Protocol Number: **HUM00047900**

Version Date: **August 10, 2017**

Title: **A Phase II Evaluation of Metformin, Targeting Cancer Stem Cells for the Prevention of Relapse in Patients with Stage IIC/III/IV Ovarian, Fallopian Tube, and Primary Peritoneal Cancer**

Principal Investigator: ***Carolyn Johnston, MD**
University of Michigan Cancer Center
Obstetrics and Gynecology
L4606 Women's Hospital
Ann Arbor, MI 48109-0276
Phone: 734-936-6884
Fax: 734-764-7261
E-mail: johnstob@med.umich.edu

Co-Investigators: ***R. Kevin Reynolds, MD**
University of Michigan Cancer Center
Obstetrics and Gynecology
L4000 Women's Hospital
Ann Arbor, MI 48109-0276
Phone: 734-764-9106
Fax: 734-764-7261
E-mail: rkr@med.umich.edu

***Rebecca Liu, MD**
University of Michigan Cancer Center
Obstetrics and Gynecology
L4000 Women's Hospital
Ann Arbor, MI 48109-0276
Phone: 734-936-3024
Fax: 734-764-7261
E-mail: rliu@med.umich.edu

***Karen Mclean, MD, PhD**
University of Michigan Cancer Center
Obstetrics and Gynecology
L4606 Women's Hospital
Ann Arbor, MI 48109-0276
Phone: 734-936-6886
Fax: 734-764-7261
E-mail: karencl@med.umich.edu

***Shitanshu Uppal**
University of Michigan Cancer Center
Obstetrics and Gynecology
L4000 Women's Hospital
Ann Arbor, MI 48109-5276
Phone: 734-936-6886
Fax: 734-764-7261

Statistician:

Kent Griffith, M.S.
Senior Research Associate
UMCCC Biostatistics Unit, 8D17
300 North Ingalls Building
Ann Arbor, MI 48109-0473
Phone: (734) 615-0601
Fax: (734) 936-9582
Email: kentg@med.umich.edu

*** Authorized to consent**

Summary

Despite 70% remission rates with surgery and chemotherapy, the majority of patients with stage III/IV ovarian cancer will relapse and die of their disease. This is consistent with a cancer stem cell (CSC) model in which a few residual treatment resistant stem cells persist and initiate disease recurrence. Laboratory studies indicate therapies targeting CSC will greatly improve cancer outcomes. We have recently characterized a population of CSC in ovarian cancer. Importantly, similar to that observed in breast cancer, we have found that the diabetes drug metformin can restrict ovarian CSC growth and proliferation. In addition metformin increases tumor cell sensitivity to chemotherapy. Consistent with this, epidemiologic studies demonstrate that diabetic patients with ovarian cancer taking metformin have better outcomes than those not taking metformin. However, metformin has not been tested as an anti-cancer stem cell agent in ovarian cancer. Thus we propose to perform a phase II clinical trial using metformin as an anti-cancer stem cell agent in ovarian cancer patients. Patients will receive metformin neoadjuvantly for two weeks prior to primary debulking surgery. Tumor specimens will be acquired for all patients at the time of primary surgery. All patients will then receive metformin concurrent with traditional neo-adjuvant or adjuvant chemotherapy. The primary objective of this study will be to determine if metformin improves the recurrence-free survival (RFS) of patients relative to historical controls. Secondary objectives of this study will be: (a) to compare the amount of CSC in primary tumor specimens in metformin treated patients versus matched controls from our tumor bank, (b) to determine if metformin improves overall survival relative to matched historical controls, (c) to confirm the safety of metformin in non-diabetic ovarian cancer patients, and (d) as laboratory studies indicate that metformin is most active in p53 mutant cells and p53 is mutated in ~50% of ovarian cancers, we will assess whether response rates correlate with p53 mutation status. If successful, this well tolerated FDA approved drug could be immediately translated into phase III trials and impact patient outcomes.

Table of Contents

1. Objectives

1.1. Clinical Objectives:

1. **Primary clinical objective:** Determine if metformin administered in combination with chemotherapy to women with advanced ovarian, primary peritoneal or fallopian tube cancer will improve recurrence-free survival (RFS) at 18 months compared to matched historical controls. We have strong preclinical evidence that metformin increases the activity of traditional chemotherapy in the treatment of ovarian cancer. Metformin is currently being used in clinical trials for patients with other types of cancer.

2. **Secondary clinical objectives:**

   a. Determine if metformin therapy is associated with an improvement in overall survival at 2 years compared to matched historical controls.

   b. Confirm the safety of metformin in non-diabetic patients with ovarian cancer. Metformin is a well-tolerated FDA approved drug used in millions of patients with and without cancer. It has been safely used in diabetic ovarian cancer patients receiving chemotherapy and in non-diabetic patients with polycystic ovarian syndrome. It has not been used in combination with chemotherapy in non-diabetic ovarian cancer patients.

1.2. Translational Objectives:

1. **Primary translational objective:** Compare the percentage and absolute number of cancer stem cells (CSC) in primary tumor specimens in metformin treated patients compared to matched controls from our tumor bank. We have demonstrated that aldehyde dehydrogenase activity (ALDH) alone and in combination with the cell surface marker CD133+ identify cancer stem cells in ovarian cancer patients. Our preliminary data suggests that metformin reduces the percentage of ALDH+ tumor cells in vitro. We hypothesize that neo-adjuvant treatment with metformin will reduce the number of CSC in ovarian cancer patient's primary tumor specimens. The primary objective of our study is to assess the percentage of CSC in the tumors of control and metformin treated patients. We further hypothesize that the number of CSC in tumors will correlate with clinical outcomes. We therefore propose to:

   a. Quantify the percentages of ALDH+ and ALDH+CD133+ cells using Fluorescence-activated cell sorting (FACS) and AQUA co-immunofluorescence.

   b. Determine if percentage of ALDH+ and/or ALDH+CD133+ CSC in primary tumor specimens is altered by treatment with metformin.

2. **Secondary translational objective:** Correlate numbers of CSC in primary tumor specimens and patient recurrence-free survival.

3. **Exploratory translational objective:** Assess p53 mutation status and response to metformin. p53 mutation status has been proposed to be a predictor of response to metformin. We therefore plan a subset analysis comparing the outcomes of metformin treated patients outcome based on p53 status (mutant in 50% of ovarian cancer patients).

2. Background and Preliminary Results

2.1. Ovarian Cancer Epidemiology: Ovarian cancer is the most deadly gynecologic malignancy, and the fifth most deadly malignancy of women in the United States. Approximately 22,000 women will be diagnosed with ovarian cancer in 2011 and approximately 15,000 will die of this disease[1]. Seventy percent of ovarian cancer patients present with late stage disease. Of the women with stage III/IV disease, 70% will have a complete response with surgical debulking and adjuvant chemotherapy. Unfortunately, the majority will relapse. Thus, even with modern chemotherapy, the majority of patients diagnosed with ovarian cancer will die of their disease. Although multimodality treatment regimens, including cytoreductive surgery and cisplatin-containing combination chemotherapy have prolonged survival, the overall cure rate of the ovarian cancer has not changed dramatically. There have been few new developments in the treatment of ovarian cancer patients since the standardization of platinum and paclitaxel which has been the mainstay of ovarian cancer adjuvant therapy for the last 20 years. For these reasons there is a clear need for novel therapeutics. Recent laboratory studies suggest that cancer stem cells may be the cause of disease recurrence and a critical target for new therapies.

2.2. Cancer Stem Cells as a Therapeutic Target: The cancer stem cell hypothesis suggests that, unlike most cancer cells within a tumor, cancer stem cells resist chemotherapeutic drugs and are able to regenerate the various cell types within a tumor, thereby causing relapse of the disease. Ovarian cancer appears to be a stem cell driven tumor. Of the 70% of women that respond to therapy, the majority will recur and die of their disease. This is consistent with the CSC theory that a small number of cells within a tumor are resistant to chemotherapy and are able to regenerate the tumor. In fact, we have recently characterized a population of cancer stem cells in ovarian cancer patients[2]. Our studies indicate that aldehyde dehydrogenase activity (ALDH) can be used to identify ovarian cancer stem cells both in human tumor cell lines and in primary tissues. Small numbers of ALDH+ cells initiate tumors in mice, while a 10-fold excess of ALDH (-) tumor cells cannot. Interestingly, ALDH used in combination with CD133 enriches CSC isolation in certain tumor specimens. These ALDH+ cells are inherently resistant to chemotherapy, with increasing percentages of ALDH+ cells observed with increasing doses of chemotherapy.

If the cancer stem cell hypothesis is correct, drugs that target cancer stem cells offer great promise in cancer treatment. Several recent studies have identified agents that appear to specifically target cancer stem cells. One compound, salinomycin, reduces the proportion of CSC by >100-fold relative to paclitaxel, a commonly used chemotherapeutic drug[3]. More recently studies in breast cancer identified IL8 signaling as critical in breast cancer stem cells. Importantly, inhibition of IL8 signaling with the CXCR1 inhibitor, repertaxin, potently restricted cancer growth and maintenance repertaxin therapy prevented cancer recurrences[4]. Similarly in ovarian cancer, the mullerian inhibitory substance was shown to inhibit ovarian cancer stem cell growth and potently augment therapy in chemoresistant tumors [5]. This data strongly suggests that targeting cancer stem cells will improve patient outcomes.

2.3. Using Metformin to Target Cancer Stem Cells: Recently metformin, a traditional type 2 diabetes medication, was found to target cancer stem cells in breast cancer. Metformin inhibited cellular transformation and selectively killed breast cancer stem cells in vitro and in vivo[6]. Another recent publication showed that metformin impedes the formation of breast cancer stem cells to form mammospheres [7]. Importantly, the combination of standard chemotherapy with metformin reduced tumor mass and prevented relapse in a xenograft mouse model[6]. Metformin has also been associated with transcriptional repression of the epithelial-mesenchymal transition, which has been specifically correlated with CSC [7]. A third study in breast cancer demonstrated that metformin synergistically interacts with trastuzumab, the anti-HER2 monoclonal antibody, to suppress self-renewal and

proliferation of cancer stem cells in HER-2 positive carcinomas [8]. These data strongly implicate metformin as a cancer stem cell targeting agent.

Metformin may have similar activity in ovarian cancer. Metformin appears to be acting via regulation of the insulin/glucose/IGF pathways. There is significant evidence that the insulin/glucose/IGF pathways are critical for ovarian cancer growth. Insulin depresses the IGF binding proteins IGF-BP-1 and 2 thereby leading to increases in IGF-I and IGF-II. IGFs in turn act via the PI3K/mTor pathway to promote ovarian tumor cell proliferation and inhibit apoptosis. IGF signaling activation has been correlated with poor outcomes in ovarian cancer patients. Metformin has been shown to inhibit several enzymes associated with this pathway and reduces levels of circulating insulin and IGF-I[9,10]. We recently demonstrated that type 2 diabetes has a negative impact on the survival of ovarian cancer patients [11]. Type 2 diabetes was an independent prognostic factor with implications as significant as debulking status—one of the primary prognostic factors in ovarian cancer patients. Importantly, patients whose diabetes was better controlled appeared to have a better outcome, suggesting that the impact of diabetes in ovarian cancer patients may be directly related to biologic alterations in diabetics' cancer cell biology. Consistent with this, diabetic patients were more likely to have a poorly differentiated histology [11]. Decreased states of tumor differentiation would be consistent with an increase in tumor "stemness."

2.4. Metformin and Cancer Patients: While currently the primary data for metformin as a potential anti-cancer agent is laboratory based, there is significant epidemiologic data supporting the possibility of metformin as an active anti-cancer agent in humans. In a variety of tumor types, metformin has been correlated with decreased rates of malignancy. Recent studies demonstrate that users of metformin are at lower risk of cancer compared with people with type 2 diabetes on other treatments[12,13]. Metformin use is also associated with a lower cancer mortality compared to nonuse of metformin[14]. Type 2 diabetics receiving neoadjuvant chemotherapy for breast cancer as well as metformin were more likely to have a complete remission than patients not receiving metformin[15]. Patients receiving metformin seem to have a lower incidence of prostate and pancreatic cancers[16,17]. Metformin has also been shown to be a potent inhibitor of cell proliferation in two endometrial cancer cell lines[18]. Epidemiological studies have confirmed that metformin significantly reduces cancer incidence and improves cancer patients' survival in type 2 diabetics [19]. Interestingly, metformin has also been demonstrated to be most active in p53 mutant cancer cells[20]. p53 is the most commonly mutated gene in ovarian cancer patients, mutated in ~50% of ovarian cancers [21]. p53 gene mutations occur more often in stage III and IV ovarian cancers when compared to stage I and II (58% in stage III/IV vs. 37% in stage I/II) [22]. There seems to be a trend that p53 mutation status, as determined by immunohistochemical (IHC) analysis and in univariate analyses, might be of prognostic value in ovarian cancer patients in relation to prognosis, prediction of response to adjuvant chemotherapy and in disease progression or survival [23].

2.5. Metformin in Non-diabetic Patients: In an early study conducted to determine metformin pharmacokinetics, there were no significant differences in metformin kinetics in patients with non-insulin dependent diabetes compared with healthy subjects. In healthy subjects, metformin showed no effect on plasma glucose, but significantly attenuated the rise in immediate postprandial insulin levels.[24] In addition, metformin has been safely used in thousands of polycystic ovarian syndrome patients and is also being explored for use in pregnancy[25,26]. Metformin is currently being investigated in a number of clinical trials involving cancer patients. Currently on clinicaltrials.gov there are 24 clinical trials using metformin involving 8 different tumor types (breast, colorectal, pancreas, head and neck, thyroid, acute lymphocytic leukemia, prostate and endometrial). There are no ongoing trials of metformin in ovarian cancer patients. Metformin is being investigated alone, in combination with surgery, in combination with chemotherapy, and in combination with surgery and chemotherapy. All doses used are within the typical range for type 2 diabetics receiving metformin.

2.6. Metformin in Ovarian Cancer Patients, the Rationale: While the majority of patients with ovarian cancer will respond to first-line chemotherapy, over 70% will relapse and ultimately succumb to the disease. This indicates a clear need for better therapy. One attractive potential mechanism to prevent tumor recurrence is to target the cancer stem cell population. In breast cancer, CSC can be targeted by metformin. One study evaluating the effect of metformin in ovarian cancer demonstrated potential anti-cancer activity of metformin alone and in combination with chemotherapy[27]. This study did not assess the impact of metformin on ovarian cancer stem cells. Another recent publication demonstrated that metformin treatment of ovarian cancer xenografts resulted in reduced tumor size and metastatic lung nodules, as well as diminished angiogenesis compared to controls[28].

1. Identification of Ovarian Cancer Stem Cells: We recently performed an extensive characterization of CSC in ovarian cancer [2]. We have demonstrated that in CD133 (-) cell lines and in human primary tumors, aldehyde dehydrogenase enzymatic activity (ALDH) acts a marker of CSC. Treatment of ovarian cancer cell lines with chemotherapy leads to an increase in the percentage of ALDH+ cells, suggesting that these cells are chemoresistant. Similarly, FACS isolated ALDH+ cells treated with cisplatin demonstrated greater survival than ALDH- cells confirming chemoresistance. In vivo, limited numbers of ALDH+ cells were able to initiate tumors, whereas ALDH- cells could not. Importantly we were able to generate tumors from ALDH+ tumors cells directly isolated from human tumors in 2/9 tumor specimens. Interestingly, in CD133+ cell lines, the combination of ALDH and CD133 identifies a population of cancer stem cells with even greater tumor initiation capacity. This was confirmed using human primary tumor cells in sphere forming assays. Importantly, we were able to generate primary tumor xenografts from 10-1000 cells from 5/10 ALDH+CD133+ tumors. We found that the presence of ALDH+CD133+ cells strongly correlated with poor outcome in ovarian cancer patients.

2. Metformin as an Anti-Neoplastic in Ovarian Cancer: We have now performed extensive studies on the activity of metformin in ovarian cancer. Results indicate that metformin alone can inhibit the proliferation of ovarian cancer cells in a dose-dependent manner. We have found that metformin treatment inhibits growth rates ~50% at 1mM, a dose easily achieved in patients with standard dosing of metformin. We observed this for both the SKOV3 and A2780 ovarian cancer cell lines. We assessed the activity of metformin when used in combination with cisplatin chemotherapy. Once again we used SKOV3 cells, a cisplatin-resistant ovarian cancer cell line, and A2780DK, a cisplatin-sensitive ovarian cancer cell line. Toxicity was dose-dependent with increasing doses of metformin while maintaining a stable dose of cisplatin. We observed this for both the SKOV3 and A2780 ovarian cancer cell lines. As to be expected, the cisplatin-sensitive cell line, A2780DK, needed much less of either medication to see the additive effect of the metformin.

3. Metformin and Ovarian Cancer Stem Cells: Given previous studies in breast cancer stem cells, we have specifically investigated the impact of metformin on ovarian cancer stem cells. As discussed above we have shown that ALDH is a CSC marker in ovarian cancer. We therefore analyzed the percent of ALDH+ SKOV3 cells treated with cisplatin and metformin. We observed that cisplatin therapy actually leads to an ***increase*** in the percentage of ALDH+ cells, i.e. standard chemotherapy enriches the stem cell population and causes these cells to become more stem-like. However, when metformin is added to the treatment regimen we see the cancer stem cell population ***decrease*** below pretreatment levels. To confirm an effect on primary human tumor specimens we performed tumor sphere assays with primary human tumor ascites cells. In the presence of metformin we observed a 2 fold decrease in the number of tumor spheres. Next we sorted the same patient's cells into ALDH+ and ALDH (-) cells. Again we performed tumor sphere assays in the presence and absence of metformin. As expected very

few spheres form in the ALDH (-) groups since the stem cells have been removed from this cell population. In ALDH+ group in the presence of metformin we observed an even greater effect of metformin, almost a 3-fold decrease in the number of tumor spheres.

4. Metformin as an Anti-Neoplastic in Ovarian Cancer in a Xenograft Mouse Model: The cancer stem cell hypothesis for the progression of human cancers is based on the differential tumor-forming properties and response to chemotherapy of cancer stem cells and non-cancer stem cells. Therefore we hypothesize that if metformin reduces the number of cancer stem cells, it will significantly reduce tumor growth rates when added to cisplatin chemotherapy. In addition we hypothesize that metformin therapy will significantly slow or even prevent recurrences after traditional platinum chemotherapy. We have performed in vivo experiments in which tumors are treated with cisplatin alone or concurrently with cisplatin and metformin. After 6 weeks of tumor growth, we clearly see the effects of metformin. The cisplatin and metformin group appears to have a greater effect than cisplatin alone with 3 of the 8 mice in this group have complete resolution of the tumors and the other mice have tumors half the size of the cisplatin group. Thus combination therapy has a dramatic effect on these otherwise treatment resistant tumors.

2.7. Our data strongly indicates that metformin is an active therapeutic in ovarian cancer and that metformin acts by targeting the ovarian cancer stem cell population. Importantly our studies observed significant activity with one quarter the dose achieved with traditional dosing in humans. When used alone, metformin slows ovarian cancer cell growth. When used in combination with chemotherapy, metformin blocks the chemotherapy induced enrichment of ALDH+ CSC. This leads to a synergistic reduction in cancer cell growth. Furthermore we have observed that metformin reduces the growth of ovarian tumor spheres, an attribute of cancer stem cells. Finally, similar to that seen in breast cancer, metformin combined with chemotherapy slows the rates of ovarian tumor growth in mice.

We hypothesize that metformin will specifically target ovarian cancer stem cells in ovarian cancer patients. Metformin is currently FDA approved for use in patients with type 2 diabetes and has proven safe for human use. It is inexpensive, readily available and well tolerated in most patients. Based on preclinical data from our laboratory, we believe that metformin used in combination with chemotherapy will improve progression free survival. Furthermore, given the oral availability of the drug and the minimal side effects, metformin represents an ideal therapy to prevent or prolong the time to disease relapse.

3. Drug Information[29]

3.1. Other Names: ActoplusMet, Avandamet, Fortamet, Glucophage, Glucophage XR, Glucovance, Glumetza, Metaglip, Riomet

3.2. Classification: Biologic therapeutic

3.3. CAS Registry Number: 1115-70-4

3.4. Description: Metformin hydrochloride is an oral anti-hyperglycemic drug used in the management of type 2 diabetes. Metformin hydrochloride (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is a white to off-white crystalline compound with a molecular formula of C4H11N5 • 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.

3.5. Mode of Action: Metformin is an anti-hyperglycemic agent which improves glucose tolerance in patients

with type 2 diabetes, lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral anti-hyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia. With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease. The mechanism of action of metformin on ovarian cancer stems cells is currently unknown.

3.6. How Supplied: Metformin is supplied as tablets for oral administration containing 500 mg, 850 mg, or 1000 mg of Metformin hydrochloride.

3.7. Source of Drug: Metformin will be purchased from the pharmacy at the University of Michigan by the study team.

3.8. Drug Accountability: Metformin tablets will be ordered by the study team and stored/dispensed by investigational drug services in accordance with applicable regulatory requirements. Medication that is returned from patients will be stored separately from medication that needs to be dispensed.

Accountability for metformin tablets at the study site is the responsibility of the investigator. The investigator will ensure that the study drug is used only in accordance with this protocol. The investigator may assign the drug accountability responsibilities to a pharmacist. Drug accountability records will indicate the drug's delivery date to the site, the inventory at the site, and the use by each patient. At a minimum, accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), patient initials and study number, and doses.

All used, unused or expired metformin tablets will be disposed of at the study site according to local regulations for the disposition of prescription drugs and documented.

3.9. Storage and Stability: Store at controlled room temperature (59° to 86°F).

3.10. Route of Administration: Oral.

3.11. Adverse reactions: In a U.S. double-blind clinical study of metformin in patients with type 2 diabetes, 141 patients received metformin therapy (up to 2550 mg per day) and 145 patients received placebo. Adverse reactions were reported in greater than 5% of the metformin patients, and were more common in metformin than placebo-treated patients, are: diarrhea (53.2% vs. 11.7%), nausea/ vomiting (25.5% vs. 8.3%), flatulence (12.1% vs. 5.5%), asthenia (9.2% vs. 5.5%), indigestion (7.1% vs. 4.1%), abdominal discomfort (6.4% vs. 4.8%), headaches (5.7% s. 4.8%) Diarrhea led to discontinuation of study medication in 6% of patients treated with metformin. Additionally, the following adverse reactions were reported in ≥ 1 to ≤ 5% of metformin patients and were more commonly reported with metformin than placebo: abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, sweating increased, taste disorder, chest discomfort, chills, flu syndrome, flushing, palpitation.

3.12. Contraindications:

1. Renal disease or renal dysfunction (as suggested by serum creatinine levels ≥ 1.5 mg/dL [males], ≥ 1.4 mg/dL [females] or abnormal creatinine clearance) which may also result from conditions such as cardiovascular collapse (shock), acute myocardial infarction, and septicemia.

2. Known hypersensitivity to metformin hydrochloride.

3. Acute or chronic metabolic acidosis, including diabetic ketoacidosis, with or without coma.

3.13. Potential Drug Interactions:

1. **Furosemide:** A single-dose, metformin-furosemide drug interaction study in healthy subjects demonstrated that pharmacokinetic parameters of both compounds were affected by co-administration. Furosemide increased the metformin plasma and blood Cmax by 22% and blood AUC by 15%, without any significant change in metformin renal clearance. When administered with metformin, the Cmax and AUC of furosemide were 31% and 12% smaller, respectively, than when administered alone, and the terminal half-life was decreased by 32%, without any significant change in furosemide renal clearance. No information is available about the interaction of metformin and furosemide when co-administered chronically.

2. **Nifedipine:** A single-dose, metformin-nifedipine drug interaction study in normal healthy volunteers demonstrated that co-administration of nifedipine increased plasma metformin Cmax and AUC by 20% and 9%, respectively, and increased the amount excreted in the urine. Tmax and half-life were unaffected. Nifedipine appears to enhance the absorption of metformin. Metformin had minimal effects on nifedipine.

3. **Cationic drugs:** Cationic drugs (e.g., amiloride, digoxin, morphine, procainamide, quinidine, quinine, ranitidine, triamterene, trimethoprim, or vancomycin) that are eliminated by renal tubular secretion theoretically have the potential for interaction with metformin by competing for common renal tubular transport systems. Although such interactions remain theoretical (except for cimetidine), careful patient monitoring and dose adjustment of metformin and/or the interfering drug is recommended in patients who are taking cationic medications that are excreted via the proximal renal tubular secretory system.

4. **Cimetidine:** Interaction between metformin and oral cimetidine has been observed in normal healthy volunteers in both single- and multiple-dose, metformin-cimetidine drug interaction studies, with a 60% increase in peak metformin plasma and whole blood concentrations and a 40% increase in plasma and whole blood metformin AUC. There was no change in elimination half-life in the single-dose study. Metformin had no effect on cimetidine pharmacokinetics.

5. **Other:** Certain drugs tend to produce hyperglycemia and may lead to loss of glycemic control. These drugs include the thiazides and other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blocking drugs, and isoniazid. When such drugs are administered to a patient receiving metformin, the patient should be closely observed for loss of blood glucose control. When such drugs are withdrawn from a patient receiving metformin, the patient should be observed closely for hypoglycemia.

3.14. Precautions:

1. **Monitoring of renal function:** Metformin is known to be substantially excreted by the kidney and the risk of metformin accumulation and lactic acidosis increases with the degree of impairment of renal function. Thus, patients with serum creatinine levels above the upper limit of normal for their age should not receive metformin. In patients with advanced age, metformin should be carefully titrated to establish the minimum dose for adequate glycemic effect, because aging is associated with reduced renal function. In elderly patients, particularly those ≥ 80 years

of age, renal function should be monitored regularly and, generally, metformin should not be titrated to the maximum dose.

2. **Radiologic studies:** This refers to radiologic studies involving the use of intravascular iodinated contrast materials (for example, intravenous urogram, intravenous cholangiography, angiography, and computed tomography (CT) scans with intravascular contrast materials). Intravascular contrast studies with iodinated materials can lead to acute alteration of renal function and have been associated with lactic acidosis in patients receiving metformin. Therefore, in patients in whom any such study is planned, metformin should be temporarily discontinued at the time of or prior to the procedure, and withheld for 48 hours subsequent to the procedure and reinstituted only after renal function has been re-evaluated and found to be normal.

3. **Hypoxic states:** Cardiovascular collapse (shock) from whatever cause, acute congestive heart failure, acute myocardial infarction and other conditions characterized by hypoxemia have been associated with lactic acidosis and may also cause prerenal azotemia. When such events occur in patients on metformin therapy, the drug should be promptly discontinued.

4. **Surgical procedures:** Metformin therapy should be temporarily suspended for any surgical procedure (except minor procedures not associated with restricted intake of food and fluids) and should not be restarted until the patient's oral intake has resumed and renal function has been evaluated as normal.

5. **Alcohol intake:** Alcohol is known to potentiate the effect of metformin on lactate metabolism. Patients, therefore, should be warned against excessive alcohol intake, acute or chronic, while receiving metformin.

6. **Impaired hepatic function:** Since impaired hepatic function has been associated with some cases of lactic acidosis, metformin should generally be avoided in patients with clinical or laboratory evidence of hepatic disease.

7. **Hypoglycemia:** Hypoglycemia does not occur in patients receiving metformin alone under usual circumstances of use, but could occur when caloric intake is deficient, when strenuous exercise is not compensated by caloric supplementation, or during concomitant use with other glucose-lowering agents (such as sulfonylureas and insulin) or ethanol.

8. **Lactic Acidosis:** Lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin; when it occurs, it is fatal in approximately 50% of cases. Lactic acidosis may also occur in association with a number of pathophysiologic conditions, including diabetes mellitus, and whenever there is significant tissue hypoperfusion and hypoxemia. Lactic acidosis is characterized by elevated blood lactate levels (>5 mM/L), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. When metformin is implicated as the cause of lactic acidosis, metformin plasma levels >5 mcg/mL are generally found. The reported incidence of lactic acidosis in patients receiving metformin hydrochloride is very low (approximately 0.03 cases/1000 patient-years, with approximately 0.015 fatal cases/1000 patient-years). In more than 20,000 patient-years exposure to metformin in clinical trials, there were no reports of lactic acidosis. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, including both intrinsic renal disease and renal hypoperfusion, often in the setting of multiple concomitant medical/surgical problems and multiple concomitant medications. Patients with congestive heart failure requiring pharmacologic management, in

particular those with unstable or acute congestive heart failure who are at risk of hypoperfusion and hypoxemia, are at increased risk of lactic acidosis. The risk of lactic acidosis increases with the degree of renal dysfunction and the patient's age. The risk of lactic acidosis may, therefore, be significantly decreased by regular monitoring of renal function in patients taking metformin and by use of the minimum effective dose. In particular, treatment of the elderly should be accompanied by careful monitoring of renal function. Metformin treatment should not be initiated in patients ≥80 years of age unless measurement of creatinine clearance demonstrates that renal function is not reduced, as these patients are more susceptible to developing lactic acidosis.

4. Staging Criteria FIGO Staging for Ovarian Cancer, Revised 1989[30]

4.1. Stage I: Growth limited to the ovaries.

1. IA: Growth limited to one ovary; no tumor on external surface; capsule intact; no ascites
2. IB: Growth limited to both ovaries; no tumor on external surface; capsule intact; no ascites
3. IC: Tumor on the surface of one or both ovaries; or ruptured capsule(s); or ascites containing malignant cells; or positive peritoneal washings

4.2. Stage II: Growth involving one or both ovaries with pelvic extension.

1. IIA: Extension and/or metastases to the uterus and/or fallopian tubes
2. IIB: Extension to other pelvic tissues
3. IIC: Tumor of stage II with tumor on the surface of one or both ovaries; or ruptured capsule(s); or ascites containing malignant cells; or positive peritoneal washings

4.3. Stage III: Tumor involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal lymph nodes. Superficial liver metastasis equals stage III.

1. IIIA: Histologically confirmed microscopic seeding of abdominal peritoneal surfaces
2. IIIB: Histologically confirmed implants of peritoneum <=2cm in diameter and nodes negative
3. IIIC: Abdominal peritoneal implants of >2cm and/or positive retroperitoneal or inguinal lymph nodes

4.4. Distant metastases. If pleural effusion is present, there must be positive cytologic test results to allow stage IV. Parenchymal liver metastasis equals stage IV.

5. Eligibility/ Exclusion Criteria

5.1. Eligibility Criteria

1. Patients with potential diagnosis of ovarian, fallopian, or primary peritoneal cancer. Care plan including surgical debulking and traditional adjuvant or neo-adjuvant chemotherapy (6-9 cycles of platinum and taxane based therapy).
2. ECOG performance status 0-2.
3. Age > 18 years or < 80 years.
4. Adequate renal function (serum creatinine <1.4mg/dL).
5. Adequate liver function (bilirubin < 1.5 times ULN).
   a. ALT or AST < 5X ULN in case of liver metastases.
   b. ALT or AST < 2.5X ULN in absence of liver metastases.
6. Ability to understand and complete written informed consent.

7. Mentally, physically, and geographically able to undergo treatment and follow up.

5.2. Exclusion Criteria

1. Patients with diabetes mellitus. (Patients with only a history of gestational diabetes will be allowed to be included in the study.)
2. Metformin use in the last 6 months.
3. A known hypersensitivity to metformin.
4. A history of metabolic acidosis, including ketoacidosis or increased risk of lactic acidosis.
5. Pregnancy or Lactation.
6. Patients who have any severe and/or uncontrolled medical conditions.
7. Patients with a history of renal disease.
8. Patients with other known active malignancy (excluding adequately treated basal cell / squamous cell skin cancer, in situ cancer, or other cancer for which the patient has been disease free for 2 years).
9. Patients receiving any other investigational agents.

6. Treatment Plan

6.1. Recruitment: We plan to recruit all patients at the University of Michigan Health System with new diagnoses of stage IIC/III/IV ovarian, fallopian tube and primary peritoneal cancers who are planned to receive both chemotherapy and surgical debulking. Patients will be included regardless of whether the patient is optimally debulked (less than 1 cm of remaining tumor) at the time of primary surgery. Eligibility and exclusion criteria are given in Section 5. All eligible patients will be given the opportunity to participate.

6.2. Agent Administration:

1. **Patients treated with primary debulking surgery:** All patients receiving primary surgical debulking followed by adjuvant chemotherapy will initiate metformin therapy 11+/- 4 days prior to primary surgery. Patients will take 500mg tablets orally once daily for 3 days then the dose will be increased to 500mg twice daily (this stepwise increase is to minimize the mild gastrointestinal side effects often associated with initiation of metformin therapy). Metformin therapy will be held 48 hours prior to surgery. Following surgery patients will be initiated on metformin 7 days (+/- 4 days) prior to the initiation of chemotherapy. Patients will begin with metformin 500mg tablets orally once daily for 3 days then the dose will be increased to 500mg twice daily. (This stepwise increase is to minimize the mild gastrointestinal side effects often associated with initiation of metformin therapy.) Metformin will be used daily concurrent with chemotherapy and discontinued after the patient's last cycle of primary adjuvant chemotherapy. Patients will be removed from the study whose surgical specimen is determined to be something other than epithelial ovarian, fallopian tube or primary peritoneal cancer or the stage is less than IIC.

2. **Patients treated with neoadjuvant chemotherapy:** Patients treated with neoadjuvant chemotherapy will be initiated on metformin with the initiation of chemotherapy. Patients will begin with metformin 500mg tablets orally once daily for 3 days then the dose will be increased to 500mg twice daily. (This stepwise increase is to minimize the mild gastrointestinal side effects often associated with initiation of metformin therapy.) Metformin will be used daily concurrent with chemotherapy (for 3-6 cycles of chemotherapy, and discontinued 48 hours prior to surgical debulking. Patients will be removed from the study whose surgical specimen is determined to be something other than epithelial ovarian, fallopian tube or primary peritoneal cancer. Following surgery, if additional adjuvant therapy is indicated, patients will be initiated on

metformin 7 days (+/- 4 days) prior to the re-initiation of chemotherapy. Patients will begin with metformin 500mg tablets orally once daily for 3 days then the dose will be increased to 500mg twice daily. (This stepwise increase is to minimize the mild gastrointestinal side effects often associated with initiation of metformin therapy.) Metformin will be used daily concurrent with chemotherapy and discontinued after the patient's last cycle of primary chemotherapy.

6.3. Dose Levels of Metformin: All patients will initiate drug at dose level 1. The dose escalation schedule for individual patients is indicated here:

Dose-Escalation Schedule	
Dose Level	**Dose of Metformin**
(-)1	500mg PO Daily
1	500mg PO BID

6.4. Dose justification: The dose of metformin that we have chosen is the typical dose used in the treatment of type 2 diabetes and polycystic ovarian syndrome. It is also the dose currently being used in many on-going clinical trials of metformin in other cancers. In our experiments in the ovarian cancer xenograft mouse, we use a dose of 150mg/kg. This dose can be translated to the human equivalent dose using the well-established method as described by Reagan-Shaw et al.[31] According to the formula, the human equivalent dose (mg/kg) = animal dose (mg/kg) x animal K_m/human K_m. Species and K_m values are based on body surface area. K_m for a 60kg human adult is 37 and a 20g mouse is 3. On the basis of this formula, the human equivalent dose of 150mg/kg in a mouse is 730mg in an average sized woman of 60kg. This suggests that the minimum efficacy is 730mg. This is less than one third the maximum dose of 2550mg/day recommend by the Food and Drug Administration[29] and less than half of the 1000mg/day our patients will ultimately receive.

6.5. Primary Chemotherapy: All neo-adjuvant and adjuvant platinum-taxane combinations of therapy and number of treatment cycles will be determined by the patients' primary oncologist. Some of the typical treatment regimens are listed below.

1. Most common: Paclitaxel (175mg/m^2) i.v. + carboplatin (AUC=5-6 mg/ml per minute) i.v. every 21 days.
2. IP Regimen 1: Docetaxel (75mg/m^2) i.v. + cisplatin i.p. (70-100mg/m^2) on day 1, paclitaxel i.p. (60mg/m^2) on day 8 every 21 days
3. IP Regimen 2: Paclitaxel i.v.(135mg/m^2) day 1, cisplatin (75mg/m^2) i.p. day 2, paclitaxel (60mg/m^2) i.p. day 8 every 21 days
4. Dose Dense Paclitaxel Regimen: Paclitaxel (80mg/m^2) i.v. on days 1,8, and 15 + carboplatin (AUC=6) on day 1 every 21 days

6.6. Frequency of Monitoring: Patients will be monitored prior to every chemotherapy cycle and after completion of chemotherapy, patients will be monitored every 12 weeks +/-2 weeks during the first year after therapy, and every 4 months +/-2 weeks in the second year, or until evidence of progression.

6.7. Radiologic studies: Metformin should be temporarily discontinued at the time of or prior to and withheld for 48 hours subsequent to any radiologic study involving the use of intravascular iodinated contrast materials (for example, intravenous urogram, intravenous cholangiography, angiography, and computed tomography (CT) scans with intravascular contrast materials). If renal compromise is a concern of the patient's treating physician, metformin should be reinstituted only after renal function has been re-

evaluated and found to be normal.

6.8. Surgical procedures: Metformin therapy should be temporarily suspended 48 hours prior to any surgical procedure (except minor procedures not associated with restricted intake of food and fluids) and should not be restarted until the patient's oral intake has resumed. In the case of the patient's primary debulking surgery, metformin will be re-started 7 days (+/- 4 days) prior to the initiation of chemotherapy as long as the patient's oral intake has resumed.

6.9. Hypoxic states: Cardiovascular collapse (shock) from whatever cause, acute congestive heart failure, acute myocardial infarction and other conditions characterized by hypoxemia have been associated with lactic acidosis and may also cause prerenal azotemia. When such events occur in patients on metformin therapy, the drug should be promptly discontinued. Metformin should be reinstituted only after renal function has been re-evaluated and found to be normal.

6.10. Laboratory Monitoring: Blood sampling will be performed prior to the first administration of metformin. For data collection purposes samples will be obtained (i) prior to surgery, (ii) prior to each chemotherapy cycle and (iii) prior to every surveillance visit after the completion of chemotherapy while on study. Delays of two weeks will be accepted. Labs to be drawn include:

1. CBCPD
2. Electrolytes (including serum creatinine and glucose levels)
3. Liver Function Tests (including AST, ALT, and Bilirubin)
4. CA-125 will be drawn prior to every surveillance visit after completion of primary chemotherapy.
5. Hemoglobin A1C will be drawn once with pre-study blood work.

Physicians will be allowed to collect additional labs at their discretion.

6.11. Duration of Therapy: In the absence of treatment delays due to adverse events, treatment may continue until after the completion of primary chemotherapy or until one of the following criteria applies:

1. Disease progression
2. Intercurrent illness that prevents further administration of treatment
3. Unacceptable adverse event(s)
4. Patient decides to withdraw from the study
5. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
6. If there is any evidence of a negative impact of metformin therapy, therapy will be discontinued (see safety sections 8.3 and 12.6).

6.12. Monitoring Compliance: Patients will be asked to bring pill bottles/blister packs to doctors' appointments. In addition patients will be asked to maintain a medication diary to share with their doctor at each visit.

6.13. Supportive Care Guidelines: Patients will be permitted to receive appropriate supportive care measures as deemed necessary by the treating physician.

6.14. Duration of Follow-up: Patients will be followed for up to 24 months after completion of chemotherapy or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event or a total of 24 months after completion of chemotherapy, whichever occurs last.

7. Toxicities/ Dosage Modifications

7.1. Toxicities. Given the various treatment protocols allowed, for uniformity, mandatory monitoring of adverse events will be performed with each primary chemotherapy appointment (every three weeks for all protocols). Physicians will be allowed to monitor interim labs at their discretion.

1. **Adverse Events That Can Be Attributed to Metformin Therapy:**[29]

a. Gastrointestinal: diarrhea, nausea and vomiting, flatulence, indigestion, abdominal discomfort
b. Neurologic: Headache, asthenia
c. Lactic Acidosis: Lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment with metformin. Lactic acidosis may also occur in association with a number of pathophysiologic conditions, including diabetes mellitus, and whenever there is significant tissue hypoperfusion and hypoxemia. Lactic acidosis is characterized by elevated blood lactate levels (>5 mM/L), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. When metformin is implicated as the cause of lactic acidosis, metformin plasma levels >5 mcg/mL are generally found. The reported incidence of lactic acidosis in patients receiving metformin is very low (approximately 0.03 cases/1000 patient-years, with approximately 0.015 fatal cases/1000 patient-years). In more than 20,000 patient-years exposure to metformin in clinical trials, there were no reports of lactic acidosis. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency, including both intrinsic renal disease and renal hypoperfusion, often in the setting of multiple concomitant medical/surgical problems and multiple concomitant medications. Patients with congestive heart failure requiring pharmacologic management, in particular those with unstable or acute congestive heart failure who are at risk of hypoperfusion and hypoxemia, are at increased risk of lactic acidosis.
d. Other: The following adverse reactions were reported in ≥ 1 to ≤ 5% of metformin patients and were more commonly reported with metformin than placebo: abnormal stools, hypoglycemia, myalgia, lightheaded, dyspnea, nail disorder, rash, sweating increased, taste disorder, chest discomfort, chills, flu syndrome, flushing, palpitation.

2. **Adverse Events That Can Be Attributed to Platinum Therapy:**[29]

a. Renal: renal insufficiency
b. GI: nausea and vomiting, diarrhea, hiccups, elevated serum amylase
c. Neurologic: sensory polyneuropathy, autonomic neuropathies, seizures, encephalopathy, myasthenic syndrome, and cortical blindness. Ototoxicity (tinnitus, high frequency hearing loss), headache, encephalopathy, and strokes
d. Hematologic: myelosuppression, Coombs' positive hemolytic anemia
e. Ocular: optic neuritis, papilledema, cortical blindness, focal deficits, cerebral blindness, blurred vision, altered color perception
f. Vascular: myocardial infarction, cerebrovascular accident, thrombotic microangiopathy (hemolytic uremic syndrome), cerebral arteritis
g. Cardiovascular: chronic lipid and blood pressure abnormalities, possible cardiotoxicity (ST-T wave abnormalities and bundle branch block), atrial fibrillation, supraventricular tachycardia
h. Endocrine: syndrome of inappropriate antidiuretic hormone
i. Hypersensitivity: anaphylactic-like reactions (facial edema, wheezing, tachycardia, and hypotension)
j. Hepatic: transient elevations of liver enzymes (SGOT and bilirubin)
k. Dermatologic: rash, alopecia

3. **Adverse Events That Can Be Attributed to Paclitaxel Therapy:**[29]

a. Hematologic: Myelosuppression
b. Gastrointestinal: Nausea and vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis, ischemic colitis, neutropenic enterocolitis
c. Heart: Arrhythmia, heart block, ventricular tachycardia, myocardial infarction (MI), bradycardia, atrial arrhythmia
d. Pulmonary: Pneumonitis
e. Blood Pressure: Hypotension, hypertension (possibly related to concomitant medication--Dexamethasone)
f. Neurologic: Sensory (taste), peripheral neuropathy, seizures, mood swings, hepatic encephalopathy, encephalopathy
g. Skin: Infiltration: erythema, induration, tenderness, rarely ulceration, radiation recall reactions, erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)
h. Allergy: Anaphylactoid and urticarial reactions (acute), flushing, rash, pruritus
i. Liver: Increased SGOT, SGPT, bilirubin and alkaline phosphatase, hepatic failure, hepatic necrosis
j. Other: Alopecia, fatigue, arthralgia, myalgia, light-headedness, myopathy
k. Other, Vision: Sensation of flashing lights, blurred vision, scintillating scotomata

4. **Amount of expected toxicities of platinum and paclitaxel therapy as determined by 3 previous clinical trials:**

Frequency of Grade 3/ 4 Events	CT IP vs. CT IV[32]	Dose Dense CT vs. CT IV[33]	ICON4: CT IV vs. Platinum alone (grades 2-4)[34]
Leukopenia/ Neutropenia	76% v 64%	92% v 88%	NR
Other heme event	94% v 90%	NR	29% v 46%
Thrombocytopenia	NR	44% v 38%	NR
Anemia	NR	69% v 44%	NR
GI event/ Nausea+Vomitting+Diarrhea	46% v 24%	16% v 18%	35% v 40%
Metabolic event	27% v 7%	NR	NR
Neuro event/ Motor + sensory	19% v 9%	12% v 10%	20% v 1%
Fatigue	18% v 4 %	5% v 3%	NR
Infection/ Febrile neutropenia	16% v 6%	9% v 9%	17% v 14%
Renal	7% v 2%	NR	8% v 9%
Hepatic	3% v <1%	NR	NR
Alopecia	NR	NR	86% v 25%

NR = not reported

a. **Intraperitoneal (CT IP)** = paclitaxel 135mg/m2 i.v. over 24 hours + cisplatin 100mg/m2 i.p. on day 2 + paclitaxel 60mg/m2 on day 8 **vs. CT IV** = paclitaxel 135mg/m2 iv over 24 hours + cisplatin 75mg/m2 iv on day 2[32]
b. **Dose Dense** = paclitaxel 80mg/m2 i.v. over 1 hour on days 1, 8, and 15 + carboplatin AUC 6mg/ml/min on day 1 of a 21 day cycle **vs. Conventional (CT IV)** = paclitaxel 180mg/m2 iv over 3h + carboplatin AUC 6mg/ml/min on day 1 of 21 days cycle[33]
c. ICON4/AGO-OVAR-2.2: **CT IV** = Paclitaxel 175mg/m2 iv over 3hr + Carboplatin AUC 5-6mg or Cisplatin 50 mg/m2 iv on day 1 ever 21 days **vs. Cisplatin** 75mg/m2 on day 1 every 21 days[34]

7.2. Dose Modifications for Metformin

1. **Toxicities** should be attributable to the study drug to constitute Dose-Limiting Toxicity (DLT).

Patients who have one DLT will be allowed to reduce the metformin dose to 500mg daily. If a second DLT occurs, the patient will be removed from the study.

Dose Modifications	
Dose level	**Metformin**
-1	500 mg PO daily
1	500 mg PO BID

2. **Hematologic toxicities** are known (expected) adverse events associated with standard adjuvant chemotherapy (platinum and paclitaxel). There are no hematologic toxicities associated with metformin; therefore there will be no metformin dose modifications due to hematologic toxicities. For Grade 4 hematologic toxicities, growth factor support is recommended. It is unexpected that metformin will exacerbate hematologic toxicities, however, if greater than 30% grade 4 hematologic toxicities are observed, new patients will be enrolled at dose level (-)1.

3. **Non-hematologic toxicity**
   a. **Diarrhea:** Grade 2 diarrhea can be managed with loperamide and i.v. fluid hydration as needed at the discretion of the patients physician. Diarrhea is expected to resolve within 2 weeks of starting metformin. If Grade 3 diarrhea persists greater than 2 weeks despite optimal medical management hold until recovery to Grade 1 and then dose reduce to Level -1. Any patient with Grade 4 diarrhea despite optimal medical management will be removed from the trial.
   b. **Nausea, Vomiting, Constipation:** It is expected that patients with nausea, vomiting, or constipation will receive appropriate medical management without dose modification. However, patients with persistent grade 3 (or greater) toxicity despite of optimal medical management require metformin dose reduction of -1 level.
   c. **Headache:** It is expected that patients with headache will receive appropriate medical management without dose modification.
   d. **Renal Toxicity:** Any patient with a creatinine >1.4 mg/dl, hold metformin until recovery to normal renal function (up to 2 weeks) then restart at dose level -1. If no recovery within 2 weeks or a second event, the patient will be removed from the trial.
   e. **Hepatic Toxicity:** Any Grade 3 (or greater) elevations in SGOT (AST), SGPT (ALT), or bilirubin due to any medication (a known toxicity of paclitaxel), hold metformin until recovery to patients baseline and restart at -1 dose reduction.
   f. **Peripheral Neuropathy:** Peripheral neuropathy is a known adverse event associated with paclitaxel therapy. It is not associated with Metformin use, therefore there will be no metformin dose modifications based on peripheral neuropathy.
   g. **Alopecia, Fatigue:** There will be no dose modifications for alopecia or fatigue.
   h. **Other Non-hematologic Toxicities:** Any other non-hematologic abnormal laboratory criteria (Grade $\geq$ 3) will be considered DLT if clinically significant and not clearly attributable to another cause. If baseline value is elevated prior to drug therapy, an increase will not be considered a DLT unless there is an elevation by more than 2 grades and it is of clinical significance. This DLT will require reduction of one dose level.

7.3. Safety Analysis: In order to protect patients in the event that metformin treatment was not a safe as we believe a priori, we have implemented a series of stopping rules that will be applied after sequential cohorts of 10 patients are accrued. The acceptable levels of toxicity for each category have been determined based upon our own experience with the chemotherapy regimens used at the University of

Michigan and from published toxicity rates in the literature (see section 7.1 #4). Section 12.6 outlines specific details for stopping the trial.

Type of Toxicity	Expected Grade 3/ 4 Events due to chemotherapy	Allowed Grade 3/ 4 Events with addition of metformin	Amount of Grade 3/ 4 Events to stop trial
Hematologic toxicities: -Neutropenia	90%	Any	>20% of patients requiring dose delays not manageable with growth factor
-Thrombocytopenia	45%	<70%	70%
-Anemia	95%	Any	>20% of patients requiring blood transfusions
Nephrotoxicity	10%	<20%	20%
Neurotoxicity	20%	<40%	40%
Hepatic toxicities	5%	<15%	15%
GI toxicities	45%	<70%	70%

7.4. Dose Escalations: Other than with the initiation of drug pre/post-surgery or post CT scans, there will be no dose escalations (or re-escalations) on this study.

8. Study Calendar A-Patients treated with primary surgical debulking and adjuvant chemotherapy.

Study Calendar																		
		Treatment										Follow Up Year 1				Follow up Year 2		
Timeline	Pre-Study	Pre- surgery	Surgery	Post-surgery	Chemotherapy[g]						Month 3[f]	Month 6[f]	Month 9[f]	Month 12[f]	Month 16[f]	Month 20[f]	Month 24[f]
					Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6							
Metformin 500mg PO		For 3 days		Resume 1 week prior to initiation of chemotherapy													
Metformin 500mg BID		Begin after 3 days of dose level -1 up until 48 hours prior to surgery			X Stop after last cycle of chemotherapy												
Adjuvant Chemotherapy					X Chemotherapy regimen and start time as determined by Gyn Onc												
Informed Consent	X																
Demographics	X																
Medical History	X																
Concurrent Meds	X[d]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs including weight	X[d]				X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X[d]				X	X	X	X	X	X							
Physical Exam	X[d]				X	X	X	X	X	X	X	X	X	X	X	X	X
Performance Status	X[d]				X	X	X	X	X	X	X	X	X	X	X	X	X
CBC (w/diff, plts)	X[d]				X	X	X	X	X	X							
Serum Chemistry[a]	X[d]				X	X	X	X	X	X							
LFTs[b]	X[d]				X	X	X	X	X	X							
CA-125	X[d]				X	X	X	X	X	X	X	X	X	X	X	X	X
PT/INR/PTT[c]	X[d]				X	X	X	X	X	X							
HgBA1C	X[d]																
Adverse Event Evaluation	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Radiologic Studies[e]	X																
Tissue Collection			X														

a: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium
b: albumin, alkaline phosphatase, total bilirubin, total protein, AST, ALT
c: for patients on Warfarin only or per surgeon's discretion
d: 14 days prior to registration
e: if clinically indicated, not required for registration, may include X-rays, CT scans, MRI, and PET scans
f: +/- 2 weeks
g: Patients will be monitored prior to every chemotherapy cycle at their pre-chemotherapy clinic appointment.

Study Calendar B—Patients treated with neoadjuvant chemotherapy

Study Calendar																					
		Treatment												Follow up							
Timeline	Pre-Study	Pre- Chemotherapy	Neo-adjuvant Chemotherapy[g]						Surgery	Pre-Adjuvant Chemotherapy	Adjuvant Chemotherapy [h]			Year 1				Year 2			
			Cycle 1	Cycle 2	Cycle 3	Cycle 4[h]	Cycle 5[h]	Cycle 6[h]			Cycle 1	Cycle 2	Cycle 3	Month 3[f]	Month 6[f]	Month 9[f]	Month 12[f]	Month 16[f]	Month 20[f]	Month 24[f]
Metformin 500mg PO for 3 days		X [i]								X [i]										
Metformin 500mg BID		Begin after 3 days of dose level -1	X Stop 48 hours prior to surgery								X stop after last cycle of chemotherapy									
Chemotherapy			X Chemotherapy regimen and start and stop time as determined by Gyn Onc								X Chemotherapy regimen and start and stop time as determined by Gyn Onc									
Informed Consent	X																			
Demographics	X																			
Medical History	X																			
Concurrent Meds	X[d]		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Vital Signs including weight	X[d]		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
Height	X[d]		X	X	X	X	X	X			X	X	X							
Physical Exam	X[d]		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
Performance Status	X[d]		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
CBC (w/diff, plts)	X[d]		X	X	X	X	X	X			X	X	X							
Serum Chemistry[a]	X[d]		X	X	X	X	X	X			X	X	X							
LFTs[b]	X[d]		X	X	X	X	X	X			X	X	X							
CA-125	X[d]		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X
PT/INR/PTT[c]	X[d]		X	X	X	X	X	X			X	X	X							
HgBA1C	X[d]																			
Adverse Event Evaluation	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Radiologic Studies[e]	X																			
Tissue Collection									X											

a: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium
b: albumin, alkaline phosphatase, total bilirubin, total protein, AST, ALT
c: for patients on Warfarin only or per surgeon's discretion
d: 14 days prior to registration
e: if clinically indicated, not required for registration, may include X-rays, CT scans, MRI, and PET scans
f: +/- 2 weeks
g: Patients will be monitored prior to every chemotherapy cycle at their pre-chemotherapy clinic appointment.
h: if indicated --3-6 cycles of neoadjuvant chemotherapy and an additional 3 cycles of adjuvant therapy are allowed as determined by the primary physician.
i: start the day of initiation of chemotherapy

9. Evaluation Criteria

9.1. Definition of progression or recurrence and survival will be defined as increasing clinical, radiological or histological evidence of disease since study entry or two serum values of CA-125 greater

than or equal to two times the upper limits of normal (ULN) performed at least one week apart, regardless of CT scan results. Since disease progression based only on rising CA-125 involves two observations on two different dates, the date of progression will be defined as the first date on which the CA-125 was greater than or equal to two times the upper limit of normal. In the event of increasing symptoms and no elevation of CA-125, a CT scan will be performed to evaluate for progression. Patients with progressing disease based on rising CA-125 must have CT scan of the abdomen and pelvis performed.

9.2. Recurrence-Free Interval will be defined as date from start of chemotherapy to the date of first clinical, biochemical, or radiological evidence of progression or death due to any cause. RFS will be censored at the last assessment of disease progression for living patients who have not progressed.

9.3. Overall Survival (OS) will be defined as observed length of life from entry onto the protocol to death due to any cause, or for living patients, date of last contact (regardless of whether or not this contact is on a subsequent protocol).

9.4. All conclusions should be based on all evaluable patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should be provided.

9.5. Evaluable patients

1. **For clinical endpoints** “evaluable patients” will be defined as all patients with a final pathologic diagnosis of at least Stage IIC/III/IV and have taken metformin for at least 1 complete cycle of chemotherapy (regardless of whether it is during neoadjuvant or adjuvant chemotherapy). Patients who enroll and are not considered evaluable will be replaced in the trial.
2. **For translational endpoints** will be defined as all patients with a final pathologic diagnosis of at least Stage IIC/III/IV and have taken metformin for at least 11+/- 4 days prior to primary surgery or at least 1 complete cycle of neoadjuvant chemotherapy.

10. Adverse Event and Reporting Definitions

In the event of an adverse event, the first concern will be for the safety of the subject. Investigators are required to report any serious adverse event, whether expected or unexpected, and which is felt by the investigator to be reasonably or possibly related to or caused by Metformin. All events meeting these criteria will be reported for the time period beginning with any amount of exposure to Metformin through the protocol-defined follow-up period. Serious criteria, definitions, and guidance for reporting follows.

10.1 Definitions

Adverse event
Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An *adverse event* (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

Adverse reaction
An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Pre-existing condition
Any medical condition or laboratory abnormality with an onset date before initial investigation agent/intervention administration is considered to be pre-existing in nature.

Suspected adverse reaction
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the protocol, informed consent, investigator brochure, or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Serious
An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor (UNIVERSITY OF MICHIGAN), it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Previously planned (prior to signing the informed consent form) surgeries should not be reported

as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

Life-threatening
An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

10.2 Reporting of Serious Adverse Events Associated with Metformin

Event reporting for ovarian cancer protocols can be complicated and confusing to investigators, data managers, and regulatory oversight bodies because patients typically develop numerous complications such as infections, chemotherapy- related organ damage, medication side effects, etc. as part of the typical course of treatment for ovarian cancer and not related to the study therapy. Furthermore, ovarian cancer- related complications often occur both simultaneously and in series, as one complication leads to a series of additional downstream events, making time-sensitive reporting of events difficult. Therefore, a well-conceived event reporting plan will separate complications that might be seen with any cancer, from study-related events that are relevant to subject safety. In order to achieve this goal, the DSM plan for this study will focus on rapid and specific identification and reporting of the following as SAEs:

a) Events which are serious and likely (probably or definitely) related to the investigational component of study therapy.
b) Events occurring at unusual frequency or severity in study subjects compared to non-study subjects undergoing similar treatment..
c) Events resulting in death regardless of attribution.
d) Events that are serious and unexpected

Therefore, we will not report as SAEs events that are expected and coincident with a typical ovarian cancer chemotherapy course unless they are either fatal or related to the investigational therapy.

- All serious adverse events (SAEs) that are definitely or probably related to Metformin (this applies to both expected and unexpected events) will be recorded on the CTO Standard Serious Adverse Event form.

- All serious adverse events that are definitely or probably related to Metformin will be reported to the IRB per current institutional standards.

Reporting Procedures

Serious adverse Events (SAE's) should be reported to:

Data Manager: Michelle Smith
Phone: (734) 232-0758
Fax: (734) 936-0645

Email: smimiche@med.umich.edu

Principal Investigator: Ronald J. Buckanovich, MD, PhD
Phone: (734) 764-2395
Fax: (734) 936-7376
E-mail:ronaldbu@umich.edu

The Clinical Trials Office (CTO) staff will coordinate the reporting process between the Investigator and the IRBMED as well as any other applicable reporting. Copies of all related correspondence and reporting documents will be maintained in the subject research chart and/or regulatory file as appropriate.

The data manager will complete the CTO SAE Report form for all adverse events meeting one or more of the serious criteria listed in Section 10.1. The SAE Report form will be submitted to the IRB within seven calendar days of the event or investigator's receipt of notification of the event.

Death: Deaths occurring **within 30 days** of the last study intervention are reportable events regardless of whether or not the investigators deem the death to be related to the study and must be submitted to the IRB within 7 calendar days of the event or investigator's receipt of notification of the event.
If death occurs later than 30 days after the last study intervention with metformin, then the death is not reportable unless related to metformin.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial.
Serious Adverse Events (SAEs) will continue to be followed until resolution or clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

The investigator is responsible for the detection, documentation, grading and assignment of attribution of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the principal investigator to ensure that all staff involved in the trial is familiar with the content of this section.

All adverse events occurring from the initial investigational agent/intervention administration through 30 days following the last dose of the investigational agent/intervention must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, severity (grade) or assessed relationship to the investigational agent/intervention.

Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history and recorded in the appropriate section of the case report form. In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins taking the investigational agent/intervention is also considered an adverse event.

10.3 Adverse Event Characteristics

CTCAE term
Adverse events (AE's) will use the descriptions and grading scales found in the revised National Cancer Institute (NCI) Common Terminology for Adverse Events Criteria (CTCAE).

A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page:
http://ctep.info.nih.gov/reporting/ctc.html

CTCAE v4.0 can also be found as a PDF file on the Cancer Center Intranet Clinical Trials Office page:
http://www.cancer.med.umich.edu/i/cto.htm

Attribution of the AE: The investigator is responsible for assignment of attribution.
Definite – The AE *is clearly related* to the investigational agent/intervention.
Probable – The AE *is likely related* to the investigational agent/intervention.
Possible – The AE *may be related* to the investigational agent/intervention.
Unlikely – The AE *is doubtfully related* to the investigational agent/intervention.
Unrelated – The AE *is clearly NOT related* to the investigational agent/intervention.

11. Data and Safety Monitoring

Scheduled meetings will occur every quarter or more frequently depending on the activity of the protocol. These meetings will include the protocol investigators and data managers involved with the conduct of the protocol. Scheduled meetings by the study specific Data and Safety Monitoring Committee (DSMC) will occur quarterly or more frequently depending on the activity of the protocol. This committee will include the protocol investigators, data manager or designee, and other members of the study team involved with the conduct of the trial.

During these regular meetings, the DSMC will discuss matters related to:

1. Safety of study participants (SAE/UaP reporting)
2. Validity and integrity of the data
3. Enrollment rate relative to expectations, characteristics of participants
4. Retention of participants, adherence to protocol (potential or real protocol deviations)
5. Data completeness

DSMC meetings will be documented by the Protocol Specific Data and Safety Monitoring Report (DSMR). The data manager or designee assigned to the trial will be responsible for completing the report. DSMRs will be signed by the Principal Investigator or by one of the co-investigators and will be kept on file in the Cancer Center CTO. (See Appendix 2 for DSMR.)

The University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Board (UMCCC DSMB) will provide independent oversight of the safety and data integrity for this trial. DSMRs and any other pertinent documents will be submitted to the UMCCC DSMB for review on a quarterly basis unless specified more frequently by a DSMB ruling.

The Principal Investigator or designee will forward all correspondence and recommendations generated by the UMCCC DSMB to the Institutional Review Board.

12. Statistical Considerations

12.1 Primary objectives: This trial has two primary objectives: 1- to determine the impact of adding metformin to traditional chemotherapy on recurrence-free survival when measured at 18 months following the initiation of adjuvant chemotherapy with metformin treatment following surgical resection, as compared to matched historical data, and 2 - to determine the impact of adding a short course of metformin prior to surgery on the proportion of cancer stem cells in the surgical specimen when compared to non-study, matched control patients.

12.2 Secondary objectives: This trial has three secondary objectives: 1 – to determine the overall survival of ovarian cancer patients from the initiation of treatment with adjuvant metformin and traditional chemotherapy following surgical resection, 2 – to confirm the safety of metformin treatment in non-diabetic ovarian cancer patients, and 3 - to correlate the number of CSC in the primary tumor specimens and recurrence-free survival.

12.3 Exploratory objectives: This trial will assess the impact of p53 status of tumor tissue on the patient's response to metformin treatment.

12.4 Power and sample size considerations: This trial will accrue 50 patients meeting the evaluable criteria (see section 9.5 for evaluable criteria). This trial will further assess the CSC proportion for 50 matched control patients for whom tissue is already stored or that will be stored by the Gynecologic Oncology Program during the conduct of this trial. The sample size for this trial was chosen after careful consideration of the statistical power to address the study's two primary objectives.

Historically 50% of ovarian cancer patients meeting our study's eligibility criterion would be alive and recurrence-free at 18 months following surgical resection. We hypothesize that with the addition of metformin to standard chemotherapy regimens, the proportion will increase to 70%. If we test this dichotomous outcome using a one-sided, one-sample exact binomial test (DTPLAN, Version 4.2) allowing at most 2.5% type I error (equivalent to 5% for two-sided test), then 49 patients are necessary for the test to have at least 80% power. As we believe adherence to protocol will be extremely high and loss to follow-up or treatment discontinuation to be extremely low, 50 patients are recommended for this trial.

The CSC proportion from surgical resection specimens after a short neoadjuvant course of metformin treatment will be compared to non-metformin treated, control ovarian cancer specimens. Control cases must meet the eligibility criteria set forth for this trial and will consist of frozen tissue samples currently available to the Gynecologic Oncology Program or from non-study patients with tumor resection at the University of Michigan. Untreated controls will be matched with primary surgery patients and controls who have received prior treatment will be matched with neoadjuvant patients. Preliminary data suggests that the ovarian cancer stem cell proportion is known to vary between 1 and 7% for resected specimens with an approximate mean of 3%. So it is expected that average CSC proportion for control cases will be 3%. With metformin treatment, it is hypothesized that the CSC proportion will be reduced by half, to 1.5%. In order to calculate power, the scale of the measurements will be transformed to their angular equivalents using an adaptation of the arcsine transformation of the square root of the proportion, in order to normalize the otherwise skewed distribution. On the transformed scale, the mean of the metformin treated cases and the control cases are expected to be 403.07 and 571.48, respectively. We expect mild variability, an assumption formalized by setting the standard deviation equal to ½ of the mean for each group. With 50 treated and 50 control cases, using a two-sample, two-sided, t-test will yield over 90% power to detect the hypothesized difference significantly, with at most 5% type I error.

12.5 Analysis Plan: The proportion of CSCs per patient will be calculated based upon the ALDH+ or ALDH+/CD133+ phenotype as measured by FACS. The proportion will be summarized graphically by histogram with the mean, median, 25^{th} and 75^{th} percentiles, and the minimum and maximum proportions reported. The results will be stratified by treatment versus control status and the p-value from the arcsine transformed t test comparison of the mean proportions reported.

The crude proportion of patients alive and recurrence-free at 18 months will be reported. Given that loss to follow-up is expected to be minimal or non-existent, the crude proportion should equal the

product-limit estimate of Kaplan and Meier which will also be used to estimate the recurrence-free survival experience over the entire follow-up period available. Point-wise confidence interval will be constructed using Greenwood's estimate of the variance using the log-log transformation of the RFS estimate.

Recurrence-free survival will be summarized for those patients with CSC proportions at or above the median study value versus below the median for a crude comparison of differences in RFS by CSC number. If visual evidence exists that patients with CSC proportion values below the median have longer RFS, then more sophisticated analyses may be explored using the Cox proportional hazard model. Categorical or continuous transformed values for the CSC proportion may be modeled along with clinical and patient characteristics known to influence RFS, including but not limited to patient's age, stage of disease, and CA125 levels.

The overall survival of the treated population will be reported by the product-limits estimates of Kaplan and Meier. Point-wise confidence intervals will be reported as described for RFS.

The safety of metformin treatment in non-diabetic ovarian cancer will be monitored throughout the conduct of the trial by the stopping rules discussed in the following section. At the conclusion of the trial, if observed toxicity has not triggered the study's stopping rules, the highest graded toxicity (CTCAE, version 4.0) will be reported for each patient in the following categories: hematologic (separated into neutropenia, anemia, and thrombocytopenia), nephrologic, neurologic, hepatic, and gastrointestinal. The number and proportion of the highest graded toxicity for each category will be reported.

The p53 mutation status will be determined for each treated patient, with patients grouped as wild-type or mutant p53. A priori we expect the patients to be equally divided, with approximately 25 patients in each group. We further expect that metformin treatment may be more likely to benefit p53 mutant patients than wild-type. RFS and OS will be summarized by group and differences tested for using the log-rank test statistic. If we accept our hypothesis that 70% of patient will be alive and recurrence-free at 18 months and that the sample size of mutant and wild-type p53 are equal, then it might be plausible to expect that 80% of p53 mutant patients are alive and recurrence-free at 18 months, while only 60% of wild-type are. The trial as constructed would only have 35% power to detect such a difference significantly, hence this objective designation as exploratory.

12.6 Safety Stopping Rule: In order to protect patients in the event that metformin treatment was not a safe as we believe a priori, we have implemented a series of stopping rules that will be applied after sequential cohorts of 10 patients are accrued. The stopping rules have been constructed based upon CTCAE version 4.0 grading of toxicity as grade 3 or 4 in 5 categories: nephrologic, neurologic, hepatic, thrombocytopenic, and gastrointestinal. The rules were created using the Bayesian beta-binomial framework, so that if at the assessment points we becoming at least 90% confident that the probability of toxicity in any one of the toxicity categories is above our acceptable level, the trial will halt for further investigation and possible changes in treatment, or trial termination. The acceptable levels of toxicity for each category have been determined based upon our own experience with the chemotherapy regimens used at the University of Michigan and from published toxicity rates in the literature (see section 7.1 #4). That information suggests that over the differing chemotherapy used, that with chemotherapy alone (without metformin treatment) that 10%, 20%, 5%, 45%, and 45% of ovarian cancer patients undergoing therapy would experience grade 3 or 4 nephrologic, neurologic, hepatic, thrombocytopenic, and gastrointestinal toxicity. The prior probabilities of toxicity with chemotherapy alone were used to create beta prior distributions for each toxicity category, minimally informative with the distribution centered at the mean, with the equivalent of 2 patients worth of information. For example, the prior beta distribution for nephrologic toxicity was B (0.20, 1.80). The following table lists the stopping rules for each toxicity category and the acceptable threshold applied. With the implementation of these rules we believe this patient population will be protected from undue increases in treatment related toxicity attributable to the addition of metformin.

Number of patients experiencing the listed toxicity (grade 3/4) that would trigger the stopping rule (acceptable threshold in parentheses)					
# patients on treated on trial	**Hepatic (15%)**	**Nephrologic (20%)**	**Neurologic (40%)**	**Thrombocytopenia (70%)**	**Gastrointestinal (70%)**
10	4	5	7	9	9
20	6	7	12	17	17
30	8	10	17	25	25
40	10	12	21	32	32

13. Registration Guidelines

13.1 When it has been verified that a patient meets the inclusion and exclusion criteria and has consented for this trial, the study data manager, Michelle Smith, should be contacted to register the patient. Please fax a completed eligibility checklist, required laboratory tests and signed consent form to Michelle Smith and the PI (Ronald Buckanovich), between 9 a.m. and 4 p.m. Monday through Friday.

Michelle Smith
Phone: (734) 232-0758
Fax: (734) 936-0645
Email: smimiche@med.umich.edu

Ronald J. Buckanovich, MD, PhD
Phone: (734) 764-2395
Fax: (734) 936-7376
E-mail: ronaldbu@umich.edu

13.2 At the time of registration, personnel will verify the following:

1. Patient eligibility checklist

2. Existence of a signed consent form
3. Existence of a signed authorization for use and disclosure of protected health information.

13.3 Treatment on this protocol must be administered under the supervision of an oncologist.

13.4 Treatment cannot begin prior to registration, and must begin within 14 days of registration.

13.5 Pre-study tests must be completed within the guidelines specified in the Study Calendar.

14. Data Forms, Submission and Distribution Information

Data Forms Submission Schedule		
Forms to be submitted	**When**	**To What Location**
Eligibility Checklist	At enrollment	CTO
Consent Form	At enrollment	CTO
Data and Safety Monitoring Reports	Quarterly	CTO
Medication Diary	Prior to each chemotherapy cycle	CTO
Response to Treatment	Every 3 cycles while on therapy Every 12 weeks post chemo year 1 and every 4 months year 2.	CTO
Adverse Events Report	As dictated in section 11.3	PI and Data Manager in CTO

15. Specific Instructions Used to describe special samples, cores or other procedures (including pathology review) if used in the protocol. For in-house protocols, defines areas of responsibility.

15.1 Personnel Responsibilities: Kun Yang (Research assistant): Dr. Yang will be responsible for all laboratory analysis of patient tissue specimens including tumor processing for FACS analysis and IHC staining. Mr. Yang will also do all batch analysis of stem cell specimens in conjunction with Dr. Shank.

15.2 Tumor Processing: At the time of primary debulking surgery we will obtain tumor specimens from all treated and non-treated patients. Kun Yang, Lula Cabrera or one of their representatives will be obtaining the tissue specimens directly from the operating room. Half of each tumor specimen will be mechanically dissected into single cell suspensions as previously described[35]. Isolated cells will then be frozen for future batch FACS analysis (see below). The remaining portion of each sample will be split again, with one portion snap frozen and the remaining piece fixed in formalin and embedded for IHC analysis

15.3 Cancer Stem Cell Assessment: Cancer stem cells in each tumor sample will be assayed in batches of 10 specimens (5 treated and 5 untreated). For each sample we will assess tumor sphere formation from whole tumor single cell suspensions as previously described [2]. Briefly, ~4,000 viable cells will be plated in triplicate in ultra-low attachment plates (Corning, Acton, MA, USA) in serum-free DMEM/F12 (Invitrogen) or EBM-2 (Lonza) supplemented with 5 µg/mL insulin (Sigma), 20 ng/mL human recombinant epidermal growth factor (EGF; Invitrogen), 10 ng/mL basic fibroblast growth factor (bFGF; Invitrogen), and 0.4% bovine serum albumin (BSA; Sigma). Sphere formation is counted 2 weeks after seeding the cells. Spheres are then photographed in overlapping high power fields (90-100% coverage). Sphere number is then compared between groups using a two-sided student's t-test. Spheres are then mechanically separated and re-plated to assess the ability to passage. This is repeated for a total of 3 passages.

For each sample we will also assess the percentage of ovarian CSC. We will use both FACS and AQUA co-immunofluorescence to determine the percentage of ALDH+ and CD133+, or ALDH+/CD133+ cells in primary ovarian tumors. We found that the presence of ALDH+CD133+ cells was strongly predictive of poor outcome. Whereas, consistent with previous studies, ALDH strong tumors were associated with a good outcome. We will therefore quantify these populations in the metformin treated and non-treated tumors to determine if metformin has an impact on the percentage of each of these putative CSC populations. FACS analysis and AQUA analysis will be performed as previously described[2].

Finally, we will confirm the functional activity of the CSC in these tumor specimens. From each of the isolated tumors will we inject tumors in NOD/Shi-scid/IL-2Rγnull (NOG) mice and assess (1) engraftment and (2) rates of growth. All tumors are weighed at the time of resection and then a portion of each tumor is snap frozen for histological analysis and another portion is processed into a single cells suspension as described above. Tumor volumes are calculated using the (L*W*W)/2 formula. Tumor weights are compared using a student's t-test. Tumor growth curves are compared using ANOVA and a student's t-test.

Appendix 1

The University of Michigan Comprehensive Cancer Center
Clinical Trials Office

Protocol Number: UMCC 2011.037
A Phase III Evaluation of Metformin, Targeting Cancer Stem Cells for the Prevention of Relapse in Patients with Stage IIC/III/IV Ovarian, Fallopian Tube and Primary Peritoneal Cancer

Registration and Eligibility Checklist

Date:_____ Patient Initials: *(first, middle, last)*_____ Date of Birth _____

Eligibility

Answers 1-10 must be 'Yes' to be eligible for this study. Record all dates in (mm/dd/yyyy) format.

1. Does the subject have a potential diagnosis of ovarian, fallopian tube or peritoneal cancer? __Yes __No

2. Does the patient's plan of care include either (1) neo-adjuvant chemotherapy and surgical debulking OR (2) primary debulking surgery and adjuvant chemotherapy? (Patients for whom surgical debulking is not planned are not eligible). __Yes __No

3. Is the ECOG performance status 0-2? __Yes __No
   ECOG:_____

4. Is the patient > 18 years or older? __Yes __No

5. Is the patient < 80 years old __Yes __No

6. Does the patient have normal organ function as defined in the protocol Section 5.1, obtained within 14 days prior to registration? (ULN = upper normal limit) __Yes __No

Date of labs: _____
Creatinine <1.4mg/dl: _____
Bilirubin <1.5x UNL: _____ ULN: _____
ALT < 2.5x UNL (<5x ULN for liver metastases): _____ULN: _____
AST < 2.5x UNL (<5x ULN for liver metastases):_____ ULN: _____

7. Is the patient willing to provide blood samples for correlative studies and paraffin blocks/slides from prior diagnostic samples? __Yes __No

8. Does the patient consent to fresh tissue sample donation? __Yes __No

9. Is the patient competent and willing to provide written documentation of informed consent? __Yes __No

10. Is the patient mentally, physically and geographically able to undergo treatment and follow up? __Yes __No

11. Does the patient have diabetes mellitus? _Yes __No __NA

12. Has the patient been treated with Metformin in the last 6 months? __Yes __No __NA

13. Does the patient have a known hypersensitivity to Metformin? __Yes __No __NA

Protocol Number: UMCC 2011.037
A Phase III Evaluation of Metformin, Targeting Cancer Stem Cells for the Prevention of Relapse in Patients with Stage IIC/III/IV Ovarian, Fallopian Tube and Primary Peritoneal Cancer

14. Does the patient have a history of metabolic acidosis, including ketoacidosis or increased risk of lactic acidosis? __Yes __No __NA

15. Is the patient pregnant or lactating? __Yes __No __NA

16. Does the patient have any severe or uncontrolled medical conditions? __Yes __No __NA

17. Does the patient have a history of renal disease? __Yes __No __NA

18. Does the patient have any other known malignancy (excluding adequately treated basal cell or squamous cell skin cancer, in situ cancer, or other cancer for which the patient has been disease free for 2 years)? __Yes __No __NA

19. Is the patient receiving any other investigational agents? __Yes __No __NA

Pretreatment Evaluations: The following tests must be performed within 2 weeks of study enrollment:

History & Physical Exam: Date: _ _ /_ _ /_ _ _ _
Height, Weight, & ECOG Status: Date: _ _ /_ _ /_ _ _ _
CBC w/differential & platelets: Date: _ _ /_ _ /_ _ _ _
HgBA1C Date: _ _ /_ _ /_ _ _ _
Chemistries: (sodium, potassium, chloride, bicarbonate, BUN, Creat, Calcium) Date: _ _ /_ _ /_ _ _ _
Liver Function Tests: (albumin, alkaline phosphatase, total bilirubin, total protein, AST, ALT) Date: _ _ /_ _ /_ _ _ _
Coagulation Profile (PT, PTT, INR) (For patients on Coumadin only or per surgeon's discretion) Date: _ _ /_ _ /_ _ _ _ or ___NA

Is the patient eligible? __Yes __No

Investigator Signature: _____ Date: _____

Appendix 2

PROTOCOL SPECIFIC DATA AND SAFETY MONITORING REPORT

UMCC PROTOCOL #: _____ **DSMC DATE:** _____

STUDY STATUS: _____ **DATE OF LAST REPORT:** _____

PROTOCOL TITLE			
ATTENDANCE			
PROTOCOL ACTIVITY			
Planned Accrual Duration:		Global Accrual to Date / Goal:	0 / 0
Date First UM Patient Enrolled:		UM Accrual to Date / Goal: List multi-stages individually	0 / 0
Consented Since Last Report:		Accrual Since Last Report:	
Eligible Since Last Report:		Ineligible Since Last Report:	
Eligibility Exceptions to Date:			

Specifically for Phase I and/or Dose Escalating Trials:	
Dose Level	Accrual
1	
2	
3	
4	

SAE/UaP REPORTING SINCE LAST REPORT♦ List event, patient study ID number, date of occurrence, and date IRB notified. Attach SAE reports. Attach the updated SAE/UaP spreadsheet summarizing ALL events that have occurred since trial initiation.
1]
2]
3]

PATIENTS COMPLETED/OFF PROTOCOL SINCE LAST REPORT ♦ Provide reason [progression, death, toxicity, completed therapy, etc]. Provide detailed supplemental information for patients off study treatment due to toxicity or death.

PROTOCOL DEVIATIONS SINCE LAST REPORT ♦ Include both *purposeful and accidental* variances in the approved procedures outlined for a study in its IRB approved protocol; provide date reported to Regulatory Affairs or IRB. Attach any protocol deviation forms.

PROTOCOL AMENDMENTS SINCE LAST REPORT♦ Include amendment summary, date submitted to regulatory bodies, and date approved.

OTHER COMMENTS

Investigator Signature:		**Data Manager Signature:**	
Date:		**Date:**	

PRE-SURGERY METFORMIN MEDICATION DIARY

•Fill out this diary every day

•Bring this diary and metformin bottles (empty or not) to your clinic appointment and give to the study team

•Remember to STOP TAKING METFORMIN 48 HOURS PRIOR TO YOUR SURGERY DATE

Day	Date	Time	Dose	CIRCLE Number of Tablets Taken
1		AM	500 mg	1
		PM	500 mg	1
2		AM	500 mg	1
		PM	500 mg	1
3		AM	500 mg	1
		PM	500 mg	1
	** INCREASE TO 500 MG TWICE DAILY UNLESS TOLD OTHERWISE BY YOUR DOCTOR**			
4		AM	500 mg	1
		PM	500 mg	1
5		AM	500 mg	1
		PM	500 mg	1
6		AM	500 mg	1
		PM	500 mg	1
7		AM	500 mg	1
		PM	500 mg	1
8		AM	500 mg	1
		PM	500 mg	1
9		AM	500 mg	1
		PM	500 mg	1
10		AM	500 mg	1
		PM	500 mg	1
11		AM	500 mg	1
		PM	500 mg	1
12		AM	500 mg	1
		PM	500 mg	1
13		AM	500 mg	1
		PM	500 mg	1
14		AM	500 mg	1
		PM	500 mg	1
15		AM	500 mg	1
		PM	500 mg	1
16		AM	500 mg	1
		PM	500 mg	1
17		AM	500 mg	1
		PM	500 mg	1
18		AM	500 mg	1
		PM	500 mg	1
19		AM	500 mg	1
		PM	500 mg	1
20		AM	500 mg	1
		PM	500 mg	1

PRE-SURGERY METFORMIN MEDICATION DIARY

Day	Date	Time	Dose	CIRCLE Number of Tablets Taken
21		AM	500 mg	1
		PM	500 mg	1
22		AM	500 mg	1
		PM	500 mg	1
23		AM	500 mg	1
		PM	500 mg	1
24		AM	500 mg	1
		PM	500 mg	1
25		AM	500 mg	1
		PM	500 mg	1
26		AM	500 mg	1
		PM	500 mg	1
27		AM	500 mg	1
		PM	500 mg	1
28		AM	500 mg	1
		PM	500 mg	1
29		AM	500 mg	1
		PM	500 mg	1
30		AM	500 mg	1
		PM	500 mg	1
31		AM	500 mg	1
		PM	500 mg	1
32		AM	500 mg	1
		PM	500 mg	1
33		AM	500 mg	1
		PM	500 mg	1
34		AM	500 mg	1
		PM	500 mg	1
35		AM	500 mg	1
		PM	500 mg	1

REMEMBER TO STOP METFORMIN 48 HOURS PRIOR TO YOUR SURGERY DATE

Patient Signature: _____ Date: _____

The following should be filled out by clinic staff only:

Number of Metformin tablet remaining in the bottle: _____

Signature of person doing the tablet count: _____ Date: _____

POST-SURGERY METFORMIN MEDICATION DIARY

• Please fill out this diary every day.
• Please bring this diary and your Metformin bottle (EMPTY OR NOT) to your clinic appointment and give them to the study team. Thank you

Day	Date	Time	Dose	CIRCLE Number of Tablets Taken
1		AM	500 mg	1
		PM	500 mg	1
2		AM	500 mg	1
		PM	500 mg	1
3		AM	500 mg	1
		PM	500 mg	1
4	** INCREASE TO 500 MG TWICE DAILY UNLESS TOLD OTHERWISE BY YOUR DOCTOR **			
		AM	500 mg	1
		PM	500 mg	1
5		AM	500 mg	1
		PM	500 mg	1
6		AM	500 mg	1
		PM	500 mg	1
7		AM	500 mg	1
		PM	500 mg	1

Patient Signature: _____ Date: _____

The following should be filled out by clinic staff only:

Number of Metformin tablet remaining in the bottle: _____

Signature of person doing the tablet count: _____ Date: _____

METFORMIN MEDICATION DIARY

•Fill out this diary every day

•Bring this diary and metformin bottles (empty or not) to your clinic appointment and give to the study team

Day	Date	Time	Dose	CIRCLE Number of Tablets Taken
1		AM	500 mg	1
		PM	500 mg	1
2		AM	500 mg	1
		PM	500 mg	1
3		AM	500 mg	1
		PM	500 mg	1
4	** INCREASE TO 500 MG TWICE DAILY UNLESS TOLD OTHERWISE BY YOUR DOCTOR**			
		AM	500 mg	1
		PM	500 mg	1
5		AM	500 mg	1
		PM	500 mg	1
6		AM	500 mg	1
		PM	500 mg	1
7		AM	500 mg	1
		PM	500 mg	1
8		AM	500 mg	1
		PM	500 mg	1
9		AM	500 mg	1
		PM	500 mg	1
10		AM	500 mg	1
		PM	500 mg	1
11		AM	500 mg	1
		PM	500 mg	1
12		AM	500 mg	1
		PM	500 mg	1
13		AM	500 mg	1
		PM	500 mg	1
14		AM	500 mg	1
		PM	500 mg	1
15		AM	500 mg	1
		PM	500 mg	1
16		AM	500 mg	1
		PM	500 mg	1
17		AM	500 mg	1
		PM	500 mg	1
18		AM	500 mg	1
		PM	500 mg	1
19		AM	500 mg	1
		PM	500 mg	1
20		AM	500 mg	1
		PM	500 mg	1

METFORMIN MEDICATION DIARY

Day	Date	Time	Dose	CIRCLE Number of Tablets Taken
21		AM	500 mg	1
		PM	500 mg	1
22		AM	500 mg	1
		PM	500 mg	1
23		AM	500 mg	1
		PM	500 mg	1
24		AM	500 mg	1
		PM	500 mg	1
25		AM	500 mg	1
		PM	500 mg	1
26		AM	500 mg	1
		PM	500 mg	1
27		AM	500 mg	1
		PM	500 mg	1
28		AM	500 mg	1
		PM	500 mg	1
29		AM	500 mg	1
		PM	500 mg	1
30		AM	500 mg	1
		PM	500 mg	1
31		AM	500 mg	1
		PM	500 mg	1
32		AM	500 mg	1
		PM	500 mg	1
33		AM	500 mg	1
		PM	500 mg	1
34		AM	500 mg	1
		PM	500 mg	1
35		AM	500 mg	1
		PM	500 mg	1

Patient Signature: _____ Date: _____

The following should be filled out by clinic staff only:

Number of Metformin tablet remaining in the bottle: _____

Signature of person doing the tablet count: _____ Date: _____

Appendix 4

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix 5

References

1. Edwards, B.K.*, et al.* Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst* **97**, 1407-1427 (2005).
2. Silva, I.B., S; McLean, K; Yang, K; Griffith, K; Thomas, D; Ginestier, C; Johnston, C; Kueck, A; Reynolds, R; Wicha, M; Buckanovich R. Aldehyde dehydrogenase and CD133 define angiogenic ovarian cancer stem cells that dramatically influence patient survival outcomes. *Cancer Research* **Accepted for publication**(2011).
3. Gupta, P.B.*, et al.* Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* **138**, 645-659 (2009).
4. Ginestier, C.*, et al.* CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. *J Clin Invest* **120**, 485-497 (2010).
5. Wei, X.*, et al.* Mullerian inhibiting substance preferentially inhibits stem/progenitors in human ovarian cancer cell lines compared with chemotherapeutics. *Proc Natl Acad Sci U S A* **107**, 18874-18879 (2010).
6. Hirsch, H.A., Iliopoulos, D., Tsichlis, P.N. & Struhl, K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* **69**, 7507-7511 (2009).
7. Vazquez-Martin, A.*, et al.* Metformin regulates breast cancer stem cell ontogeny by transcriptional regulation of the epithelial-mesenchymal transition (EMT) status. *Cell Cycle* **9**, 3807-3814 (2010).
8. Vazquez-Martin, A., Oliveras-Ferraros, C., Barco, S.D., Martin-Castillo, B. & Menendez, J.A. The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. *Breast Cancer Res Treat* (2010).
9. Becker, S., Dossus, L. & Kaaks, R. Obesity related hyperinsulinaemia and hyperglycaemia and cancer development. *Arch Physiol Biochem* **115**, 86-96 (2009).
10. Abe, R. & Yamagishi, S. AGE-RAGE system and carcinogenesis. *Curr Pharm Des* **14**, 940-945 (2008).
11. Bakhru, A., Buckanovich, R.J. & Griggs, J.J. The impact of diabetes on survival in women with ovarian cancer. *Gynecol Oncol* (2011).
12. Libby, G.*, et al.* New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* **32**, 1620-1625 (2009).
13. Evans, J.M., Donnelly, L.A., Emslie-Smith, A.M., Alessi, D.R. & Morris, A.D. Metformin and reduced risk of cancer in diabetic patients. *BMJ* **330**, 1304-1305 (2005).
14. Landman, G.W.*, et al.* Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* **33**, 322-326 (2010).
15. Jiralerspong, S.*, et al.* Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* **27**, 3297-3302 (2009).
16. Wright, J.L. & Stanford, J.L. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. *Cancer Causes Control* **20**, 1617-1622 (2009).
17. Li, D., Yeung, S.C., Hassan, M.M., Konopleva, M. & Abbruzzese, J.L. Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* **137**, 482-488 (2009).
18. Cantrell, L.A.*, et al.* Metformin is a potent inhibitor of endometrial cancer cell proliferation--implications for a novel treatment strategy. *Gynecol Oncol* **116**, 92-98 (2010).
19. Martin-Castillo, B., Vazquez-Martin, A., Oliveras-Ferraros, C. & Menendez, J.A. Metformin and cancer: Doses, mechanisms and the dandelion and hormetic phenomena. *Cell Cycle* **9**(2010).
20. Buzzai, M.*, et al.* Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* **67**, 6745-6752 (2007).
21. Dobrzycka, B.*, et al.* Circulating free DNA and p53 antibodies in plasma of patients with ovarian epithelial cancers. *Ann Oncol* (2010).
22. Shelling, A.N., Cooke, I.E. & Ganesan, T.S. The genetic analysis of ovarian cancer. *Br J Cancer* **72**, 521-527 (1995).
23. Schuijer, M. & Berns, E.M. TP53 and ovarian cancer. *Hum Mutat* **21**, 285-291 (2003).
24. Sambol, N.C.*, et al.* Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. *J Clin Pharmacol* **36**, 1012-1021 (1996).
25. McCarthy, E.A., Walker, S.P., McLachlan, K., Boyle, J. & Permezel, M. Metformin in obstetric and

gynecologic practice: a review. *Obstet Gynecol Surv* **59**, 118-127 (2004).
26. Eyal, S.*, et al.* Pharmacokinetics of metformin during pregnancy. *Drug Metab Dispos* **38**, 833-840 (2010).
27. Gotlieb, W.H.*, et al.* In vitro metformin anti-neoplastic activity in epithelial ovarian cancer. *Gynecol Oncol* **110**, 246-250 (2008).
28. Rattan, R., Graham, R.P., Maguire, J.L., Giri, S. & Shridhar, V. Metformin suppresses ovarian cancer growth and metastasis with enhancement of Cisplatin cytotoxicity in vivo. *Neoplasia* **13**, 483-491 (2011).
29. http://www.drugs.com/pro/metformin.html.
30. Odicino, F., Pecorelli, S., Zigliani, L. & Creasman, W.T. History of the FIGO cancer staging system. *Int J Gynaecol Obstet* **101**, 205-210 (2008).
31. Reagan-Shaw, S., Nihal, M. & Ahmad, N. Dose translation from animal to human studies revisited. *FASEB J* **22**, 659-661 (2008).
32. Armstrong, D.K.*, et al.* Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* **354**, 34-43 (2006).
33. Katsumata, N.*, et al.* Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. *Lancet* **374**, 1331-1338 (2009).
34. Parmar, M.K.*, et al.* Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. *Lancet* **361**, 2099-2106 (2003).
35. Pulaski, H.L.*, et al.* Identifying alemtuzumab as an anti-myeloid cell antiangiogenic therapy for the treatment of ovarian cancer. *J Transl Med* **7**, 49 (2009).