Mayo Clinic Cancer Center

Phase II Trial to Evaluate the Efficacy of Auranofin and Sirolimus in Serous Ovarian Cancer Patients with Recurrent Disease

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√Study contributor(s) not responsible for patient care

Funded in part by Mayo Clinic Ovarian SPORE

Drug Availability
Supplied Commercial Agents: auranofin and sirolimus (IND 136802)

Document History	**(Effective Date)**
Activation	30Mar2018
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Protocol Resources

Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion and submission	[redacted] Phone: E-mail:
Study Coordinator - Clinical	[redacted] Phone:
Study Coordinator - Data	[redacted] Phone:
Protocol document, consent form, regulatory issues	[redacted] Phone:
Non-paraffin biospecimens	[redacted] Phone:
Serious Adverse Event Reporting	[redacted] Phone:

*No waivers of eligibility allowed

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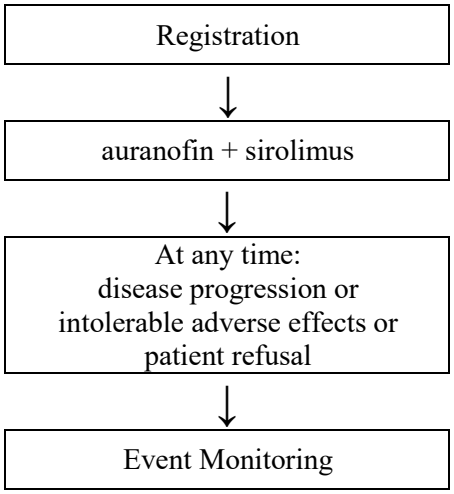
Schema



Cycle = 28 days

Generic name: auranofin Brand name(s): Ridura® Mayo Abbreviation: AURANOFIN Availability: Supplied Commercial	Generic name: sirolimus Brand name(s): Rapamune® Mayo Abbreviation: RAPA Availability: Supplied Commercial

1.0 Background

1.1 The Need for Studying Novel Approaches in Ovarian Cancer.

Three decades have yielded only two new classes of drug for treating epithelial ovarian cancer: angiogenesis inhibitors and poly-ADP ribose polymerase (PARP) inhibitors [1]. In contrast, in the past three years, 40 drugs have been approved for other cancer indications. This sluggish progress in ovarian cancer treatment underscores the urgent need for improved therapy.

1.2 PKCι as a Therapeutic Target in Ovarian Cancer.

The Fields' Laboratory has demonstrated that PKCι plays a critical role in ovarian cancer tumorigenesis. **As a result, patients with serous ovarian cancer appear to be logical candidates for mechanism-based therapies that target oncogenic PKCι signaling** [2-15]. In fact, PKCι is the first and, to date, the only PKC isozyme to be identified as an oncogene in human cancer; it is overexpressed in ovarian cancer, particularly in the serous histology. Importantly, PKCι expression in ovarian cancers correlates with tumor stage and thereby suggests an association between PKCι expression and tumor aggressiveness [12,16-18]. The PKCι gene (*PRKCI*) sits on chromosome 3q26 a region of DNA frequently amplified in human cancers [5-13]. Tumor PKCι mRNA and protein expression correlate with gene copy number gains of the *PRKCI* gene [2]. *PRKCI* gene amplification drives PKCι overexpression in primary ovarian cancer, particularly **ovarian serous tumors which harbor 3q26 amplification in approximately 80%** [5-13] (**Figure 1**). Decreased PKCι expression inhibits anchorage-independent growth of ovarian cancer cells, whereas overexpression of PKCι promotes murine ovarian surface epithelium transformation, showing further that PKCι is a gene target of 3q26 amplification in serous ovarian cancer [13-15].

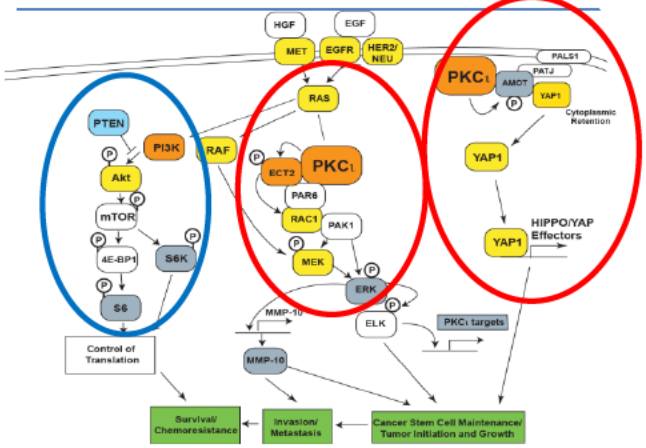


Figure 1: This proposal aims to inh bit 2 independent but synergistic pathways – the PKCι pathway and the HIPPO/YAP pathway which is unique to ovarian cancer (red circles) and the mTOR pathway (blue circle) -- in a clinical trial to treat ovarian cancer patients.

1.3 Further Preclinical Justification for Targeting PKCι.

First, PKCι-Par6 binding recruits the Rho family GTPase guanine nucleotide exchange factor Ect2 to the complex (**Figure 2**) [8-11]. Ect2 activates the Rho family GTPase Rac1 which in turn activates a Rac1-Pak-Mek-Erk signaling cascade [8-11]. PKCι directly phosphorylates Ect2 at T328, an event that is required for efficient binding of Ect2 to the PKCι-Par6 complex, activation of Rac1, and transformed growth and invasion in cancer cells. Interestingly, *PRKCI* and *ECT2* both reside at chromosome 3q26, and these genes are coordinately amplified and over-expressed in tumors harboring 3q26 amplification such as ovarian cancer of serous histology [8-11]. Thus, *PRKCI* and *ECT2* are genetically, biochemically and functionally linked in tumors harboring 3q26 amplification and together drive oncogenesis. The Fields Laboratory has also shown that **1)** *PRKCI* and *ECT2* are genetically, biochemically, and functionally linked in ovarian serous tumors and that **2)** the PKCι-Par6-Ect2-Mek-Erk signaling axis is active in ovarian tumor cells [8]. Second, the Fields Laboratory has shown that activation of the HIPPO/YAP pathway results

in ovarian cancer initiation and growth. PKCι directly phosphorylates AMOT at Thr750, thereby directly inhibiting YAP1 binding; conversely, inhibition of **PKC**ι decreases YAP1 nuclear localization and blocks ovarian tumor growth both *in vitro* and *in vivo* [13] (**Figure 1**). Third, from a practical standpoint, the Fields' Laboratory has used high throughput screening to identify gold compounds as inhibitors of PKCι [21,22]. Auranofin have been available for years, can be purchased for clinical use, and can now be **"repurposed"** for further testing in the clinic as an antineoplastic agent.

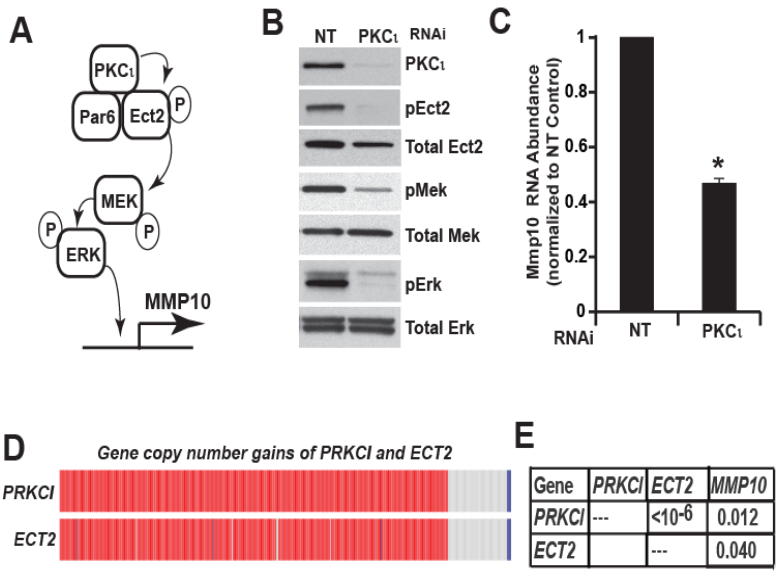


Figure 2: A) PKCι launches the PKCι-Par6-Ect2-Mek-Erk signaling pathway in ovarian cancer, as shown with a schematic overview of this pathway. **B)** PKCι leads to a decrease in Ect2 phosphorylation as well as a decrease in pMek and pErk. **C)** PKCι inh bits MMP10 (*p<0.05 compared to no treatment (NT) controls). **D)** Of note, PRKCI and ECT2 are co-amplified in ~80% of primary ovarian serous carcinomas. **E)** Also, *PRKCI*, *ECT2* and *MMP10* mRNA are strongly expressed in primary ovarian serous tumors.

1.4 The Addition of mTOR Inhibition.

mTOR is downstream of PI3K and is often co-amplified (*PIK3CA*) with *PRKCI* on 3q26 in high grade ovarian serous carcinoma, as per data from the Cancer Genome Atlas (TCGA). The Fields Laboratory has shown that inhibition of the mTOR pathway with clinically available agents such as sirolimus **1)** invokes inhibition of a separate, independent antineoplastic pathway for treating ovarian cancer; **2)** is synergistic (**Figure 1**); and **3)** is feasible in view of the clinical availability of two drugs – auranofin and sirolimus – both of which lend themselves to drug repurposing and provide a **scientifically compelling** argument for testing these drugs in combination.

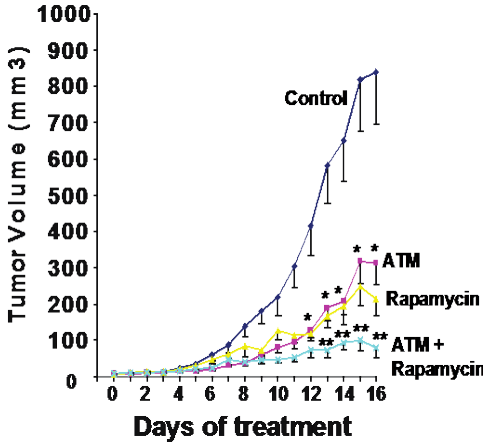


Figure 3: The combination of a gold compound (in this instance, ATM) and an mTOR inhibitor (in this instance, Rapamycin) yielded synergistic tumor kill that exceeded what was observed with either drug alone (unpublished).

This approach of combining drugs is very much in keeping with the widespread use of multi-drug regimens in cancer treatment. For this reason, our group sought synergy between inhibition of the PKCι pathway and inhibition of the mTOR pathway. The latter was chosen because it represents a distinctly independent pathway that leads to tumor cell survival and chemoresistance (**Figure 1**). The gold compound, aurothiomalate (ATM), in combination with an mTOR inhibitor (Rapamycin) yields synergistic tumor kill and marked reduction in tumor

volumes (**Figure 3**). Such data further suggest a need to study this drug combination in a clinical setting and provide justification for this clinical trial described in this protocol.

1.5 Preliminary Clinical Trial Data.

Indeed, our group has taken auranofin into the clinic. In a feasibility trial in heavily pre-treated ovarian cancer patients, we reported on a patient who had **baseline and monthly CA-125 levels of 5,570; 6,085; 3,511; and 2,230 U/ml, respectively, stopped auranofin because of radiographic progression at 3 months, and manifested an increase in CA-125 to 7,168 U/ml 3 months later** – a pattern indicative of the therapeutic benefits of auranofin for ovarian cancer patients [19,20].

PATIENT	FISH RATIO	PRKCι AMPLIFICATION	PKCι IMMUNO-HISTOCHEMISTRY	TUMOR RESPONSE
1	2.4	yes	3+	yes
2	2.8	yes	3+	yes
3	1.6	no	1+	no
4	2	no	1+	no
5	4.7	yes	3+	no
6	1.1	no	2+	yes
7	2.2	yes	2+	yes
8	1.1	no	1+	no
9	1.4	no	0	no

Additionally, a 9-patient trial in non-ovarian cancer patients yielded 3 findings: **1)** safe dosing consists of auranofin 6 mg/day orally and sirolimus 5 mg/day orally; **2)** a strong correlation exists between PKCι amplification/expression and tumor response (for example, p=0.048 for expression) -- observations that provide the scientific underpinnings for a clinical trial; and **3)** this combination yields tumor responses (**Figure 4)** and in the accompanying table, providing further rationale for a phase 2 trial in ovarian cancer.

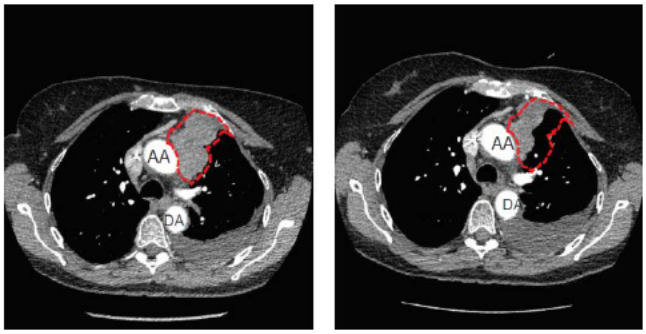


Figure 4: This Mayo Clinic patient had a non-ovarian tumor that overexpressed PKCι, received auranofin and sirolimus and manifested a partial tumor response after 6 weeks of therapy; this response was confirmed with a later scan (unpublished data).

1.6 Dosing discussion

Based on a small cohort of lung cancer patients that included 6 patients who were treated with the current dose of auranofin and sirolimus (per Section 7.0) on MC1125, no patients were found to have dose limiting toxicity. In other words, no patient experienced a Grade 3 or worse adverse event that was thought related to the study agents. For this reason, this same dose is being used in the current trial.

We have allowed for a larger dose modification to sirolimus if the initial dose is not well-tolerated. Because of the combination with auranofin, we choose to drop back on the sirolimus dose to this extent because we believe it will be safer for patients. The trial cited above (MC1125) used the same dose and schedule as the current trial. In another IIT using a combination of

auranofin and sirolimus in NSCLC (MC1213) started at 3mg and reduced to 1mg but also increased to 6mg and 9mg if tolerated.

1.7 Summary

This protocol represents the culmination of a well-delineated approach that **builds on decades of preclinical data from the Fields' Laboratory, is eminently feasible with 2 clinically available drugs that can be repurposed to treat cancer, and has already shown clinical signs of therapeutic promise.** The foregoing justifies the study of this combination in patients with ovarian cancer.

NOTE: While patient care fot this trial is being done in Rochester, MN, the Fields' Laboratory in Jacksonville, FL will receive available patient specimens from this trial for analysis as delineated on the title page.

2.0 Goals

2.1 Primary Goal

To estimate the overall tumor response rate (ORR, that is, complete response (CR) + partial response (PR)) of the combination of auranofin and sirolimus in the setting of metastatic serous ovarian cancer across all patients

2.2 Secondary Goal

2.21 Key secondary endpoint: To estimate the overall tumor response rate (ORR, that is, complete response (CR) + partial response (PR)) of the combination of auranofin and sirolimus in the setting of metastatic serous ovarian cancer within patients that have overexpression of PKCι.

2.22 To estimate progression-free survival, overall survival, and adverse events from the combination of auranofin and sirolimus

2.3 Correlative Research

To explore whether PKCiota-relevant biomarkers in serous ovarian cancer tumors are associated with treatment response patterns, such as ORR, progression free survival, and overall survival

3.0 Registration Patient Eligibility

3.1 Inclusion Criteria

3.11 Age ≥18 years.

3.12 Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1 (see Appendix I)

3.13 Subjects must have histologically confirmed high grade ovarian, fallopian tube, or primary peritoneal carcinoma of serous histology (any of these three are referred to in this protocol as "ovarian cancer")

3.14 Incurable cancer

3.15 Willingness to provide paraffin-embedded tissue blocks of ovarian cancer

3.16 Measurable disease (see Section 11.0)

3.17 The following laboratory values obtained ≤14 days prior to registration:

- ANC ≥1500 μL
- PLT ≥100,000 μL
- Hgb ≥9 g/dL
- Total bilirubin ≤1.5 x upper limit of normal (ULN) or direct bilirubin ≤ULN
- SGOT (AST) and SGPT (ALT) ≤3 x ULN or SGOT (AST) and SGPT (ALT) ≤5 x ULN is acceptable if liver has tumor involvement
- Creatinine ≤1.5 x ULN
- Fasting serum glucose ≤1.5 x ULN
- Total cholesterol ≤1.5 x ULN
- Triglycerides ≤1.5 x ULN

3.18 Life expectancy ≥12 weeks

3.2 Exclusion criteria

3.21 Platinum-sensitive disease (exceptions allowed: patient has had a hypersensitivity reaction to platinum or the treating oncologist thinks that further platinum therapy is not in the patient's best interest).

3.22 Morbidities or concurrent major illness (for example, bowel obstruction or a second active malignancy) that, in the opinion of the treating healthcare provider, would make participation in the trial problematic

3.23 Leptomeningeal disease or uncontrolled brain metastasis.

3.24 Failure to recover from acute, reversible effects of prior therapy regardless of interval since last treatment.
NOTE: Patients can have peripheral (sensory) neuropathy.

3.25 History of hypertriglyceridemia or hypercholesterolemia and currently on medication(s).

3.26 Use of St. John's Wort ≤7 days prior to registration.

3.27 Unable to discontinue use of a strong CYP3A4 inhibitor (see Appendix III).

4.0 Study Calendar

4.1 Test schedule for ovarian cancer

Tests and procedures	≤28 days prior to registration	≤14 days prior to registration	≤7 days prior to registration	Prior to the start of each treatment cycle, Cycle 2 and beyond	End of all protocol treatment for any reason
window				±3 days	±7 days
History and exam, weight, PS	X			X	X
Height	X				
Adverse event assessment		X		X	X
Urine pregnancy test[1]			X		
Hematology group: CBC with differential		X		X	X
Chemistry panel Alanine aminotransferase (ALT) Aspartate aminotransaminase (AST) Alkaline phosphatase Total or direct bilirubin Creatinine		X		X	X
Lipid profile including cholesterol, triglycerides Fasting serum glucose		X		X	
Urinalysis/urine protein dipstick		X		X[2]	
CA-125[3]	X			X	
Tumor measurement[4]	X			X	X
Patient Medication Diary (Appendix II)[5]				X	X
Mandatory submission of archived research tissue specimens (see Section 17.0) [R]	X[6]				
Optional submission of tissue obtained at the time of progression (See Section 17.0)[R]					X

[1] For persons of childbearing potential only. NOTE: Most patients (99%) will be unable to bear children due to the cancer being studied.
[2] If nephrotic range proteinuria is suspected, then a 24-hour urine collection is suggested.
[3] Use same timing as tumor measurements: to be done at the completion of Cycles 2, 4, 6, etc., until disease progression or treatment discontinuation.
[4] Tumor measurements (CT or MRI) are to be done at the completion of Cycles 2, 4, 6, etc., until disease progression or treatment discontinuation. Use same imaging throughout the study.
[5] The diary must begin the day the patient starts taking the medication and must be completed per protocol and returned to the treating institution OR compliance must be documented in the medical record by any member of the care team.
[6] If the patient makes every effort to submit a tissue block, but this material is unable to be obtained, this mandatory submission can be waived.

Cycle = 28 days; R=Research funded (see Section 19.0)

4.2 Event Monitoring/Survival Follow-up

	Event Monitoring Phase[1]				
	q. 6 months until PD	At PD	After PD q. 6 months	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

1. If a patient is still alive 3 years after registration, no further follow-up is required.

5.0 Grouping Factors: None

6.0 Registration Procedures

6.1 Registration

6.11 Patient registration

Access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page (http://hsrwww.mayo.edu/ccs/training) and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual and institution registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted]. If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.12 Verification

Prior to accepting the registration, the registration application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.2 Verification

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval of the study
- Patient eligibility
- Grouping factor

6.3 Documentation of IRB approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office (fax: [redacted]). If the necessary documentation is not submitted in advance of attempting

patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Correlative studies

6.41 A mandatory correlative research component is part of this study; the patient will be automatically registered onto this component.

6.42 An optional correlative research component is part of this study, there will be an option to select if the patient is to be registered to this component.

- Patient has/has not given permission to give tissue specimens for research purposes

At the time of registration, the following will also be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future research of cancer at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.5 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic under the supervision of a medical oncologist or endocrinologist

6.6 Treatment start

Treatment cannot begin prior to registration and must begin ≤14 days after registration.

6.7 Baseline symptoms

All required baseline symptoms must be documented and graded.

6.8 Study drug is available on site

Study drug is available on site for this patient.

7.0 Protocol Treatment

7.1 Treatment Schedule

7.11 Treatment medication table

Agent	Dose Level	Route	Day	ReRx
auranofin	6 mg/day	PO	1-28 (continuous)	every 28 days
sirolimus	5 mg/day	PO	1-28 (continuous)	every 28 days

7.12 For the purposes of this study, a treatment cycle is 28 days.

7.2 Auranofin and sirolimus

Take both drugs together as one dose. Each dose is to be taken at the same time of the day under the same conditions, i.e., if fasting, then should be fasting every time; if taken with food, should be taken with food every time. Each dose should be separated by 24 hours (±4 hours).

Definition of fasting conditions: At least 2 hours after last food consumption and at least 1 hour before next food consumption.

If patient misses a dose and it is more than 5 hours after usual time, then patient should skip that dose and resume regular dose the next day.

7.3 Treatment by local medical doctor (LMD)

Treatment by a local medical doctor (LMD) is not allowed.

8.0 Dosage Modification Based on Adverse Events

Strictly follow the modifications in this table for the first **two** cycles, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose.

⟶ ***ALERT:*** ***ADR reporting may be required for some adverse events (See Section 10.0)*** ⟵

Use the following to describe actions in the Action column:

- **Omit** = The current dose(s) for the specified drug(s) during a cycle is skipped. The patient does not make up the omitted dose(s) at a later time.
- **Hold/Delay** = The current dose(s) of all drugs during a cycle is delayed. The patient does make up the delayed dose(s) when the patient meets the protocol criteria to restart drugs.
- **Discontinue** = The specified drug(s) are totally stopped.

NOTE: If the patient experiences a significant adverse event requiring a dose reduction at the start of the next cycle, then the dose will remain lowered for that entire subsequent cycle.

8.1 Dose Levels (Based on Adverse Events in Tables 8.2 and 8.3)

Dose Level	Auranofin	Sirolimus
0*	6 mg/day	5 mg/day
-1	6 mg/day	2 mg/day
-2	3 mg/day	2 mg/day

*Dose level 0 refers to the starting dose.

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) current version 4.03* unless otherwise specified*** ← ←

Located at http:/ctep.cancer.gov/protocolDevelopment/electronic_applications.ctc.htm

8.2 Dose Modifications for Auranofin and Sirolimus

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
Investigations	Cholesterol high Grade 2	auranofin sirolimus	Institute appropriate lipid/cholesterol lowering therapy Hold auranofin and sirolimus until levels return to Grade 1 If unresolved by four weeks, discontinue auranofin and sirolimus and go to event monitoring
	Grade 3 or 4		Discontinue auranofin and sirolimus and go to event monitoring
	Neutrophil count decreased Grade 4 Platelet count decreased Grade 4		Delay therapy until ANC ≥1500 and/or PLT ≥100,000, and decrease by 1 dose level Discontinue auranofin and sirolimus if AE persists beyond 14 days or if patient is already at dose level -2 and go to event monitoring
	Neutrophil count decreased Grade 3 Platelet count decreased Grade 3		Delay therapy until ANC ≥1500 and/or PLT ≥100,000 If delay is 1 week or less, resume at prior dose If delay exceeds 7 days but is not more than 14 days, decrease by 1 dose level Discontinue auranofin and sirolimus if AE persists beyond 14 days or if subject is already at dose level -2 and go to event monitoring
Metabolism and nutrition disorders	Hypertriglyceridemia Grade 2		Institute appropriate lipid/cholesterol lowering therapy Hold auranofin and sirolimus until levels return to Grade 1 If unresolved by four weeks, discontinue auranofin and sirolimus therapy and go to event monitoring
	Grade 3 or 4		Discontinue auranofin and sirolimus and go to event monitoring.
Respiratory, thoracic and mediastinal disorders	Interstitial pneumonitis any grade		Discontinue auranofin and sirolimus and go to event monitoring
Other non-hematologic adverse events (includes gastrointestinal symptoms)	Grade 3 or 4		Discontinue auranofin and sirolimus and go to event monitoring If patient is deriving benefit, then discuss further treatment with PI
Renal and urinary disorders	Proteinuria	auranofin	Hold auranofin if 1-3.4 grams of protein are detected in a 24 hour urine collection For Grade 3 proteinuria (>3.5 grams) discontinue auranofin and sirolimus and go to event monitoring.

9.0 Ancillary Treatment/Supportive Care

Full supportive care
Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

10.0 Adverse Event (AE) Monitoring and Reporting

The site principal investigator is responsible for reporting any/all serious adverse events and adverse events of special interest to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor/sponsor-investigator is responsible for notifying FDAand all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	Will automatically be sent to [redacted]

Definitions

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

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

Unanticipated Adverse Device Event (UADE)
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site:

[redacted]

a. Identify the grade and severity of the event using the CTCAE version 4.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.
NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME (Combination) Arm

NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the **entire combination (arm) is then considered an investigational intervention for reporting.**

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for **investigational** agents/interventions.
- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report, unless hospitalization is required. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

- An increased incidence of an expected adverse event (AE) is based on the patients treated for this study at their site. A list of known/expected AEs is reported in the package insert or the literature, including AEs resulting from a drug overdose.
- Commercial agent expedited reports must be submitted to the FDA via MedWatch 3500A for Health Professionals (complete all three pages of the form).

[redacted]

or

[redacted]

Instructions for completing the MedWatch 3500A:

[redacted]

10.32 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will <u>not</u> be reported in an expedited manner[1]
General disorders and administrations site conditions	Fatigue	≤Grade 3
	Malaise	≤Grade 3
Skin and subcutaneous tissue disorders	Alopecia	Any Grade

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.
Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.32 for exceptions to Expedited Reporting

10.42 **General reporting instructions**

The Mayo IND Coordinator will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form [redacted] for investigational agents or commercial/investigational agents on the same arm.

For commercial agents:

Submit form MedWatch 3500A to the FDA, [redacted], by fax at [redacted] or online at [redacted]

Submit SAEs to Industry Partner Contacts:

10.43 **Reporting of re-occurring SAEs**

ALL SERIOUS adverse events that meet the criteria outlined in table 10.41 MUST be immediately reported within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 **Other Required Reporting**

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form [redacted] The Mayo Regulatory Affairs Office will review and process the submission to the Mayo Clinic IRB.

10.52 **Death**

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 **Secondary Malignancy**

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND/IDE will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy

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

10.54 **Second Malignancy**

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

10.55 **Pregnancy, Fetal Death, and Death Neonatal**

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form:

[redacted]

10.551 Pregnancy

Pregnancies occurring in the patient while she is receiving study drug or within 3 months after the patient's last dose of study drug will not be considered serious, but are to be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Study drug must be discontinued immediately in the event of a pregnancy in the patient. The patient should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. Pregnancy should be followed until the outcome is known and must be reported to the Medical Monitor within 5 days.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Any fetal death should be reported expeditiously, as **Grade 4 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)"** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the study drug should also be reported.

A neonatal death should be reported expeditiously as **Grade 4 "General disorders and administration - Other (neonatal loss)"** under the General disorders and administration SOC.

10.6 Required Routine Reporting

10.61 **Baseline and Adverse Events Evaluations**

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation. Grading is per CTCAE v4.03 **unless** alternate grading is indicated in the table below:

CTCAE System/ Organ/ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Blood and lymphatic system disorders	Anemia	X	X
Gastrointestinal disorders	# of stools	X	
	Diarrhea		X
	Nausea	X	X
	Vomiting	X	X
	Mucositis Oral	X	X
General disorders and administration site conditions	Fever	X	X
	Fatigue	X	X
Immune system disorders	Allergic reaction	X	X
Investigations	Platelet Count Decreased	X	X
	Neutrophil Count Decreased	X	X
	White Blood Cell Decreased	X	X
Musculoskeletal and connective tissue disorders	Myalgia	X	X
	Arthralgia	X	X
Nervous system disorders	Dizziness	X	X
Renal and urinary disorders	Proteinuria	X	X
Respiratory, thoracic and mediastinal disorders	Pneumonitis	X	X
Skin and subcutaneous tissue disorders	Palmar-plantar erythrodysesthesia syndrome	X	X
	Rash maculo-papular	X	X

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation/Measurement of Effect

This protocol uses RECIST 1.1 criteria {Eisenhauer & Therasse 2009} [26]

11.1 Schedule of Evaluations

Evaluations using RECIST will then be performed at completion of Cycles 2, 4, 6, etc until disease progression or discontinuation of all protocol treatment.

11.2 Definitions of Measurable and Non-Measurable Disease

11.21 Measurable Disease

11.211 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

11.212 A malignant lymph node is considered measurable if its short axis is >1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: Tumor lesions in a previously irradiated area are not considered measurable disease.

11.22 Non-Measurable Disease

11.221 All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥1.0 to <1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/ pericardial effusions, lymphangitis cutis/pulmonis, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. In addition, lymph nodes that have a short axis <1.0 cm are considered non- pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.31 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.

The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up.

11.32 Acceptable Modalities for Measurable Disease:

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

- As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.
- PET-CT: CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

11.33 Measurement at Follow-up Evaluation:

- A subsequent scan must be obtained at least 4 weeks following initial documentation of an objective status of either complete response (CR) or partial response (PR).
- In the case of stable disease (SD), follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks (see Section 11.44).
- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.
- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in ATC after chemoradiation can represent fibrotic tissue)

11.4 Measurement of Effect

11.41 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

  Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.
- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.
- Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.
- The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.42 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with 11.433.

11.43 Response Criteria

11.431 All target lesions and target lymph nodes followed by CT/MRI/PET-CT must be measured on re-evaluation Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment,

11.432 Evaluation of Target Lesions

Complete Response (CR):All of the following must be true:

a. Disappearance of all target lesions.

b. Each target lymph node must have reduction in short axis to <1.0 cm.

Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (*see* Section 11.41).

Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.

b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.433 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

Complete Response (CR): All of the following must be true:

a. Disappearance of all non-target lesions.

b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.

c. Tumor markers, such as Ca125, if followed, has normalized.

Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.

Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (<1.0 cm short axis) and increased to ≥1.0 cm short axis during follow-up.

b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)

11.44 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following table:

11.441 For Patients with Measurable Disease

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

11.45 Symptomatic Deterioration

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration.

12.0 Descriptive Factors

Tumor appears to overexpress PKC iota: Yes vs. no

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Continuation of treatment

Patients who have not had disease progression and have experienced acceptable toxicity are to continue treatment per protocol until documentation of progressive disease, unacceptable toxicity or refusal.

13.2 Criteria for discontinuation of all protocol treatment

- Disease progression
- Request by patient to discontinue all protocol treatment
- Unacceptable toxicity
- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or require discontinuation of all protocol treatment
- Administration of radiotherapy, non-protocol chemotherapy, immunotherapy, biological agents, or an experimental drug
- Development of new primary cancer
- Ineligibility

Patients who discontinue protocol treatment due to any of the above reasons will proceed to event monitoring phase of the trial where patient and disease status until death or a maximum of 3 years post-registration. Further treatment is at the discretion of the patient's medical team.

13.3 Definition of ineligible

A patient is deemed ineligible if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry.

- If the patient received protocol treatment and is deriving benefit, the patient may continue on study per protocol as if the patient was eligible. If the patient chooses to end protocol treatment, then all data up until the point of treatment discontinuation must be submitted. No further data submission is necessary.
- If the patient never received any protocol treatment, on-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.4 Definition of cancel

A patient who cancels before any study treatment is given does not need to be followed further. On-study material (except biospecimens) and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Biospecimen Collection: None

15.0 Drug Information

15.1 Auranofin for Oral Administration (Ridaura®)

15.11 **Background**: Auranofin is a gold compound which has anti-inflammatory effects. Gold compounds can alter the immune response and have been shown to inhibit prostaglandin synthesis.

15.12 **Formulation**: Commercially available for oral administration as: Capsules: 3 mg [gold 29%]

15.13 **Preparation, storage, and stability**: Refer to package insert for complete preparation and dispensing instructions. Store oral tablets at room temperature. Dispense in a tight, light-resistant container.

15.14 **Administration:** Refer to the treatment section for specific administration instructions.

15.15 **Pharmacokinetic information:**
Absorption: ~25% of the gold in a dose of Auranofin is absorbed from the gastrointestinal tract
Protein binding: Moderate. In blood, approximately 60% of the gold is bound to plasma proteins; the remainder is present in red blood cells
Metabolism: Rapidly metabolized (the intact molecule has not been detected in blood)
Half-life elimination: Blood: 21 – 31 days. Tissue: 42 -128 days **Excretion**: 60% of the absorbed gold (15% of the administered dose) is excreted in the urine; the remainder of the dose is excreted in the feces

15.16 **Potential Drug Interactions:**
In a single patient-report, there is the suggestion that concurrent administration of Auranofin and phenytoin may have increased phenytoin blood levels.

15.17 **Known potential adverse events:**
Consult the package insert for the most current and complete information. Auranofin is contraindicated in patients with a history of any gold-induced disorders: anaphylactic reactions, necrotizing enterocolitis, pulmonary fibrosis, exfoliative dermatitis, bone marrow aplasia or other severe hematologic disorders.

Common known potential toxicities, >10%:
Dermatologic: pruritus, skin rash (acneiform or macular popular)
Gastrointestinal: diarrhea, loose stools, stomatitis

Less common known potential toxicities, 1% - 10%:
Dermatologic: Alopecia, urticaria
Gastrointestinal: Nausea, vomiting, anorexia, flatulence, dyspepsia, constipation, dysgeusia, glossitis
Genitourinary: Proteinuria, hematuria
Hematologic and oncologic: Anemia, eosinophilia, leukopenia, thrombocytopenia
Heaptic: Increased serum transaminases
Ophthalmic: Conjunctivitis

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Agranulocytosis, aplastic anemia, angioedema, bronchitis (gold), corneal deposits, dysphagia, exfoliative dermatitis (other gold compounds), fever, gastrointestinal hemorrhage, gingivitis, hepatotoxicity, interstitial pneumonitis, jaundice, metallic taste, melena, neutropenia, pancytopenia, peripheral neuropathy, pure red cell aplasia, ulcerative enterocolitis

15.18 Drug procurement
Commercial supplies: Study will purchase for enrolled patients.

15.19 Nursing Guidelines:

15.191 Auranofin is contraindicated in patients with history of gold- induced disorders. Assess patients for any of these conditions, hold drug and notify study team.

15.192 Dermatitis is common and is exacerbated by sunlight. Instruct patients to avoid prolonged sun exposure without protection (i.e sunscreen SPF 30+ and covering up with clothing).

15.193 Patients may experience hair loss. Warn patients of this possibility.

15.194 Gastrointestinal side effects are common (diarrhea, constipation, nausea, vomiting, etc.) Treat symptomatically and assess for effectiveness.

15.195 Monitor LFTs as elevated liver enzymes have been seen. Report elevations to study physician.

15.196 Cytopenias can be seen. Rarely these can be serious. Monitor CBC w/differential. Instruct patients to report any excessive bruising or bleeding and/or signs/symptoms of infection to study team.

15.197 Instruct patients to report any hematuria to the study team.

15.198 Pneumonitis has been reported rarely, but can be serious and life-threatening. Instruct patients to report any cough, shortness of breath, and/or chest pain to the study team immediately.

15.2 Sirolimus for Oral Administration (Rapamune®)

15.21 Background: Sirolimus inhibits T-lymphocyte activation and proliferation in response to antigenic and cytokine stimulation and inhibits antibody production. Its mechanism differs from other immunosuppressants. Sirolimus binds to FKBP-12, an intracellular protein, to form an immunosuppressive complex which inhibits the regulatory kinase, mTOR (mammalian target of Sirolimus). This inhibition suppresses cytokine mediated T-cell proliferation, halting progression from the G1 to the S phase of the cell cycle. It inhibits acute rejection of allografts and prolongs graft survival.

15.22 Formulation: Commercially available for oral administration as: Solution, oral: 1 mg/mL (60 mL) Tablet: 1 mg, 2mg

15.23 Preparation, storage, and stability: Refer to package insert for complete preparation and dispensing instructions. Store oral solution under refrigeration and protect from light. Store tablets at room temperature and protect from light.

15.24 **Administration:** Refer to the treatment section for specific administration instructions.
Tablet: Administer consistently either with or without food. Do not crush, split or chew.
Solution: Mix with at least 2 ounces (60mL) of water or orange juice. No other liquids should be used for dilution. Patient should drink diluted solution immediately. The cup should then be refilled with an additional 4 ounces (120mL) of water or orange juice, stirred vigorously, and the patient should drink the contents at once.

15.25 **Pharmacokinetic information**: (Note: Sirolimus tablets and oral solution are not bioequivalent, due to differences in absorption)
Absorption: Rapid
Distribution: 12 L/kg (range: 4-20 L/kg)
Protein binding: 92%, primarily to albumin **Bioavailability:** Oral solution: 14%; Oral tablet:18% **Time to peak, serum:** 1-2 hours
Metabolism: Extensively via P-glycoprotein and hepatic via CYP3A4; to 7 major metabolites **Half-life elimination:** 62 hours (range: 46-78 hours); extended in hepatic impairment (Child-Pugh class A or B) to 113 hours
Excretion: Feces (91% due to P-glycoprotein-mediated efflux into gut lumen); urine (2%)

15.26 Potential Drug Interactions:

Cytochrome P450 Effect: Substrate of CYP3A4 (major), P-glycoprotein;

Inhibits CYP3A4 (weak)

Increased Effect/Toxicity: Cyclosporine may increase sirolimus concentrations during concurrent therapy; Sirolimus should be taken 4 hours after cyclosporine oral solution (modified) and/or cyclosporine capsules (modified). CYP3A4 inhibitors may increase the levels/effects of Sirolimus. Refer to package insert for a list of CYP3A4 inhibitors.

Concurrent use of ACE inhibitors may increase the risk of angioedema. Concurrent live organism vaccines may increase the adverse/toxic effect of the vaccine; vaccinial infections are possible (avoid concurrent use).

Voriconazole may decrease the metabolism (via CYP isoenzymes) of sirolimus (avoid concurrent use). Concurrent therapy with calcineurin inhibitors (cyclosporine, tacrolimus) may increase the risk of HUS/TTP/TMA.

Decreased Effect: CYP3A4 inducers may decrease the levels/effects of sirolimus. Vaccination (dead organisms) may be less effective with concurrent sirolimus (monitor).

Ethanol/Nutrition/Herb Interactions:

Food: Do not administer with grapefruit juice; may decrease clearance of sirolimus. Ingestion with high-fat meals decreases peak concentrations but increases AUC by 23% to 35%. Sirolimus should be taken consistently either with or without food to minimize variability.

15.27 **Known potential adverse events:** Consult the package insert for the most current and complete information.

Warnings:
- Anaphylactic or hypersensitivity reactions

- Angioedema
- Infections (immunosuppressive agents, including sirolimus, increase the risk of infection)
- Interstitial lung disease
- Hyperlipidemia
- Lymphocele/fluid accumulation
- Malignancy (immunosuppressive agents including sirolimus, may be associated with the development of lymphoma and other malignancies including skin cancer)
- Proteinuria
- Renal effects (increased serum creatinine and decreased GFR)
- Wound dehiscence/healing

Common or severe toxicity: Hypertension, edema, capillary leak syndrome, hypertriglyceridemia, hypokalemia, constipation, diarrhea, dyspepsia, nausea and vomiting, deep venous and arterial thrombosis, abnormal liver function tests, sepsis, arthralgia, headache, renal failure, pulmonary hemorrhage, thrombocytopenia and leukopenia.

Note that abnormal liver function tests could rarely lead to severe liver damage associated with pulmonary edema, ascites and/or pericardial effusion.

Note that rarely thrombocytopenia can be prolonged for more than 2 weeks.

15.28 Drug procurement: Commercial supplies. Study will purchase for enrolled patients.

15.29 Nursing Guidelines:

15.291 Patients should be instructed that tablets should be swallowed whole, and not crush, split or chewed. If solution is used, it should be mixed with at least 2 ounces of water or orange juice. No other liquids should be used for dilution. Mixed solution should be drunk immediately, then add an additional 4 ounces of water or juice to the cup, stir vigorously and again drunk immediately.

15.292 Patients should not receive vaccination with live vaccination preparations while on study medication. Patients should be instructed to check with the study team prior to receiving any vaccine.

15.293 Hypertension is a known side effect. Monitor patients BP as outlined in the protocol or as symptoms dictate.

15.294 Elevated lipid levels, specifically triglycerides can be seen. Monitor lipid levels as outlined in the protocol.55 MC1111

15.295 Thrombosis (venous and arterial) can be seen. Instruct patient in the warning signs of this and to seek out proper medical treatment.

15.296 Cytopenias (thrombocytopenia/leukopenia) can be a side effect of agent. Instruct patient in signs and symptoms of infection and to report any unusual bruising or bleeding to the study team.

15.297 Infection (including sepsis) and delayed wound healing are possible. Instruct patients to check with the study team prior to having any

invasive procedure done. Instruct patients to report any fever or infection symptoms to the study team immediately.

15.298 Pneumonitis is seen with this agent. Instruct patients to report any cough or shortness of breath to the team.

15.299a Headache is a known side effect. Administer analgesics as ordered and assess for their effectiveness.

15.299b GI disturbances are common and vary (diarrhea, constipation, nausea, vomiting, dyspepsia). Treat symptomatically and assess for effectiveness.

16.0 Statistical Considerations and Methodology

16.1 Overview:

This is a phase 2 trial that seeks to obtain tumor response data and other clinically relevant data on the novel combination of auranofin and sirolimus in patients with metastatic serous ovarian cancer. The primary endpoint is the confirmed response rate, with secondary endpoints of progression-free survival, overall survival, and adverse events from the combination of auranofin and sirolimus.

16.11 Primary Endpoint

The primary endpoint of this trial is the proportion of patients with a confirmed tumor response (PR or CR at least 4 weeks apart). All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for the confirmed response rate. This response rate primary endpoint will be estimated across all patients.

16.12 Sample Size

The 1-stage study design with an interim analysis is fully described in Section 16.2. 44 evaluable patients will be accrued onto this phase II study unless undue toxicity is encountered. We anticipate accruing an additional 4 patients to account for ineligibility, cancellation, major treatment violation, or other reasons. Therefore, maximum accrual is expected to be 48 patients.

16.13 Accrual Time and Study Duration

The anticipated accrual rate is approximately 2 patients per month. Therefore, the accrual period for this phase II study is expected to be approximately 24 months. The final analysis can begin approximately 36 months after the trial begins, i.e. as soon as the last patient has been followed for 8 months plus time for data entry and clean-up.

16.2 Statistical Design

16.21 Decision Rule

The largest success proportion where the proposed treatment regimen would be considered ineffective in this population is 15%. The smallest response rate that would warrant further subsequent studies is 35%. The following Simon optimal 2-stage design (i.e. one-stage design with an interim analysis) uses 44 evaluable patients to test the null hypothesis that the true confirmed response rate in this patient population is at most 15%.

16.211 Final Analysis Decision Rule (full 44 eligible patients):
Enter 44 evaluable patients. If 10 or fewer patients have a confirmed response, we will consider this regimen ineffective in this patient population. If 11 or more patients have a confirmed response (25%), we may recommend further testing of this regimen in subsequent studies in this patient population. If the study is negative based on this endpoint, but the key secondary endpoint is positive (see 16.321), we may still recommend further testing of this regimen in subsequent studies.

16.212 Interim Analysis
An interim analysis will be performed after enrollment of the first 19 eligible patients. If 3 or fewer responses are observed in these 19 eligible patients, the trial will be stopped early for negative results. Otherwise, we will continue to full accrual.

16.213 Over Accrual

If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making process. Analyses involving over accrued patients is discussed in Section 16.35.

16.214 Data and Safety Monitoring:

The principal investigator(s) and the study statistician will review the study at least twice a year to identify accrual, adverse event, and any endpoint problems that might be developing. The trial is monitored continually by the study team who are notified of every grade 4 and 5 event in real time. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office. Any safety issues requiring protocol changes are communicated through protocol amendments.

Adverse Event Stopping Rule: Based on previous experience with this disease, we expect approximately 30% of patients to experience Grade 4+ adverse events. If at any time, 4 of the initial 10 patients or 40% of all patients (i.e., when accrual is greater than 10 patients), have experienced any Grade 4 or 5 adverse event (at least possibly related to the study treatment), accrual to the study will be suspended to allow for a full review of the data. Each grade 5 event will be reviewed on a case by case basis in a real time fashion to determine whether study accrual should be suspended. After consideration by the study team [ie, Study Chair(s), Statistician, Operations Office, etc] and consultation with representatives at the primary Internal Review Board (IRB) affiliated with the Operations Office, a decision will be made as to whether and how the study will proceed.

16.22 Power and Significance Level

Assuming that the number of responses is binomially distributed, the significance level is 5% when the true confirmed response rate is 15% and the power is 90% when the true confirmed response rate is 35%. See table below.

If the true success proportion (p) is	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
then the probability of declaring that the regimen warrants further studies is	0.05	0.22	0.50	0.76	0.90
and the probability of stopping at the interim analysis is.	0.68	0.46	0.26	0.13	0.06

16.23 Other Considerations

Toxicity, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries or changes in standard care will be taken into account in any decision to terminate the study.

16.3 Analysis Plan

16.31 Primary Endpoint

The proportion of responses will be estimated by the number of successes divided by the total number of evaluable patients. Ninety-five percent confidence intervals for the true success proportion will be calculated according to the exact binomial method.

16.32 Definitions and Analyses of Secondary Endpoints

16.321 Key Secondary Endpoint

The key secondary endpoint of this trial is the proportion of patients with a confirmed tumor response (PR or CR at least 4 weeks apart) in the subset of patients that have overexpression of PKCι. The hope is that in this subset, we'll see a higher response rate than across all patients. We expect that around 60% of patients will have overexpression of PKCι, so we expect that around 30 patients will be positive for this marker (27 or so eligible). The hypothesis is that the observed response rate in this subgroup should be around 40% or higher. With 27 eligible patients, we'd have 88% power to detect a true response rate of 50%, assuming a 5% significance level if the true response rate is 25%. To consider this subgroup significant and positive, we need to observe 11+ responses in the first 27 eligible patients (41%). If the primary endpoint is negative, but this key secondary endpoint is positive, we will still consider moving this treatment regimen forward into future trials.

16.322 Progression-Free Survival

Progression-free survival (PFS) is defined as the time from registration to the first of either disease progression or death from any cause. Patients who receive the study drug, but then never return for an evaluation will be censored on their last follow-up date. PFS will be estimated using the method of Kaplan-Meier.

16.323 Overall Survival

Overall survival (OS) is defined as the time from registration to death from any cause. OS will be estimated using the method of Kaplan-Meier.

16.33 Adverse Events

All patients that have intitiated treatment will be considered evaluable for adverse event (AE) analyses. The maximum grade for each type of AE will be recorded for each patient, and frequency tables will be reviewed to determine AE patterns.

16.34 Analysis of Translational Component

We will use previously-described immunohistochemistry methods and expression assessment methods [2-4]. Overexpression of PKCι will be compared to response using 2x2 tables and chi-square or Fisher exact tests. Assuming around 60% of patients have overexpression of PKCι, we expect around 27 eligible patients with overexpression and 17 eligible without (44 eligible total); if the tumor response rate across all patients is around 30%, we expect around 12 responses. The trial has 86% power at an alpha of 10% to declare a significant association between PKCι and response if most of the responses occur within the PKCι overexpression group (11/27 (41%)) vs. 6% in the unexpressed group

(1/17). This is based on a 2-sided Chi-square test of equal proportions using nQuery Advisor 7.0.

We will also correlate overexpression of PKCι with PFS and OS using Kaplan-Meier methods and the log-rank test. This translational study is considered exploratory and hypothesis generating due to the small proposed sample size for this study.

16.4 Inclusion of Women and Minorities

16.41 Study availability

This study will be available to all eligible women, regardless of race or ethnic origin.

16.42 Differential effects by race or ethnicity

There is no information currently available regarding differential effects of this regimen in subsets defined by race or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.43 Study population

Based on prior studies involving similar disease sites, we expect about 20% of patients will be classified as minorities by race and all will be women. Expected sizes (per study design) of racial by gender subsets are shown in the following table:

Accrual Estimates by Gender/Ethnicity/Race

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	5	0	5
Not Hispanic or Latino	43	0	43
Ethnic Category: Total of all subjects	**48**	**0**	**48**
Racial Category			
American Indian or Alaskan Native	2	0	2
Asian	2	0	2
Black or African American	4	0	4
Native Hawaiian or other Pacific Islander	2	0	2
White	38	0	38
Racial Category: Total of all subjects	**48**	**0**	**48**

Ethnic Categories: **Hispanic or Latino –** a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens

17.1 Summary Table of Research Tissue Specimens to be Collected for this Protocol

Correlative Study (Section for more information)	Mandatory or Optional	Block, Slides, Core, etc. (# of each to submit)	Baseline	At disease progression	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping
Archival Tissue	Mandatory*	FFPE Blocks or Slides[1]	X[2]		No	Ambient/ Ambient
Tissue	Optional	FFPE Blocks or Slides[1]		X[3]	No	Ambient/ Ambient

1. Formalin Fixed Paraffin Embedded (FFPE). If a block is not available, then please submit 1 H&E and 10 unstained slides
2. Submit Operative and Pathology reports with archival tissue sent to the MCF Biospecimen Facility.
3. For those subjects in Event Monitoring for reasons other than PD (e.g. adverse event, refusal), optional tissue will not be collected

*If the patient makes every effort to submit a tissue block, but this material is unable to be obtained, this mandatory submission can be waived

17.2 Tissue Collection and Processing

Tissue will be obtained from previous biopsy material or from biopsy of accessible tumor sites at protocol entry, along with operative and pathology reports. Patients who consent and who have accessible disease may have tissue samples obtained by standard needle core biopsy at the time of disease progression as well. (NOTE: PI approval is required prior to research biopsy.)

These samples will be analyzed by [redacted] as described in Section 17.3.

Forward FFPE tissue to the following laboratory:

[redacted]

Telephone: [redacted]

For quality assurance purposes, biopsy tissues will be fixed immediately in 10% phosphate-buffered formalin and fixed tissues will be embedded in paraffin.

17.3 Background

Genomic DNA will be extracted from 5-3µm sections from the biopsy and subjected to *PRKCI* gene copy number analysis as described previously. Sections (3µm) from the biopsy will be processed for IHC using established protocols as described previously. Tissue sections will be stained with antibodies specific to PKCι, phospho-Ser298- Mek, phospho-Thr202/Tyr204-Erk, phospho-Ser473-Akt, phospho-Thr70-4E- BP1 and phospho-Ser240/244-S6 (Cell Signaling Technology Inc.) as previously described.

Expression of these markers will be evaluated as described below for correlation with response to Auranofin/Sirolimus therapy with the primary clinical endpoint being progression free survival.

Standardization of IHC scoring: A drawback of IHC staining for detection and quantification of biomarkers is the inherent variability in IHC staining from analysis to analysis and from sample to sample. We will minimize this variability by constructing a "standard curve" of staining intensity on a scale from 0 to +3 for each of the potential biomarkers using well-characterized archival primary NSCLC tumor tissues. We have already constructed such a standard curve TMA for PKCι as follows: First, we performed immunohistochemical analysis for PKCι on 4 tissue microarrays (TMAs) containing ~100 well-characterized archived primary NSCLC cases. Each of the 100 samples were scored on a 0 to+3 scale based on PKCι staining intensity by a board certified pathologist. A 0 score corresponds to tumor PKC staining equivalent to the surrounding stroma, and scores of +1, +2 and +3 correspond to increasing intensity of PKCι staining in the tumor specimen compared to the surrounding stroma.

Tissue blocks from cases corresponding to each numerical value on the intensity scale were retrieved and used to construct a new TMA. This "standard curve" TMA contains 4 NSCLC cases that exhibit a gradient of PKCι staining representative of the full range of staining intensity across the original 100 samples (***Figure 1***). The standard curve will be validated by a second pathologist. For our proposed analysis, tissue sections from each enrolled patient will be subjected to IHC for PKCι in parallel with a section from this "standard curve" TMA. The sections will be analyzed at the same time using the same reagents and protocol to ensure uniformity of scoring across samples.
Each experimental sample will be assigned a score, based on the standard curve on a 0 to +3 scale, by two pathologists independently with joint review and resolution of discrepancies as necessary. Additionally, image analysis will be performed using ImageScope and associated image quantification software.

A similar approach will be used to generate "standard curve" TMAs for each of the potential predictors of response, phospho-Ser 298-Mek, phospho- Thr202/Tyr204-Erk, phospho-Ser473-Akt, phospho-Thr70-4E-BP1 and phospho- Ser240/244-S6. IHC scoring criteria and interpretation will be guided by published reports evaluating these markers in NSCLC cases, and will take into account, as appropriate, not only staining intensity, but also percent of tumor cells stained positively and nuclear versus cytoplasmic distribution of phospho- antigens. Phospho-Erk scoring will follow the criteria established by Cappuzzo et al. Specifically, sections will be scored on the following scale: 0=no tumor cells stain positively; +1= more than 10% of the tumor cells stained weakly; +2= more than 10% of the tumor cells stained moderately; and +3=more than 10% of the tumor cells stained strongly. Phospho-Akt staining will be evaluated based on the criteria established by David et al. Scoring will be on the following scale: 0=no staining in tumor cells; +1=slightly elevated staining in cytoplasm and/or nucleus as compared with stromal elements; +2=moderate staining in tumor cell cytoplasm and/or nucleus; and +3=dark staining in tumor cells completely obscuring cytoplasm and/or nucleus.

Phospho-S6 staining will be scored using the criteria established by Conde et al. Specifically, samples will be scored for staining intensity as follows: 0=no tumor staining; +1=weak cytoplasmic staining; +2=moderate cytoplasmic staining; and +3=strong cytoplasmic staining. Extent of staining will also be scored according to the percentage of tumor cells that stain positively for antigen as follows: 0=0% of cells staining; +1=≤50% staining; +2=51-75% cells staining; +3=76-85% cells staining; and +4=>85% cells staining. The sum of the intensity and extent of scoring will be used as

the final staining score (0-7). In their study, Conde et al. considered tumors with a final score of ≥5 as being positive or high-intensity, whereas those with a final score of <5 were negative or low-intensity. This binary model will be considered in our patient sample as described below. No scoring scale has been published for phospho-Ser298-Mek or 4E-BP1 staining in primary NSCLC tumors. However, we will consider the criteria established by Akcakanat et al. for analysis of 4E-BP1 immunohistochemical staining in primary breast cancer tumors. We will establish such interpretation criteria based on the staining patterns exhibited by our analysis of the TMA containing our 100 archival NSCLC cases. Staining intensity, percent positive cells, staining relative to surrounding stroma and nuclear/cytoplasmic distribution will be considered as potential criteria in devising standard curve TMAs and scoring paradigms for these antigens. Data from IHC scoring will be analyzed for correlation with clinical response using standard statistical treatments by our staff statistician.

Specifically, the association of the endpoint of PFS at 4 months with the predictor variables *PRKCI* gene copy number (presence of ≥1 extra copy of the gene) and IHC scoring for the given marker will be evaluated using logistic regression models, where an odds ratio and 95% confidence interval will be estimated. A separate logistic regression model will be utilized for each predictor variable. For each marker, IHC scores will be considered as a numerical variable to assess evidence of a linear association, and also as a binary categorical variable based on the sample median IHC score for the given marker. Given availability of information regarding PFS beyond the time point of interest of 4 months, in exploratory analyses, associations of the aforementioned predictor variables with PFS at any time point will be evaluated using the Kaplan-Meier method and Cox proportional hazards models (censoring at the date of last follow-up), which will account for the varying lengths of follow-up between patients.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Event monitoring

See Section 4.0 and data submission table for the event monitoring schedule.

18.3 CRF completion

This study will use Medidata Rave for remote data capture (rdc) of all study data.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for a cancer diagnosis prior to study entry. It also requires documentation (to explain why the patient has come off study therapy. These documents should be submitted within 14 days of registration (for prior to study entry materials) or within 14 days after the patient comes off study.

18.6 Labelling of materials

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.7 Incomplete data

Any data entered into a form will result in that form being marked as "received." However, missing data will be flagged by edit checks in the database.

18.8 Overdue lists

A list of overdue materials is automatically available to each site at any time. A list of overdue materials and forms for study patients will be generated monthly. The listings will be sorted by location and will include the patient study registration number. The appropriate co-sponsor/participant will be responsible to obtain the overdue material.

18.9 Data corrections

If a correction is necessary the QAS/DM will query the site. The query will be sent to the appropriate site to make the correction in the database and respond back to the QAS/DM.

19.0 Budget

19.1 Costs charged to patient: Routine clinical care including tumor assessment scans and blood work (labs) related to adverse event assessment

19.2 Tests to be research funded: Research studies on paraffin-embedded tissue

19.3 Other budget concerns: Supplies of auranofin and sirolimus will be purchased with study funds

20.0 References

1. https://www.cancer.gov/about-cancer/treatment/drugs/ovarian; last accessed February 10, 2017

2. Regala RP, Weems C, Jamieson L, Copland JA, Thompson EA, Fields AP. Atypical protein kinase Ciota plays a critical role in human lung cancer cell growth and tumorigenicity. J Biol Chem. 2005;280(35):31109-15. PubMed PMID: 15994303.

3. Regala RP, Weems C, Jamieson L, Khoor A, Edell ES, Lohse CM, Fields AP. Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res. 2005;65(19):8905-11. PubMed PMID: 16204062.

4. Erdogan E, Lamark T, Stallings-Mann M, Jamieson L, Pellechia M, Thompson EA, Johansen T, Fields AP. Aurothiomalate inhibits transformed growth by targeting the PB1 domain of atypical protein kinase Ciota. J Biol Chem. 2006. PubMed PMID: 16861740.

5. Han Y, Wei F, Xu X, Cai Y, Chen B, Wang J, Xia S, Hu H, Huang X, Han Y, Wu M, Wang M. [Establishment and comparative genomic hybridization analysis of human esophageal carcinomas cell line EC9706]. 2002;19(6):455-7. PubMe2-4d PMID: 12476413.

6. Balsara BR, Sonoda G, du Manoir S, Siegfried JM, Gabrielson E, Testa JR. Comparative genomic hybridization analysis detects frequent, often high-level, overrepresentation of DNA sequences at 3q, 5p, 7p, and 8q in human non-small cell lung carcinomas. Cancer Res. 1997;57(11):2116-20. PubMed PMID: 9187106.

7. Brass N, Racz A, Heckel D, Remberger K, Sybrecht GW, Meese EU. Amplification of the genes BCHE and SLC2A2 in 40% of squamous cell carcinoma of the lung. Cancer Res. 1997;57(11):2290-4. PubMed PMID: 9187134.

8. Frederick LA, Matthews JA, Jamieson L, Justilien V, Thompson EA, Radisky DC, Fields AP. Matrix metalloproteinase-10 is a critical effector of protein kinase Ciota-Par6alpha-mediated lung cancer. Oncogene. 2008;27(35):4841-53. Epub 2008/04/23. doi: 10.1038/onc.2008.119onc2008119 [pii]. PubMed PMID: 18427549; PubMed Central PMCID: PMC2750877.

9. Yang YL, Chu JY, Luo ML, Wu YP, Zhang Y, Feng YB, Shi ZZ, Xu X, Han YL, Cai Y, Dong JT, Zhan QM, Wu M, Wang MR. Amplification of PRKCI, located in 3q26, is associated with lymph node metastasis in esophageal squamous cell carcinoma. Genes Chromosomes Cancer. 2008;47(2):127-36. Epub 2007/11/09. doi: 10.1002/gcc.20514. PubMed PMID: 17990328.

10. Regala RP, Davis RK, Kunz A, Khoor A, Leitges M, Fields AP. Atypical protein kinase C{iota} is required for bronchioalveolar stem cell expansion and lung tumorigenesis. Cancer Res. 2009;69(19):7603-11. Epub 2009/09/10. doi: 0008-5472.CAN-09-2066 [pii]

    10.1158/0008-5472.CAN-09-2066. PubMed PMID: 19738040; PubMed Central PMCID: PMC2756303.
11. Fields AP, Frederick LA, Regala RP. Targeting the oncogenic protein kinase Ciota signalling pathway for the treatment of cancer. Biochem Soc Trans. 2007;35(Pt 5):996-1000. PubMed PMID: 17956262.

12. Fields AP, Regala RP. Protein kinase C iota: human oncogene, prognostic marker and therapeutic target. Pharmacol Res. 2007;55(6):487-97. PubMed PMID: 17570678.

13. Wang Y, Justillien V, Brennan KI, Jamieson L, Murray NR, Fields AP. PKCι regulates nuclear YAP1 localization and ovarian cancer tumorigenesis. Oncogene 2017; 36:534-545.

14. Justilien V, Fields AP. Ect2 links the PKCiota-Par6alpha complex to Rac1 activation and cellular transformation. Oncogene. 2009. PubMed PMID: 19617897.

15. Patel R, Win H, Desai S, Patel K, Matthews JA, Acevedo-Duncan M. Involvement of PKC-iota in glioma proliferation. Cell Prolif. 2008;41(1):122-35. PubMed PMID: 18211289.

16. Eder AM, Sui X, Rosen DG, Nolden LK, Cheng KW, Lahad JP, Kango-Singh M, Lu KH, Warneke CL, Atkinson EN, Bedrosian I, Keyomarsi K, Kuo WL, Gray JW, Yin JC, Liu J, Halder G, Mills GB. Atypical PKCiota contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102(35):12519-24. PubMed PMID: 16116079.

17. Weichert W, Gekeler V, Denkert C, Dietel M, Hauptmann S. Protein kinase C isoform expression in ovarian carcinoma correlates with indicators of poor prognosis. Int J Oncol. 2003;23(3):633-9. PubMed PMID: 12888898.

18. Zhang L, Huang J, Yang N, Liang S, Barchetti A, Giannakakis A, Cadungog MG, O'Brien-Jenkins A, Massobrio M, Roby KF, Katsaros D, Gimotty P, Butzow R, Weber BL, Coukos G. Integrative genomic analysis of protein kinase C (PKC) family identifies PKCiota as a biomarker and potential oncogene in ovarian carcinoma. Cancer Res. 2006;66(9):4627-35. PubMed PMID: 16651413.

19. Jatoi A, Radecki Breitkopf C, Foster NR, Block MS, Grudem M, Wahner Hendrickson A, Carlson RE, Barrette B, Karlin N, Fields AP. A mixed methods feasibility trial of protein kinase C iota inhibition with auranofin in asymptomatic ovarian cancer patients. Oncology 2015; 88:208-13.

20. Mansfield AS, Fields AP, Jatoi A, Qi Y, Adjei AA, Erlichman C, Molina JR. Phase 1 dose escalation study of the PKC iota inhibitor aurothiomalate for advanced non-small cell lung cancer, ovarian cancer, and pancreatic cancer. Anticancer Drugs 2013; 24:1079-83.

21. Regala RP, Thompson EA, Fields AP. Atypical protein kinase C iota expression and aurothiomalate sensitivity in human lung cancer cells. Cancer Res. 2008;68(14):5888-95. Epub 2008/07/18. doi: 68/14/5888 [pii] 10.1158/0008-5472.CAN-08-0438. PubMed PMID: 18632643; PubMed Central PMCID: PMC2662432.

22. Stallings-Mann M, Jamieson L, Regala RP, Weems C, Murray NR, Fields AP. A novel small-molecule inhibitor of protein kinase Ciota blocks transformed growth of non-small-cell lung cancer cells. Cancer Res. 2006;66(3):1767-74. PubMed PMID: 16452237.

23. Patel K, Foster NR, Farrell A, Le-Lindqwister NA, Mathew J, Costello B, Reynolds J, Meyers JP, Jatoi A. Oral cancer chemotherapy adherence and adherence assessment tools: a report from the North Central Cancer Treatment Group N0747 and a systematic review of the literature. J Cancer Educ 2013; 28:770-6.

24. Halfdanarson TR, Jatoi A. Oral chemotherapy: the critical interplay between patient education and patient safety. Curr Oncol Rep 2010; 12:247-52.

25. Jatoi A, Smith EL, Gunderson HD, Hartgers ML, Looker SA, Santana-Davila R, McWilliams RR. Capecitabine and temezolomide: design, implementation, and preliminary outcomes from a pilot project to ensure safe prescribing of oral chemotherapy. J Oncol Pract 2010; 6:210-2.

26. Eisenhauer EA, Therasse P, Bogaert J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 45(2): 228-247, 2009.

Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From [redacted]

Appendix II Patient Medication Diary

Patient Name _____ **Study ID Number** _____

Please complete this diary on a daily basis. Write in the dose of auranofin and sirolimus that you took in the appropriate "Day" box.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Take both drugs together as one dose. Each dose is to be taken at the same time of the day under the same conditions, i.e., if fasting, then should be fasting every time; if taken with food, should be taken with food every time. Each dose should be separated by 24 hours (±4 hours).

Definition of fasting conditions: At least 2 hours after eating and at least 1 hour before eating.

If you miss a dose and it is more than 5 hours after your usual time, then skip that dose and resume your regular dose the next day.

Both your medications must be stored at room temperature. Keep out of reach of children. The study medication may not be taken by anyone but you.

Return any unused medications your dispensed at the time of your next visit.

Week of:

Study Drug	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
auranofin							
sirolimus							

Week of:

Study Drug	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
auranofin							
sirolimus							

Week of:

Study Drug	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
auranofin							
sirolimus							

Week of:

Study Drug	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
auranofin							
sirolimus							

Patient signature: _____

Date: _____

Health or medical complaints during this time:

My next scheduled visit is: _____

If you have any questions, please call: _____

Study Coordinator Use Only

Number of pills returned _____ Number of vials returned: _____

Discrepancy Yes____/No____ Verified by _____ Date_____

Appendix III Strong CYP3A4 Inhibitors

Strong CYP3A4/5 inhibitors:

Macrolide antibiotics	Antivirals (Protease inhibitors)	Antifungals	Others
clarithromycin	indinavir	itraconazole	conivaptan
telithromycin	lopinavir	ketoconazole	elvitegravir
troleandomycin	nelfinavir	posaconazole	mibefradil
ritonavir	ritonavir	voriconazole	nefazodone
	saquinavir		saquinavir
			tipranavir