPL) experience however, a cut-off of at least 1% positive cells with automated analysis is advisable to exclude false positive signal due to slide artifact such as small folds or surgical dyes. However, a 1% cut-off is just a starting point, and can be adjusted based on statistical analysis of metrics between groups of interest (i.e. (1) obese and non-obese women and (2) responders and non-responders to metformin plus paclitaxel/carboplatin). H Score data (which takes into account both intensity and positivity) can also be evaluated in this manner.

Antigen Stability Evaluation Protocol

It is anticipated that some GOG tissue slides will have been stored at 4°C under vacuum for 18 months or more prior to analysis. To ensure that these sections will be useful for IHC, this pilot study was conducted to investigate the stability of target antigens that will be used in this trial. While TPL does not routinely store sections at 4°C/vacuum, we do utilize two other commonly used methods of storage that can be evaluated.

Staining and analysis: TMA sections containing endometrial tissue were stored for 18 months at

-20°C or at room temperature in a nitrogen gas chamber (RT/N₂). Fresh sections from these TMAs were cut and stained at the same time as aged sections using Bond autostainers. Antibodies and staining conditions have been described previously (see lab appendix).

After staining, slides were digitally imaged at 20X magnification and individual TMA cores were analyzed using Aperio analysis algorithms. Analysis results for each TMA core were compared by paired t-test using GraphPad Prism software (version 5.01, GraphPad Software Inc., La Jolla, CA).

Results and recommendations for further antibody use and further study: MATE1, MATE2 and phosphorylated (p)-S6 antigens were essentially stable for 18 months under both of the storage conditions tested (see supplemental Table 1). OCT3 and p-4E-BP1 were stable at -20°C, but had some loss of antigenicity at RT/N₂. Adjustment of positivity thresholds during automated analysis can compensate for this loss, so that older sections can be compared to freshly cut sections with the same staining conditions.

Antigenicity for p-IGF-1R was stable at -20°C, but significantly decreased under room temperature/N₂ conditions. Given these equivocal results, we plan to further test this antigen with aged slides stored at 4°C under vacuum.

PMAT had loss of antigenicity under both storage conditions, although the decrease at -20°C was less substantial. This antibody will also be evaluated at 4°C/vacuum.

Two antibodies gave abnormal results with the slides stored at -20°C in that there was an increase in signal on the aged slides as compared to the freshly cut sections. Antigen signal for p-AKT for the slide stored at -20°C was much higher than that for the freshly cut slide while the slide stored at RT/N₂ had a small decrease in antigenicity. It would be difficult to predict whether p-AKT would be a reliable marker for the IHC evaluation of tissues stored at 4°C/vacuum. Results with p-IRS1 also showed an increase in signal under storage at -20°C, but was almost completely decayed at RT/N₂. This antibody should not be used for aged slides.

OCT1 was not tested in this stability study. We found that the antibody itself (Santa Cruz Biotechnology, sc-133866) was not stable, and that the cell compartment that was stained (cytoplasmic, membrane or nuclear staining) varied with each lot of this antibody. Other commercial antibodies for OCT1 are now available from other companies and we will conduct tests for both antibody and antigen stability at 4°C to determine the viability of OCT1 as an IHC marker for this investigation.

Given these results, we have removed IHC analysis from this protocol for OCT1, phosphorylated-Akt and phosphorylated-IRS-1. PMAT and phosphorylated-IGF-1R will be re-tested under 4°C/vacuum. In addition, we will re-test phosphorylated-IGF-1R, using a monoclonal antibody as opposed to a polyclonal antibody which should be more stable and help with more reproducible results.

Table 1. Results from paired t-tests from antigen stability study.

Stain	% Positive cells -20°C	% Positive cells RT/N ₂
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MATE1	no significant difference, p= 0.1946	no significant difference, p= 0.1127
MATE2	no significant difference, p= 0.6403	mean aged = 48%, mean fresh =52%
pS6	no significant difference, p=0.7354	mean aged = 6%, mean fresh = 9%
OCT3	no significant difference, p=0.9949	mean aged = 41%, mean fresh = 52%
p-4E-BP1	no significant difference, p= 0.654	mean aged = 8.4%, mean fresh = 17%
p-IGF-1R	no significant difference, p= 0.6244	mean aged = 12%, mean fresh = 45%
PMAT	mean aged = 68%, mean fresh = 76%	mean aged = 63%, mean fresh = 81%
p-AKT	mean aged = 56%, mean fresh = 32%	mean aged = 18%, mean fresh = 22%
p-IRS1	mean aged = 45%, mean fresh = 27%	mean aged = 1.4%, mean fresh = 19%
OCT1	Not tested	Not tested

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TARGETED DNA SEQUENCING ANALYSIS

DNA Sequencing and Quantification: The analysis will be done in collaboration with the UNC-CH High Throughput Sequencing Facility. DNA will be extracted from archival formalin-fixed, paraffin-embedded (FFPE) tumor specimens using the Qiagen DNEasy column. RNA will also be collected and stored for future sequencing if funding becomes available. Given that the samples will be received as paraffin sections, there is an opportunity to dissect the tissue, which further assures the histopathologic features of tissue input into the assay.

Targeted tumor DNA sequencing will be performed using a hybrid capture approach and the Agilent custom SureSelect protocol in conjunction with next generation sequencing using the Illumina HiSeq 2000 platform to specifically assess (1) PIK3CA mutations/amplifications, PTEN mutations, PIK3R1/PIK3R2 mutations and (2) the MATE1/2 and OCT1/3 transporters. For exon capture and targeted sequence construction, we have constructed more than 1000 libraries using the Agilent SureSelect capture system. During library preparation, sequence indexes are added to the captured libraries so that multiple libraries can be sequenced on the same lane using multiplexed sequencing. FFPE samples will be sequenced using the paired end protocol with PCR duplicates removed to address the greater problem of lower complexity and PCR artifacts in paraffin samples. Additionally, performance is improved when matched normal is also embedded in paraffin. This accommodation normalizes hybridization and sequencing artifacts that can otherwise emerge (primarily C to A transversions). Currently, we are using a 50 base paired-end sequencing strategy, which provides ample gene coverage while also making it possible to identify potential gene fusions and alternative gene transcripts. To accurately identify somatic gene variants we sequence cancer samples to an average depth of 200x coverage. With current computational protocols, this depth achieves 100% sensitivity for SNPs and small indels, and >90% sensitivity for large indels, assuming the allele is present in at least 10% of the sample. At this depth, specificity for somatic events is also very high (>99.999%) when paired

normal is available. With current instrument capabilities, we can usually generate this coverage running 8 multiplexed samples per lane. Targeted sequencing in this manner is fast, reproducible, economical, and has excellent performance characteristics (sensitivity and specificity).

Data Analysis: SeqWare initiates processing of the raw data based on the protocols specified in LIMS. Extensive repeatability and accuracy studies are performed prior to final workflow specification to ensure computational protocols provide expected performance criteria. These studies, in addition to published work, resulted in an alignment and somatic mutation calling workflow optimized for detecting somatic mutations and copy number aberrations. Initial alignment to the genome is performed using Stampy, a highly sensitive DNA alignment algorithm⁶⁹. This alignment alone generates a relatively high false positive rate due to overly sensitive alignment. This issue is mitigated by our local Assembly Based ReAlignment (ABRA) algorithm, which reassembles the initial alignments in each targeted region of interest. The resulting contigs are used as estimates of the individual's genome, and all reads are re-aligned with this reference. This procedure generates significantly better alignment accuracy than alignment or assembly alone, aligns complex structural changes or mutations in close proximity, greatly reduces reference bias, and yields improved sensitivity and specificity for any subsequent genotyping procedure. Currently, the resulting alignments are then processed with UNCseq^R to generate somatic mutation calls⁷⁰. Somatic copy number aberrations are detected in targeted regions using a negative binomial model of counts for each gene to test for differences between tumor and normal. Any detection of candidate viral RNAs are further processed to ensure adequate signal above background is available. Once mutation, structural variation, copy number and viral variation is detected, these are annotated as appropriate for the study and reported back to the project specific database.

Sequencing quality is assessed to ensure that performance of an individual assay meets the expected performance criteria. Quality measures include fraction of sequence in the targeted region, overall depth of coverage, depth at each locus of interest, and adequate complexity as measured by fraction of duplicate sequences and fraction of duplicate start positions. Thresholds for each measure vary by protocol, and are determined with control samples when incorporating new protocols. The sample identity is verified by targeting and genotyping the loci in the identity panel described above.

C. References

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