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# A Phase I-II Trial of Combined PKCι and mTOR Inhibition for Patients with Advanced or Recurrent Lung Cancer (NSCLC and SCLC) without Standard Treatment Options

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Mayo Clinic Cancer Center

**A Phase I-II trial of combined PKCι and mTOR inhibition for patients with advanced or recurrent lung cancer (NSCLC and SCLC) without standard treatment options.**

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**Drug Availability**

**Commercial Agents:** Sirolimus, Auranofin

***Investigator having NCI responsibility for this protocol*, *if applicable***

√Study contributor(s) not responsible for patient care.

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| Activation | November 29, 2012 |
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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 | Lisa Finstuen, Quality Assurance Specialist<br>Phone: 507-284-1328<br>E-mail: finstuen.lisa@mayo.edu |
| Protocol document, consent form, regulatory issues | David Harwood/ Research Protocol Specialist Phone: (904) 953-0558<br>Email: Harwood.David@mayo.edu |
| Non-paraffin biospecimens | Laura Pacheco-Spann<br>Phone: (904) 953-6015<br>Email: flaprc@mayo.edu |
| Serious Adverse Events | Patricia G. McNamara, SAE C<br>Phone: (507) 266-3028<br>E-mail: mcnamara.patricia@mayo.edu |

*No waivers of eligibility per NCI

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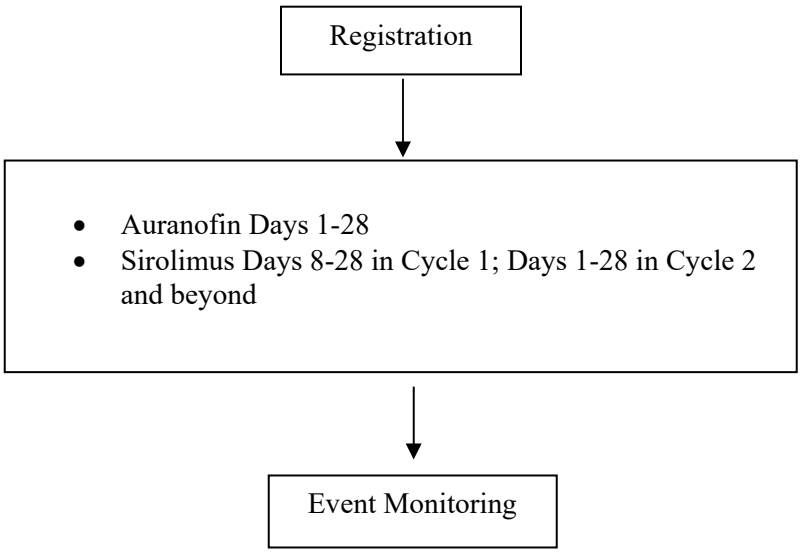
**Schema**

**PHASE I only: Prior to discussing protocol entry with the patient, call the Registration Office (507-284-2753) to ensure that a place on the protocol is open to the patient.**




**-PD at any time →**
**-Unacceptable adverse events →** **Event Monitoring**
**-Patient refusal →**

If a patient is deemed ineligible or a cancel, please refer to Section 13.0 for follow-up information.

Cycle length=28 Days

| | |
|---|---|
| Generic name: Sirolimus<br>Brand name(s): Rapamune<br>Mayo Abbreviation: RAPA<br>Availability: Commercial supply | Generic name: Auranafin<br>Brand name(s): Ridaura<br>Mayo Abbreviation: Auranofin<br>Availability: Commercial supply |

## 1.0 Background

### 1.1 Treatment

Lung cancer
Lung cancer is the most common cause of cancer death in the United States with estimated annual mortality in excess of 160,000 as of 2012.The vast majority of patients with lung cancer are not cured and the overall 5-year survival rate is a disappointing 16%. More than 80% of lung cancer patients have non-small cell lung cancer (NSCLC) and the mainstay of curative treatment for these patients is surgical resection, but only 25% to 30% of patients with NSCLC have resectable disease (Stage I or II) at diagnosis. Patients with Stage III lung cancer are treated with chemoradiotherapy with curative intent with long term survivals for a minority of patients. Survival for localized or locally advanced disease range from 25-70% at five years depending on stage. For most patients, however, diagnosis is made too late for curative therapy. Chemotherapy is the mainstay of palliative treatment and produces survivals in the 12 month range for patients with good performance status {Scagliotti, 2008 #24}. For small cell lung cancer, surgery is not a standard part of treatment for any stage and chemoradiotherapy cures about 25% of patients with localized cancer and a few of those with more advanced extensive stage cancer. In both cases, relapse is frequent and standard treatment options are few.

1.11 Palliative treatment (radiation and/or chemotherapy with supportive symptom management) is the main option for most patients with stage IV NSCLC. Response rates in the 20-30% range are expected with standard chemotherapy. Optimal therapy consists of a platinum doublet for good PS patients. Selected patients may benefit from treatment with targeted agents such as erlotinib or crizotinib in addition to or instead of chemotherapy {Sandler, 2007 #37}.

1.12 Until recently, it was felt that all commonly used chemotherapy regimens were equivalent in efficacy. The Eastern Cooperative Oncology Group (ECOG) tested four common chemotherapy regimens for Stage IIIB and IV NSCLC {Schiller, 2002 #40}. These regimens (24-hour paclitaxel/cisplatin; 3-hour paclitaxel/carboplatin; gemcitabine/cisplatin and docetaxel/cisplatin) yielded response rates (15 – 21%) and median survival rates (7.4 – 8.2 months) that were not statistically different. The poor outlook for patients with advanced lung cancer despite the best available therapy has prompted a search for new treatment options. ECOG tested carboplatin and paclitaxel with or without bevacizumab and showed that addition of the VEGF inhibitor improved overall survival in good PS patients with adenocarcinomas despite increase in bleeding {Sandler, 2006 #37}. Patients with squamous cancers were not enrolled due to excess bleeding risk discovered in earlier trials. Post hoc subset analysis suggested the majority of benefit in males and younger patients. The

subsequent AvAIL trial with cisplatin gemcitabine plus or minus bevacizumab produced superior response and survival in the control arm than the paclitaxel carboplatin backbone used in E4599 and did not show a survival benefit in any subset for addition of bevacizumab to chemotherapy {Reck, 2009 #43}. Scagliotti et al studied pemetrexed or gemcitabine with cisplatin in good PS patients with previously untreated advanced NSCLC. While the regimens were equivalent overall, a small but significant survival advantage was seen for pemetrexed in patients with adenocarcinomas and large cell cancers while gemcitabine had a similar advantage in patients with squamous cancers. {Scagliotti, 2008 #24} Analyses of previous trials with pemetrexed confirms the superior outcomes in non-squamous NSCLC {Scagliotti, 2009 #8} Histology must now be considered in choice of chemotherapy regimens for patients with stage IV NSCLC.

Maintenance:
Recent studies have shown a small but reproducible survival benefit to maintenance or early second line therapy with pemetrexed or erlotinib in NSCLC. These two agents have expected benefit primarily for non-squamous lung cancers ({Ciuleanu, 2009 #44}, Cappuzzo et al 2009). Agents with expected efficacy in squamous cancers are under active investigation but no standard maintenance is in common use for patients with squamous NSCLC although erlotinib is sometimes prescribed.

Small cell lung cancer:
Chemotherapy with etoposide and a platinum is standard for both limited and extensive stage with addition of radiation to the chest and/or prophylactic brain radiation recommended in the appropriate clinical circumstances. While topotecan or combination cyclophosphamide, doxorubicin and vincristine have been given in the second line setting, responses are modest and short lived. There is no standard maintenance or third line therapy for small cell patients.

**1.2** Protein Kinase Cι is a Novel Target

1.21 Protein Kinase Cι is an oncogene that can mediate K-ras induced lung carcinogenesis. The protein kinase C (PKC) family of serine/threonine kinases has been implicated in many forms of cancer, including lung cancer, and is therefore a potential therapeutic target for cancer treatment.

We recently demonstrated that the atypical protein kinase C, PKCι, is an oncogene in non-small cell lung cancer (NSCLC) {Regala, 2005 #50}. PKCι is overexpressed in NSCLC and SCLC tumors and tumor PKCι expression is predictive of poor clinical outcome independent of tumor stage {Regala, 2005 #50 & personal communication, Dr. Alan Fields}. The PKCι gene is a target of frequent, tumor-specific alteration by gene

amplification in a significant subset of NSCLCs {Stallings-Mann, 2006 #49}. Genetic disruption of PKCι signaling blocks the transformed growth of NSCLC cells in vitro and in vivo {Regala, 2005 #51}. Oncogenic PKCι signaling requires the N-terminal PB1 domain on PKCι {Stallings-Mann, 2006 #49}.

Using a high throughput screen, we identified the anti-arthritic gold salt aurothiomalate (ATM) and subsequently a similarly acting oral agent, Auranofin (Ridaura), as potent inhibitors of oncogenic PKCι signaling. ATM and auranofin both inhibit transformed growth of NSCLC cells by binding to the PB1 domain of PKCι, and blocking the interaction between PKCι and downstream adaptor molecules {Erdogan, 2006 #52}.

These agents exhibit potent anti-tumor activity against NSCLC in vitro and in vivo. We have demonstrated a correlation between the sensitivity of NSCLC cells to the anti-tumor effects of ATM, and the level of expression of PKCι. NSCLC cells that express high levels of PKCι are more sensitive to ATM than those with relatively low levels of PKCι expression. A phase I clinical trial conducted at our institution demonstrated feasibility of the approach. Because of drug shortages, we were unable to obtain ATM for the clinical trial described here. We therefore tested the oral, similarly acting agent auranofin in the laboratory. This drug is FDA approved for arthritis with the same indications as ATM. In vitro, its activity is at least equal to that of ATM at concentrations mirroring those found in human serum with standard clinical use. We hypothesize that auranofin will be an effective therapy for the treatment of NSCLC, especially those subsets of NSCLC exhibiting PKCι over-expression.

1.22 Gold compounds

The use of gold compounds for the treatment of rheumatoid arthritis (RA) was pioneered in 1929. Gold has also been used in some patients with psoriatic arthritis, ankylosing spondylitis with polyarthritis, and juvenile rheumatoid arthritis. Despite extensive clinical experience and an abundance of experimental data, the mechanism of action of gold compounds is poorly defined. The use of gold has declined since 1990 due to the increasing popularity of methotrexate and more recent immunomodulator therapies such as infliximab. Gold compounds occur in several oxidation states, with only complex gold compounds in clinical use. Most are administered only by intramuscular injection, however in this study an oral drug will be used: auranafin (Ridaura).

1.23 Pharmacokinetics of Gold

After intramuscular injection, gold salts are rapidly absorbed with maximal plasma levels at two hours {Forestier, 1935 #53}. Gold levels gradually rise with treatment until a plateau is reached after six to eight

weeks. The serum gold concentration correlates with the dose of gold administered; maintenance doses of 50 mg every four weeks result in a serum concentration ranging from 75 to 125 μg/dL. Intramuscular gold is retained in the body to a greater degree than oral gold. Approximately 40 percent of the dose of intramuscular gold is eliminated, either in the urine (70 percent) or in the feces (30 percent). As an example, about 300 mg of elemental gold is retained following 20 weekly injections of 50 mg, while only 73 mg of gold is retained after 20 weeks of oral gold doses of 6 mg per day. The kidneys, adrenals, and reticuloendothelial system achieve the highest gold concentrations. Intracellularly, gold attaches to organelle membranes in the nuclear, mitochondrial, and lysosomal fractions.

Auranofin is less bioavailable than injectable gold salts with about 15-25% absorbed and with less whole body retention at six months. The serum half-lives are similar and the total body half- life or auranofin is about 69 days, while it is three times longer for injectable ATM. Auranofin is excreted primarily by the fecal route {Furst, Dromgoole, 1984}. Oral administration is well tolerated and produces serum levels in the effective range based on both arthritis treatment and on our preclinical assessments of PKCiota inhibition.

Mammalian Target of Rapamycin (mTOR)

mTOR is a member of a family of protein kinases called phosphoinositide 3-kinase (PI3-K)-related kinases, which is involved in the regulation of many intracellular functions such as the control of G1 cell cycle protein synthesis. The main upstream regulator of mTOR is the phosphatidylinositol 3-kinase (PI3K) /protein kinase B (Akt) (PI3K/Akt), which activates mTOR in response to growth factor stimuli. PI3K seems to play a central role in cellular proliferation and is clearly upregulated in cancer cells. Phosphorylation of mTOR, in response to the activation of a growth receptor by its ligand, leads to the modulation of 2 different pathways: the eukaryotic initiation factor 4E binding protein-1 (4E-BP1) and the 40S ribosomal protein S6 kinase (p70s6k) {Sabatini, 2006 #55}. 4E-BP1 inhibits the initiation of translation through its binding with eIF-4E, the mRNA cap-binding sub-unit of the eukaryotic initiation factor-4 (eIF-4F) complex. The phosphorylated form of 4E-BP1 induced by mTOR has a lower affinity for eIF-4E, which increases its availability and promotes the translation initiation of mRNA 5’ cap in the G1 phase. The second downstream target modulated by mTOR is p70s6k. The major function of this protein is the phosphorylation of 40S ribosomal protein S6, which finally leads to the translation of mRNA.

1.24 Sirolimus

Rapamycin is a macrolide fungicide isolated from the bacteria Streptomyces hygroscopicus and is a potent antimicrobial, immunosuppressant and anti-tumor agent {Huang, 2003 #81}. After binding intracellularly the immunophilin FK506 binding protein 12 (FKBP12), rapamycin is capable of stopping cell proliferation and inducing apoptosis through the inhibition of mTOR and its downstream targets {Rao, 2004 #78}. This antiproliferative activity has been confirmed in preclinical experiments. mTOR inhibition has enhanced the efficacy of chemotherapy and radiation in preclinical models and early clinical trials in a variety of solid malignancies including NSCLC (reviewed in Pal S, et al *Clinical Lung Cancer,* Vol. 9, No. 6, 340-345, 2008).

Clinical trials of oral and IV inhibitors of mTOR have suggested an efficacy signal in lung cancers but have been disappointing as single agents with response endpoints. Their effects on relevant molecular pathways are clear from preclinical and animal models and their efficacy is likely to be more apparent to maintain responses initiated by cytotoxic agents than as primary therapy. A phase I study of everolimus suggested activity with disease stabilization in four of 14 heavily pretreated NSCLC pts treated with RAD001 {O'Donnell, 2003 #85}. A phase II study presented at ASCO in 2007 and subsequently published in 2009 in the Annals of Oncology showed PFS in the 3 month range in 85 advanced NSCLC patients treated in the second and third line settings with this agent {Soria, 2009 #86}. Most studies show stabilization of disease with mTOR inhibitors rather than measurable response, hence our interest in utilizing the drugs in studies in the maintenance or early second line setting as discussed below.

Sirolimus, a structural analog of rapamycin, is converted in vivo to rapamycin and has antiproliferative effects in cell culture and animal models as well as having antitumor effects in several solid tumors including NSCLC. Some data suggest that sirolimus may reduce the risk of cancer in renal transplant recipients. Additionally, phase I studies of the drug in cancer patients have not shown significant immunosuppression. Sirolimus eluting stents have been used to reduce endothelial proliferation contributing to restenosis after angioplasty. Sirolimus has been combined safely with chemotherapy and radiation in a phase I trial in NSCLC patients with no evidence of immunosuppressive effects and with serum levels compatible with mTOR pathway inhibition (*Sarkaria J et al, JTO 2:751-7, 2007*).

Sirolimus is FDA approved for organ transplants to mediate rejection.

1.25 Rationale for the Clinical Trial

PKCι is required for cellular transformation mediated by oncogenic Ras, which is expressed in approximately 40% of all NSCLC, and up to 70% of NSCLC in smokers. PKCι is overexpressed in NSCLC and tumor PKCι expression is predictive of poor clinical outcome independent of tumor stage. The PKCι gene is a target of frequent, tumor-specific alteration by gene amplification in a significant subset of NSCLCs {Regala, 2009 #45}. Genetic disruption of PKCι signaling blocks the transformed growth of NSCLC cells in vitro and in vivo {Stallings-Mann, 2006 #49}. Gold salts are potent inhibitors of oncogenic PKCι signaling inhibiting transformed growth of NSCLC cells by binding to the PB1 domain of PKCι, and blocking the interaction between PKCι and downstream adaptor molecules {Regala, 2008 #46} {Erdogan, 2006 #52}. We have demonstrated a correlation between the sensitivity of NSCLC cells to the anti-tumor effects of ATM and auranofin and the level of expression of PKCι. We hypothesize that auranofin will be an effective therapy for the treatment of NSCLC, especially those exhibiting elevated PKCι expression by virtue of PKCι gene amplification. We conducted a phase I trial of ATM in patients with advanced NSCLC and ovarian and pancreatic cancers, both of which also express high PKCι levels. The results of this trial demonstrated the safety of ATM and established a phase II dose of 50-75 mg IM weekly. No safety signals were identified in the first data analysis and the doses used mirror those used for the FDA approved indication of arthritis. There were no responses in the first 12 patients on study in this heavily pretreated population and one patient had stable disease for one cycle. ATM has become unavailable. We tested the oral gold salt aurothiomalate and found that it is equal to or better than ATM in preclinical studies at inhibiting PKCiota and tumor growth. Serum levels in RA patients mirror effective concentrations in our preclinical studies.

1.26 Rationale for combination of auranofin with sirolimus for patients with progression of disease after standard chemotherapy:
We were unable to obtain ATM for this study due to drug shortages. We have replicated the most important background data with ATM referenced above with auranofin in the laboratory and have demonstrated that auranofin shows highly synergistic anti-tumor activity with the mTOR inhibitor rapamycin in pre-clinical NSCLC models in vitro and in vivo as described in the translational research background below. Sirolimus is converted to rapamycin in vivo and is used clinically in transplant patients to manage organ rejection with overall good tolerance. Based on these data, we are pursuing a phase I/II trial to assess the efficacy of combined chemotherapy using auranofin and the mTOR inhibitor sirolimus in

advanced stage NSCLC patients and SCLC patients with residual or progressive disease after standard chemotherapy.

**Brief summary of scientific rationale:**

- Molecular targeted therapies have introduced clinically significant improvements in the therapeutic outcomes for NSCLC within the past 5 years. Bevacizumab, an anti-angiogenesis monoclonal antibody that targets VEGF (vascular endothelial growth factor), is approved in combination with front-line therapy using paclitaxel and carboplatin in patients with non-squamous NSCLC, based on results of E4599 (Sandler et al 2006). Inhibition of the epidermal growth factor pathway has also demonstrated clinical benefit particularly among patients with adenocarcinoma (Cappuzzo et al ASCO 2009 Abs).
- More recently, the combination of pemetrexed with a platinum agent as first-line treatment for NSCLC has been compared with gemcitabine in combination with a platinum agent (Scagliotti et al 2008). Pre-planned subset analysis in the phase III study reported by Scagliotti et al. suggested better survival with pemetrexed combination in patients with adenocarcinoma and in large cell histology and with gemcitabine for squamous NSCLC.
- Currently, there exists an apparent preponderance of novel agents that seem to provide benefit favoring the non-squamous subset of NSCLC.
- Patients with Ras mutated non-squamous NSCLC have worse prognosis and respond less well to chemotherapy than those with wild type Ras and present treatment challenges as a result.
- Patients with Ras mutated non-squamous NSCLC frequently exhibit PKCι gene and protein overexpression.
- Patients with squamous histology and Ras mutated adenocarcinoma comprise approximately 40-45% of NSCLC in the United States and have similar treatment outcomes. While to this date there are no novel therapeutic combinations that clearly demonstrate improved outcomes over existing standard therapies in this patient population, emerging data suggest potential targeted therapeutic options that merit further investigation.
- PKCι is of particular relevance in squamous NSCLC as it is expressed strongly in this population. Auranofin inhibits PKCι activity and slows the growth of squamous NSCLC in vitro and in vivo in animal models.
- Preclinical results with Ras mutant non-squamous NSCLC and small cell lung cancer (SCLC) are similar to those for the squamous NSCLC cells.
- Small cell lung cancer responds to first line therapy most of the time but relapses quickly and responds poorly to second line treatment.

- Small cell lung cancer overexpresses PKCι and in the laboratory responds to auranofin with PKCι inhibition and antiproliferative effects.
- Synergy between inhibition of the PKCι and mTOR pathways has been shown in recent laboratory studies in our and other laboratories.
- A phase I clinical trial has demonstrated the safety of PKCiota inhibition with gold salts (ATM) in patients with pretreated advanced NSCLC.
- A phase II study of mTOR inhibition in the second and third line setting in non-selected NSCLC showed a suggestion of activity, primarily stabilization of disease.

Based on the above, we wish to test the hypothesis that the auranofin/sirolimus combination will be effective and well-tolerated in patients with NSCLC of squamous histology, Ras mutated lung cancer and relapsed small cell lung cancer with no standard treatment options.

**1.3** Translational Research

1.31 **Background:**

We have demonstrated that the atypical PKC (PKCι) is an oncogene in lung cancer (Regala *et al.*, 2005a; Regala *et al.*, 2005b). PKCι is overexpressed in a majority of non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) tumors. PKCι expression is driven in a significant subset of NSCLC tumors as a result of tumor-specific amplification of the PKCι gene, which is part of the 3q26 amplicon. PKCι gene amplification is most prominent in lung squamous cell carcinoma (LSCC) tumors, where it is present in ~70% of tumors (Regala *et al.*, 2005b). Functionally, PKCι is required for NSCLC cell transformed growth and invasion in vitro and tumorigenicity in vivo (Regala *et al.*, 2005a). PKCι drives transformed growth through activation of a PKCι-Rac1-Pak-Mek-Erk proliferative signaling pathway. PKCι activates this pathway by forming an oncogenic complex with the adaptor protein Par6, which serves to couple PKCι to Rac1 (Frederick *et al.*, 2008). The PKCι-Par6 complex is required for oncogenic PKCι signaling. Using a high throughput screen, we identified ATM and other gold salts such as auranofin as a potent and selective small molecule inhibitors of the PKCι-Par6 interaction (Stallings-Mann *et al.*, 2006). ATM inhibits the PKCι-Par6 interaction in a dose dependent fashion, and exhibits potent anti-tumor activity against NSCLC cell proliferation in vitro and tumorigenicity in vivo (Erdogan *et al.*, 2006; Stallings-Mann *et al.*, 2006). Biochemical studies have demonstrated that the mechanism of action for the anti-tumor activity of ATM is to bind to a specific region of PKCι

causing disruption of Par6 binding to PKCι (Erdogan *et al.*, 2006). ATM is currently unavailable due to drug shortages in the United States. Auranofin (Ridaura) is FDA-approved for the treatment of rheumatoid arthritis (RA). A phase I dose escalation study of ATM in advanced NSCLC has established a well-tolerated dosing regimen for ATM in the treatment of NSCLC patients. Auranafin also shows highly synergistic anti-tumor activity with the mTOR inhibitor rapamycin in pre-clinical NSCLC models.

**Auranofin is an FDA-approved anti-rheumatoid drug in the same chemical class as aurothiomalate (ATM) and aurothioglucose**

The original protocol proposed a phase I/II clinical trial of aurothiomalate (ATM) in combination with mTOR inhibitor rapamycin in the maintenance therapy of advanced lung cancer patients. Recent drug shortages of ATM have prompted us to assess the efficacy of auranofin, another gold compound in the same chemical class as ATM as a substitute for the proposed clinical trial. Auranofin (Ridaura) is FDA-approved for treatment of RA and has been in clinical testing for treatment of acute lymphocytic leukemia. The drug has not been a subject of drug shortages and is accessible for the trial. **Figure 1** shows the chemical structure of the gold compounds ATM, ATG and auranofin. Note that all compounds belong to the same chemical class with the active pharmacophore being a gold thiolate moiety (**Figure 1**).

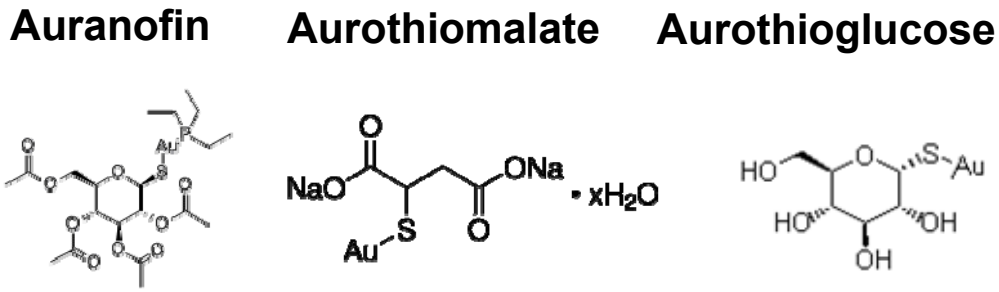



**Figure 1: Structures of the major anti-rheumatoid gold compounds in clinical use**. Note the presence of the same gold thiolate pharmacophore in each compound.

In order to determine whether, like ATM, auranofin exhibits anti-tumor activity against NSCLC cell growth, we conducted a dose response experiment to assess the effect of auranofin on the growth of H1703 lung squamous cell carcinoma cell growth. As can be seen, auranofin exhibits good dose-dependent inhibition of the transformed growth of these cells with an apparent IC50 of ~0.3 < M (**Figure 2**). This IC50 is actually better than that of ATM against this cell line (~ 1< M). Therefore, these data indicate that auranofin is a suitable substitute for ATM in the proposed clinical trial.

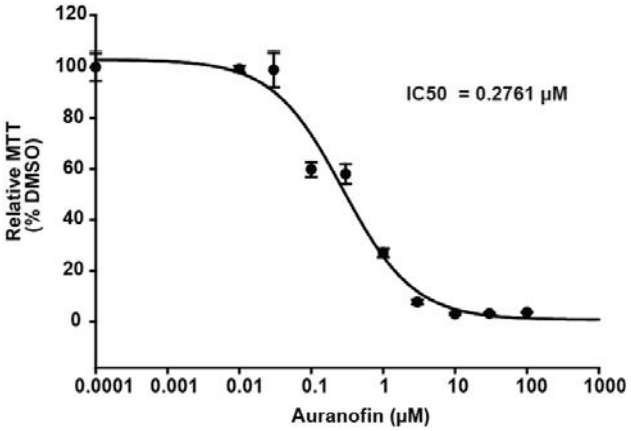



**Figure 2: Auranofin exhibits dose-dependent inhibition of growth of H1703 human lung squamous cell carcinoma cells**. Highly tumorigenic H1703 cancer stem cells were isolated from parental H1703 cell cultures, and assessed for transformed growth in the presence of the indicated concentrations of auranofin. Results are expressed as % growth relatively to DMSO negative control.

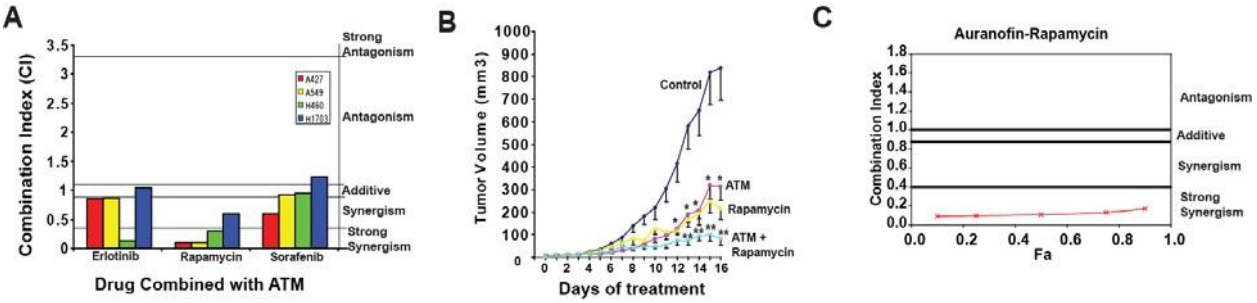



Figure 3: ATM and auranofin exhibit synergistic anti-tumor activity in combination with rapamycin. A. combination index of ATM with combination with the indicated targeted therapeutics. B. ATM and rapamycin show enhanced anti-tumor activity in vivo. C. Auranofin synergizes with rapamycin.

We also demonstrated that ATM exhibits synergistic anti-tumor activity in vitro (Figure 3A) and in vivo (Figure 3B) when combined with the mTOR inhibitor rapamycin. Auranofin exhibits similar synergy when combined with rapamycin, indicating that these agents act similarly (Figure 3C). Based on these data and on existing safety data sirolimus and other mTOR inhibitors in human use, we propose this phase II trial to assess the efficacy of combined therapy with auranofin and the sirolimus in advanced stage NSCLC patients in the adjuvant setting.

**Associated Laboratory Studies:**

**Genetic and Molecular Markers of ATM Sensitivity:**

Our recent studies have demonstrated that the level of PKC□ expression in NSCLC tumor cells correlates with response to the anti-tumor effects of ATM in vitro and in vivo (Regala *et al.*, 2008). Furthermore, we demonstrated that tumor-specific PKC□ gene amplification is a major mechanism driving PKC□ expression in primary tumors (Regala *et al.*, 2005b). These preclinical data suggest that assessment of PKC□ expression and PKC□ gene amplification in a patient's tumor may be predictive of response to ATM or auranofin therapy. Therefore, we wish to assess whether there is a similar correlation between tumor PKC□ expression and auranofin response clinically. For this purpose, we propose to obtain pre- treatment biopsies of patients' tumors prior to enrollment on the clinical trial. These biopsy samples will be used to extract genomic DNA for analysis of PKC□ gene copy number (PKC□ gene amplification) which is a major mechanism driving the overexpression of PKC□ in NSCLC tumors, and for immunohistochemical analysis of tumor tissue for elevated PKC□ protein expression within the tumor. In addition, we propose to assess whether levels of markers of PKC□ and mTOR signaling activity correlate with subsequent response. The techniques for this analysis are well established in our laboratory.

**A gene panel to assess auranofin-mediated inhibition of PKC□ signaling in vivo:**

We have conducted pre-clinical studies to identify genes that can be used as molecular markers for assessing auranofin mediated inhibition of PKC□ signaling.

## 2.0 Goals

**2.1** Primary

2.11 Phase I: To establish the maximum tolerated dose of auranofin plus sirolimus after at least one line of platinum based chemotherapy for stage IV squamous histology NSCLC for lung cancer (squamous, Ras-mutated adenocarcinoma, or small cell lung cancer) patients with no acceptable standard treatment options.

2.12 Phase II: To assess the progression-free survival at four months of patients treated with auranofin after at least one line of platinum based chemotherapy for lung cancer (squamous, Ras-mutated adenocarcinoma, or small cell lung cancer) patients with no acceptable standard treatment options.

**2.2** Secondary

2.21 To assess the overall survival in this population in comparison to recent historical controls.

2.22 To determine the Adverse Events (AE) profile and safety of the regimen.

2.23 To determine the overall response rate, per RECIST criteria, and duration of tumor response in those patients with measurable disease.

**2.3** Correlative Research

2.31 To assess the relationship between molecular correlates and PFS, OS, response and adverse events.

## 3.0 Patient Eligibility

**3.1** Inclusion Criteria

3.11 Age ≥ 18 years.

3.12 Histologic or cytologic confirmation of lung cancer (squamous, Ras-mutated adenocarcinoma or small cell lung cancer).

3.13 Patients must have received at least one course of chemotherapy consisting of a platinum doublet and must have no acceptable standard treatment options.

3.14 Prior radiation therapy is permitted as long as:

- Recovered from the toxic effects of radiation treatment before study entry, except for alopecia.

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

- ANC ≥1500 □L
- PLT ≥100,000 □L
- 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

3.16 ECOG performance status (PS) 0, 1, 2. (Appendix I)

3.17 Negative serum pregnancy test done ≤7 days prior to registration, for women of childbearing potential only.

3.18 Ability to provide informed consent.

3.19a Life expectancy □12 weeks.

3.19b Willing to return to Mayo Clinic enrolling institution for follow-up.

3.19c Willing to provide tissue samples for correlative research purposes (see Sections 6, 17).

**3.2** Exclusion Criteria

3.21 Any of the following because this study involves an agent that has known genotoxic, mutagenic and teratogenic effects.
- □ Pregnant women
- □ Nursing women
- □ Men or women of childbearing potential who are unwilling to employ adequate contraception

3.22 Symptomatic, untreated, or uncontrolled CNS metastases or seizure disorder. NOTE: Patients with treated CNS metastases without evidence of progression and without uncontrolled symptoms or need for steroids may enroll.

3.23 HIV-positive patients receiving combination anti-retroviral therapy are excluded because of possible pharmacokinetic interactions with oral investigational agents.

3.24 Unwilling or unable to, comply with the protocol.

3.25 Any of the following prior therapies:
- □ Radiation to ≥25% of bone marrow (see appendix III)

- □ Major surgery (i.e., laparotomy), open biopsy, or significant traumatic injury ≤4 weeks prior to registration. Minor surgery ≤2 weeks prior to registration. Insertion of a vascular access device is not considered major or minor surgery in this regard.

3.26 Any of the following concurrent severe and/or uncontrolled medical conditions:
- □ Hypertension, labile hypertension, or history of poor compliance with antihypertensive medication
- □ Angina pectoris
- □ History of congestive heart failure ≤3 months, unless ejection fraction >40%
- □ Myocardial infarction ≤6 months prior to registration
- □ Cardiac arrhythmia
- □ Poorly controlled diabetes
- □ Interstitial pneumonia or extensive and symptomatic interstitial

fibrosis of the lung

- Active or recent history of hemoptysis. If hemoptysis has resolved with measures such as palliative radiation therapy (e.g. 3000 cGy over 10 fractions), arteriographic embolization or endobronchial interventions (e.g. photodynamic therapy, brachytherapy), etc. for >14 days, patients may be considered for participation in this study.
- ≥ Grade 2 hypertriglyceridemia
- ≥ Grade 2 hypercholesterolemia
- Any illness that in the opinion of the investigator would compromise the ability of the patient to participate safely in the clinical trial.

3.27 Use of St. John's Wort because of its effects on hepatic drug metabolism.

3.28 Other active malignancy.
EXCEPTIONS: Non-melanoma skin cancer, localized prostate cancer, or carcinoma-in-situ of the cervix. NOTE: If there is a history or prior malignancy, patient must not be receiving other cytotoxic or molecularly targeted therapeutics treatment for their cancer. Patients receiving certain hormonal manipulations as part of their treatment may be allowed to continue at the discretion of the PI (e.g. LHRH analogs for prostate cancer).

3.29 Unable to discontinue use of potent CYP3A4 inhibitors/inducers (see appendix IV).

## 4.0 Test Schedule

| Tests and procedures | ≤14 days prior to registration | Weekly (± 2 days) | Cycle 1 | Prior to subsequent cycles (± 2 days) |
|---|---|---|---|---|
| History and exam, wt, PS | X | | X | X |
| Height | X | | | |
| Adverse event assessment | X | | | X |
| Tumor measurement (CT, PET/CT) | X[1] | | | X[2] |
| Hematology group<br>WBC, ANC, Hgb, PLT | X | X[4] | X | X |
| Chemistry group<br>Na, K, chloride, glucose, creatinine, total bilirubin, albumin, alkaline phosphatase, SGOT (AST), SGPT (ALT) | X | X[4] | X | X |
| Lipid Panel | X[R] | | | X[8] |
| Urinalysis/urine protein dipstick | X | X[4] | X | X |
| Pregnancy Test | X[3] | | | |
| Patient Medication Diary for Sirolimus and Auranofin (Appendix II)[5] | | | X | X |
| Mandatory research tissue (see Section 17)[6,7] | X[R] | | | |

1. Imaging studies such as chest x-ray, CT scans, and MRIs can be performed ≤30 days prior to registration. Use same imaging throughout the study. The CT portion of a PET CT can be considered interchangeable with a CT scan.
2. Tumor assessment must be performed at least every other cycle after completing Cycle

2;
i.e. after Cycles 2, 4, 6, etc. Tumor assessment should be performed at any time during the treatment cycle that disease progression is clinically suspected so that patient can go off treatment and receive other therapy.

3. Women of childbearing potential only. Must be done ≤7 days prior to registration.
4. Weekly during cycle 1 only.
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. Tissue specimens must be collected and submitted *once at baseline.*
7. Optional repeat biopsy of accessible tumor at response and/or disease progression.
8. Lipid panel is performed at baseline, prior to cycle 2, every other cycle through cycle 6, and then as clinically indicated (per Standard of Care) thereafter.
9. R Research funded (see Section 19.0).

## 5.0 Grouping Factor:

**5.1** Phase: Phase I vs Phase II.

## 6.0 Registration/Randomization Procedures

**6.1** Phase I

**Prior to discussing protocol entry with the patient, call the MCCC Registration Office (507-284-2753) for dose level and to insure that a place on the protocol is open to the patient.**

6.11 Registration Procedures

6.111 To register a patient, fax (507-284-0885) a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

**6.2** Phase II

6.21 Registration Procedures

6.211 To register a patient, 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 (507) 284-2753 between the hours of 8 a.m. and 5:00 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 (507) 284-2753. 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."

**6.3** Phase I and II

6.31 Correlative Research

Mandatory

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.19c and 17.0).

Optional

An optional correlative research component is part of this study: there will be an option to select if the patient is to be registered onto this component (see Section 17.0).

- Patient has/has not given permission to give his/her *tissue* sample for research testing as a part of this study (see section 17.0).

6.32 Prior to accepting the registration, registration/randomization 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

6.33 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: 507-284-0885). 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.

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

- Patient has/has not given permission to store and use his/her sample(s) for future research of Non-small cell lung 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.

6.35 Treatment on this protocol must commence at Mayo Clinic Arizona institution under the supervision of a medical oncologist.

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

6.37 Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.38 All required baseline symptoms (see Section 10.3) must be documented and graded.

## 7.0 Protocol Treatment

### 7.1 Treatment Schedule

7.11 Phase I Cohort:

| Agent | Dose Level | Route | Day | ReRx |
|---|---|---|---|---|
| Auranofin | As assigned by Registration office | PO | Daily | Q28 days |
| Sirolimus | 5 mg | PO | Daily (cycle 1 only, begin day 8, subsequent cycles begin day 1) | Q28 days |

7.12 Phase 2 Cohort (at MTD from Phase I):

| Agent | Dose Level | Route | Day | ReRx |
|---|---|---|---|---|
| Auranofin | As determined by studies in Phase I | PO | Daily | Q28 |
| Sirolimus | 5 mg | PO | Daily (cycle 1 only, begin day 8, subsequent cycles begin day 1) | Q28 days |

**7.2** Phase I Dose Escalation and Determination of MTD

7.21 Dose Escalation Guidelines

| Dose Level | Auranofin |
|---|---|
| 0* | 3 mg daily |
| 1 | 6 mg daily |

* Starting dose level.

7.211 Three patients will be treated at each dose level and observed for a minimum of 1 cycle, to assess toxicities, before new patients are treated.

7.212 Investigators are to contact the Study Chair as soon as any dose-limiting toxicity occurs.

7.213 For this protocol, dose-limiting toxicity (DLT) will be defined as an adverse event attributed (definitely, probably, possibly) in the first cycle to the study treatment and meeting the following criteria:

| Toxicity | DLT Definition |
|---|---|
| Hematologic | Grade 4 Neutropenia |
| | Grade 4 Thrombocytopenia |
| Non-hematologic | ≥Grade 3 Anaphylaxis |
| | ≥Grade 3 Proteinuria |
| | ≥Grade 3 Hematuria |
| | ≥Grade 3 Rash acneiform |
| | ≥Grade 3 Diarrhea |
| | ≥Grade 3 Mucositis oral |

7.22 MTD Determination

MTD is defined as the dose level below the lowest dose that induces dose-limiting toxicity in at least one-third of patients (at least 2 of a maximum of 6 new patients).

7.221 Three patients will be treated at a given dose level combination and observed for at least 1 cycle to assess toxicity (accrual will be suspended while the 3 patients are treated and observed).

7.222 If dose-limiting toxicity (DLT) is not seen in any of the 3 patients, 3 new patients will be accrued and treated at the next higher dose level. If DLT is seen in 2 or 3 of 3 patients treated at a given dose level, then the next 3 patients will be treated at the next lower dose level, if only 3 patients were enrolled and treated at this lower dose level.

7.223 If DLT is seen in 1 of 3 patients treated at a given dose level, up to 3 additional patients will be enrolled and treated at the same dose level (accrual will be suspended while the 3 patients are treated and observed). If DLT is seen in at least one of these additional three patients (≥2 of 6), the MTD will have been exceeded and further accrual will cease to this cohort. If DLT is not seen in any of the three additional patients, 3 new patients will be accrued and treated at the next higher dose level.

7.224 After enrolling 6 patients on a specific dose level, if DLT is observed in at least 2 of 6 patients, then the MTD will have been exceeded and defined as the previous dose unless only 3 patients were treated at the lower dose level. In that case, 3 additional patients will be treated at this lower dose level.

7.23 Dose Escalation: Doses will not be escalated in any individual patient.

7.24 If a patient fails to complete Cycle 1 for reasons other than toxicity, the patient will be regarded as treatment intolerant and an additional patient will be treated at the current dose level.

**7.3** Patients can be instructed in administration techniques for both sirolimus and auranofin and granted treatment independence.

**7.4** Patient must return to Mayo Clinic for evaluation at least every week during the first cycle of treatment and then at least every 4 weeks.

**7.5** Study 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*

***8.1*** Dose Levels (Based on Adverse Events in Tables 8.*2)*

| **Dose Level** | Auranofin | **Sirolimus** |
|---|---|---|
| +1 | 6 mg po daily | Not applicable |
| 0 | 3 mg po daily | 5 mg po daily |
| -1 | 3 mg po qOd | 3 mg po daily |
| -2 | None | 2 mg po daily |

**8.2** Dose modification Table

| □ □ ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified*** □ □ | | | |
|---|---|---|---|
| **CTCAE System/Organ/ Class (SOC)** | **ADVERSE EVENT** | **AGENT** | **ACTION** |
| ***BASED ON INTERVAL ADVERSE EVENT*** | | | |

| □ □ *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified* □ □ | | | |
|---|---|---|---|
| **CTCAE System/Organ/ Class (SOC)** | **ADVERSE EVENT** | **AGENT** | **ACTION** |
| Investigations | Neutrophil count decreased Grade 4<br><br>Platelet count decreased Grade 4 | Auranofin Sirolimus | Delay therapy until ANC ≥ 1500 and/or<br>PLT ≥ 100,000, and decrease by 1 dose level; discontinue treatment if toxicity persists beyond 14 days or if subject is already at dose level -2 and go to event monitoring phase per Section 18. |
| | Neutrophil count decreased Grade 3<br><br>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 treatment if toxicity persists beyond 14 days or if subject is already at dose level -2 and go to event monitoring phase per Section 18. |
| | Cholesterol High Grade 2 | | Institute appropriate lipid/cholesterol lowering therapy. Hold treatment until levels return to Grade 1. If unresolved by four weeks, discontinue auranofin and sirolimus therapy and go to event monitoring. |
| | Grade 3-4 | | Discontinue auranofin and sirolimus and go to event monitoring. |
| **Respiratory, thoracic and mediastinal disorders** | Interstitial pneumonitis any grade | Auranofin Sirolimus | Discontinue and go to event monitoring |

| □ □ *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified* □ □ | | | |
|---|---|---|---|
| **CTCAE System/Organ/ Class (SOC)** | **ADVERSE EVENT** | **AGENT** | **ACTION** |
| **Metabolism and nutrition disorders** | Hypertriglyceridemia Grade 2<br><br>Grade 3-4 | Auranofin Sirolimus | Institute appropriate lipid/cholesterol lowering therapy. Hold treatment until levels return to Grade 1. If unresolved by four weeks, discontinue auranofin and sirolimus therapy and go to event monitoring.<br><br>Discontinue auranofin and sirolimus and go to event monitoring. |
| **Other non-hematologic adverse events** | Non-hematologic Grade 3 or 4 | Auranofin Sirolimus | Delay therapy until severity decreases to grade 0-1 and decrease dose by one level; discontinue treatment if toxicity persists beyond 14 days or if subject is already at dose level -2 and go to event monitoring phase per Section 18.. |

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

**NOTE:** Adverse events requiring a dose-reduction step for any or all drugs beyond the two dose-reduction steps (levels –1 and –2) will result in the patient coming off study.

## 9.0 Ancillary Treatment/Supportive Care

**9.1** Antiemetics may be used at the discretion of the attending physician.

**9.2** Radiation therapy for symptom palliation is allowed. Radiation should be to non-target sites (i.e. painful pre-existing bony metastasis) in patients who are benefiting from therapy.

**9.3** Corticosteroids for treatment of adrenal insufficiency or severe nausea and vomiting are permitted.

**9.4** Blood products should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors is not permitted during study treatment.

**9.5** 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.

**9.6** Bisphosphonates: Patients with lytic metastatic bone disease may receive bisphosphonates at the discretion of the treating physician.

**9.7** Rash: The use of corticosteroid creams, and/or antihistamines are allowed at physician discretion.

**9.8** Diarrhea: This could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

## 10.0 Adverse Event (AE) Reporting and Monitoring

**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 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/forms/default.htm).

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE v4.0. Next, determine whether the event is expected or unexpected (refer to Sections 10.12 and 15.0) and if the adverse event is related to the medical treatment or procedure (see Section 10.13). With this information, determine whether an adverse event should be reported to the NCI as an expedited report (see Section 10.2) or as part of the routinely reported clinical data. Important: All AEs reported via

expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Section 10.3 and 18.0).

Expedited and/or routine reports are to be completed within the timeframes and via the mechanisms specified in Sections 10.2 and 10.3. All expedited AE reports must also be sent to the local Institutional Review Board (IRB) according to local IRB's policies and procedures.

10.12 Expected vs. Unexpected

- The determination of whether an AE is expected is based on agent-specific adverse event information provided in Section 15.0 of the protocol.
- Unexpected AEs are those not listed in the agent-specific adverse event information provided in Section 15.0 of the protocol.

10.13 Assessment of Attribution

*When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:*

Definite - The adverse event *is clearly related* to the investigational agent(s).
Probable - The adverse event *is likely related* to the investigational agent(s).
Possible - The adverse event *may be related* to the investigational agent(s).
Unlikely - The adverse event *is doubtfully related* to the investigational agent(s).
Unrelated - The adverse event *is clearly NOT related* to the investigational agent(s)

**10.2** Expedited Adverse Event Reporting Requirements

10.21 Paper reporting will be done via the AdEERS form found at Adverse Event Expedited Report – Single Agent or Multiple Agents

| | Grade 4 or 5[1] Unexpected/ Expected Regardless of Attribution | Grade 3 Unexpected/ Expected Regardless of Attribution | Other Grade 4 or 5 Events and/or any Hospitalization During Treatment Not Otherwise Warranting an Expedited Report |
|---|---|---|---|
| Submit written report within 5 working days[2] | X | X | |
| Submit Grade 4 or 5 Non-AER Reportable Events/Hospitalization in EDC within 5 working days.[3] | | | X[3] |

1. Includes **all deaths within 30 days of the last dose of agent** regardless of attribution or **any death attributed to the agent(s)** (possible, probable, or definite) **regardless of timeframe**.
2. Use paper *Adverse Event Expedited Report – Single Agent or Multiple Agents* report available within the study forms packet

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Mayo Clinic Cancer Center (MCCC) Institutions: Provide copies, along with the UPIRTSO cover sheet, by fax (507-538-7164) to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist who will determine and complete IRB reporting. The RAU will submit to the MCCC SAE Coordinator and the MCCC IND Coordinator to determine if FDA submission is needed.

3. Complete a Notification Form: Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form electronically via the Data Entry System within 5 working days of the date the clinical research associate (CRA) is aware of the event(s) necessitating the form. If expedited written report was submitted, this form does not need to be completed.

**10.3** Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per the CTCAE v4.0 grading unless otherwise stated in the table below:

| System Organ Class (SOC) | Adverse event/Symptoms | Baseline | Each evaluation |
|---|---|---|---|
| General disorders and administration site conditions | Fever | X | X |
| Blood and lymphatics system disorders | Anemia | X | X |
| Investigations | Neutrophil count decreased | X | X |
| | Platelet count decreased | X | X |
| | Creatinine increased | X | X |
| | Cholesterol high | X | X |
| | Blood bilirubin increased | X | X |
| Vascular disorders | Hypotension | X | X |
| | Flushing | X | X |
| Skin and Subcutaneous tissue orders | Rash Acneiform | X | X |
| Gastrointestinal disorders | # of stools | X | |
| | Diarrhea | | X |
| | Nausea | X | X |
| | Vomiting | X | X |
| | Mucositis, oral | X | X |

| | | | |
|---|---|---|---|
| Infections and Infestations | Bladder Infection | X | X |
| | Bronchial Infection | X | X |
| | Skin Infection | X | X |
| | Lung Infection | X | X |
| Renal and urinary disorders | Proteinuria | X | X |
| Metabolism and Nutrition disorders | Hyperglycemia | X | X |
| | Hypertriglyceridemia | X | X |
| Musculoskeletal and connective tissue disorders | Myalgia | X | X |
| | Arthralgia | X | X |
| Nervous system disorders | Dizziness | X | X |
| Immune system disorders | Allergic Reaction | X | X |

10.31 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 10.3:

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

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

10.313 Grade 5 AEs (Deaths)

10.3131 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.3132 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.

10.32 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 Using RECIST Guideline

NOTE: This study uses protocol RECIST v1.1 template dated 2/16/2011. See the footnote for the table regarding measureable disease in Section 11.44, as it pertains to data collection and analysis.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline.

**11.1** Schedule of Evaluations: For the purposes of this study, patients should be reevaluated every 8 weeks.

**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 □2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.
Tumor lesions in a previously irradiated area are considered measurable disease under the following conditions:

a. The radiation completion date is ≥ 90 days from the registration date.

b. There is objective evidence of disease progression after completion of the radiation therapy.

11.212 A superficial non-nodal lesion is measurable if its longest diameter is ≥ 1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

11.213 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).

11.214 Clinical Lesions: Clinical lesions will only be considered measurable when they are superficial (e.g. skin nodules and palpable lymph nodes) and >1.0 cm diameter as assessed using calipers (e.g. skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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, inflammatory breast disease, 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. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.

- Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

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: If the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the 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.

- Physical Examination: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

- FDG-PET: FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A ‘positive’ FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered ‘negative.’ New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

    a. Negative FDG-PET at baseline with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

    b. No FDG-PET at baseline and a positive FDG-PET at follow-up:

        i. If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

ii. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal PDG-PET scan.

iii If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, it is not classified as PD.

11.33 Measurement at Follow-up Evaluation:

- 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 8 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 tumor types such as germ cell tumors, where known residual benign tumors can remain.)

## **11.4** Measurement of Effect

11.41 Target Lesions & Target Lymph Nodes

- All measurable lesions (as defined in Section 11.21-11.22) up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. If the protocol specified studies are performed, and there are fewer than 5 lesions 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 should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

  Baseline Sum of Diameters (BSD): A sum of the diameters [longest for non-nodal target lesions (see Section 11.211), short axis for target lymph nodes see Section 11.212)] for all target lesions will be calculated and reported as the baseline sum of diameters. 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 Diameters (PBSD): A sum of the diameters [longest for non-nodal target lesions (see Section 11.211), short axis for target lymph noted (see Section 11.212)] for all target lesions will be calculated and reported as the post-baseline sum of diameters. If the radiologist is able to provide an actual measure for the target lesion, that should be recorded, even if it is below 0.5 cm. If the target lesion 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 lesion has likely disappeared, the measurement should be recorded as 0 cm.

- The minimum sum of the diameters (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/physical examination must be measured on re-evaluation at evaluation times specified in Section 11.1. 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, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

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.

c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

- 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.

- 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.)

c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

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 tables:

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<br>Non-CR/Non-PD | No | PR |
| CR/PR | Not All Evaluated* | No | PR** |
| SD | CR<br>Non-CR/Non-PD<br>Not All Evaluated* | No | SD |
| Not all Evaluated | CR<br>Non-CR/Non-PD<br>Not All Evaluated* | No | Not Evaluated (NE) |
| PD | Unequivocal PD<br>CR<br>Non-CR/Non-PD<br>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<br>Non-CR/Non-PD<br>Not All Evaluated* | Yes | PD |

*See Section 11.431

** NOTE: This study uses the protocol RECIST v1.1 template dated 2/16/2011. For data collection and analysis purposes the objective status changed from SD to PR in the MCCC protocol RECIST v1.1 template as of 2/16/2011 and to match RECIST v1.1 requirements.

For Patients with Non-Measurable Disease Only:

| Non-Target Lesions & Non-Target Lymph Nodes | New Sites of Disease | Overall Objective Status |
|---|---|---|
| CR | No | CR |
| Non-CR/Non-PD | No | Non-CR/Non-PD |
| Not All Evaluated* | No | Not Evaluated (NE) |
| Unequivocal PD | Yes or No | PD |
| Any | 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. A patient is classified as having PD due to "symptomatic deterioration" if any of the following occur that are not either related to study treatment or other medical conditions:

- Weight loss >10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

## 12.0 Descriptive Factors

**12.1** Brain metastasis at on-study: Yes vs. no.

**12.2** Prior palliative radiation: Yes vs. no.

**12.3** Phase I dose level: 0 vs. 1

## 13.0 Treatment/Follow–up Decision at Evaluation of Patient

**13.1** Patients who are CR, PR, or SD will continue treatment per protocol.

**13.2** Patients who develop PD while receiving therapy will go to the event-monitoring phase.

**13.3** Patients who go off protocol treatment for reasons other than PD will go to the event-monitoring phase per Section 18.0.

**13.4** Patients who develop PD in the CNS only should receive resection and/or brain radiationand continue treatment on study after completion of CNS treatment.

**13.5** Patients who develop non-CNS PD at any time should go to event monitoring. These patients should be treated with alternative chemotherapy if their clinical status is good enough to allow further therapy.

**13.6** 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. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly to the event-monitoring phase of the study (or off study, if applicable).

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section
  18.0 of the protocol.
- If the patient never received treatment, on-study material must be submitted. Event monitoring will be required per Section 18.0 of the protocol.

**13.7** A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. Event monitoring will be required per Section 18.0 of the protocol.

13. 8 A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

## 14.0 Body Fluid Biospecimens: 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: Dermatitis (aggravated by sunlight), pruritus, actinic rash, hair loss, urticaria
Gastrointestinal: Nausea, vomiting, abdominal cramps, diarrhea, loose stools, constipation, dysgeusia, stomatitis, glossitis
Hematologic: Anemia, leukopenia, thrombocytopenia, Eosinophilia
Hepatic: Elevated liver enzymes
Renal: Hematuria

**Less common known potential toxicities, 1% - 10%**:
Gastrointestinal: Anorexia, flatulence, dyspepsia
Ocular: Conjunctivitis
Renal: Proteinuria

**Rare known potential toxicities, <1% (Limited to important or life-threatening):**
Dermatologic: Generalized exfoliative dermatitis, angioedema

Gastrointestinal: Dysphagia, gastrointestinal bleeding, melena, positive stool for occult blood, ulcerative enterocolitis
Mucous membrane: Gingivitis
Hematologic: Aplastic anemia, neutropenia, agranulocytosis, pure red cell aplasia, pancytopenia
Hepatic: Jaundice
Neurologic: Peripheral neuropathy
Respiratory: Interstitial pneumonitis

**15.18 Drug procurement:**
Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

**15.19 Nursing Guidelines:**

15.191 Auranofin is contraindicated in patients with history of gold-induced disorders (see section 15.17). 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 LFT's 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:** Do not crush, split or chew.
**Solution:** Mix with at least 2 ounces 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 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 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.
**Common or severe toxicity**: Hypertension, edema, capillary leak syndrome, poor wound healing, hypertriglyceridemia, hypercholesterolemia, hypokalemia, constipation, diarrhea, dyspepsia, nausea and vomiting, deep venous and arterial thrombosis, abnormal liver function tests, hypersensitivity reaction, infection, sepsis, lymphoma, arthralgia, headache, renal failure, interstitial pneumonitis, pulmonary hemorrhage, thrombocytopenia and leukopenia.

15.28 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

**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 bee 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 protocol will assess the efficacy of maintenance auranofin plus sirolimus after at least one line of platinum based chemotherapy for lung cancer (squamous, Ras-mutated adenocarcinoma, or small cell lung cancer) patients with no acceptable standard treatment options , using a one-stage phase II study design. A run-in phase I study (described in Section 7.0) will be conducted to determine the MTD for the phase II portion.

16.11 Phase I Study

This run-in phase I study is a cohorts of 3 design. For a subject to be considered evaluable for dose-limiting toxicity (DLT) the subject must have received at least one dose of each drug in Cycle 1. If a subject withdraws from the study within the first cycle of treatment for reasons other than adverse events, the subject will be replaced; and only DLTs occurring in Cycle 1 will be used for establishing safety during the run-in phase I study. However, all adverse event data will be summarized in the final analysis. For this run-in phase I study, DLT the study design is defined in Section 7.0. The patients treated at the MTD within the phase I will be included in the phase II efficacy analysis. Safety for all patients (including the patients in the run-in phase I study) will continue to be monitored via the Adverse Event Stopping Rule.

16.12 Sample Size: Phase I: The study may involve a maximum of 12 patients (6 for each of 2 dose levels). Phase II: This study will accrue an additional 35 patients to have a total of 41 patients accrued to the efficacy portion of this study (6 patients at the MTD in the phase I portion plus 35 patients accrued during the phase II portion). This includes 10% overaccrual of patients during the phase II portion to allow for cancellation, ineligibility, and major treatment violations (i.e., accrual of 41 patients to have 37 evaluable patients as required in 16.41). Overall: Therefore, overall, the study will accrue a **maximum of 47 patients**.

16.13 Accrual Time and Study Duration: The anticipated accrual rate is approximately 2 patients per month, based on physician estimate. This corresponds to a requirement of approximately 17 weeks to enroll (12 weeks), treat (4 weeks), and evaluate (1 week to submit data) 6 patients. Therefore, the accrual time for the phase I portion will be a maximum of 34 weeks. The overall study duration will be 34 weeks (phase I component) + 70 weeks (accrual of an additional 35 patients) + 16 weeks (4 months to evaluate the primary endpoint) = 120 weeks (about 28 months). The final analysis can begin approximately 28 months after activation, i.e., as soon as the last patient has been observed for 4 months and data have been submitted.

16.14 Operating Characteristics: The following table gives the probability of dose escalation at a single dose level as a function of the true probability of DLT at that level using the cohorts of 3 design described in Section 7.0.

| True Rate of DLT (%) | Probability of Dose Escalation |
|---|---|
| 10 | 0.91 |
| 20 | 0.71 |
| 30 | 0.49 |
| 40 | 0.31 |
| 50 | 0.17 |

**16.2** Phase I Analysis Plans: All the relevant phase I results pertaining to adverse events, MTD, clinical endpoints, and laboratory correlates will be examined in an exploratory and hypothesis generating fashion.

16.21 Adverse Events Profile

The number and severity of all adverse events (overall and by dose level) will be tabulated and summarized.

16.22 Clinical Endpoints

Overall survival, progression-free survival, and response will be summarized descriptively (overall and by dose level).

**16.3** Phase II Design and Analysis

16.31 Primary Endpoint: The primary endpoint of this trial is the 4-month progression-free survival rate. Throughout Section 16.0, "4-month progression-free survivor" will be considered synonymous with "success", unless otherwise specified. A patient is considered to be a 4-month progression-free survivor if the patient is 4 months from registration without a documentation of disease progression (note, the patient need not be on study treatment at 4 months to be considered a success). All patients meeting the eligibility criteria who have signed a consent form and have initiated treatment will be evaluable for the primary endpoint.

**16.4** Statistical Design

16.41 Decision Rule: The largest success proportion where the proposed treatment regimen would be considered ineffective in this population is 35%, and the smallest success proportion that would warrant subsequent studies with the proposed regimen in this patient population is 55%. The following one-stage design based on properties of the binomial distribution uses 37 patients to test the null hypothesis that the true success proportion in a given patient population is at most 35%.

The null 4-month progression-free survival rate of 35% is based on Figure

2 of Scagliotti et al (2008). In the subgroup of patients with squamous cell carcinoma on the cisplatin / gemcitabine arm, approximately 65% of patients were progression-free 4 months after randomization (i.e., at or near completion of first-line therapy). Four months subsequent to this time point, the PFS rate was approximately 25%. Thus, among those who were progression-free at or near completion of first-line therapy, the subsequent 4-month PFS rate for patients receiving no maintenance therapy following first-line cisplatin / gemcitabine is approximately 35% (≈0.25/0.65).

Enter 37 patients into the study. If 16 or fewer successes are observed in the first 37 evaluable patients, we may consider this regimen ineffective in this patient population. If 17 or more successes are observed in the first 37 evaluable patients, we may recommend further testing of this regimen in subsequent studies in this population.

16.411 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 16.34.

16.42 Power and Significance Level: Assuming that the number of successes is binomially distributed, the significance level is ≤0.12 and the probability of declaring that this regimen warrants further studies (i.e., statistical power) under various success proportions can be tabulated as a function of the true success proportion as shown in the following table.

| If the true success proportion is . . . . . | 0.35 | 0.40 | 0.45 | 0.50 | 0.55 |
|---|---|---|---|---|---|
| then the probability of declaring that the regimen warrants further studies is . . . . . | 0.11 | 0.28 | 0.52 | 0.74 | 0.90 |

16.43 Other Considerations: Adverse events, 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.

**16.5** Analysis Plan: The analysis for this trial will commence at planned timepoints and at the time the patients have become evaluable for the primary endpoint. Such a decision will be made by the Statistician and Study Chair, in accordance with Mayo Clinic Cancer Center (MCCC) Standard Operating Proceedures, availability of data for secondary endpoints (e.g., laboratory correlates), and the level of data maturity.

16.51 Primary Endpoint

16.511 Definition: The primary endpoint of this trial is the proportion of 4-month progression-free survivors as defined in Section 16.31.

16.512 Estimation: The proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients. Confidence intervals for the true success proportion will

be calculated using the properties of the binomial distribution. Additionally, if some patients are lost to follow-up not having been observed for at least 4 months, an estimate and confidence interval for the 4-month progression-free survival rate incorporating censoring will be computed using the method of Kaplan-Meier (1958).

16.52 Definitions and Analyses of Secondary, Exploratory, and Correlative Endpoints

Survival time is defined as the time from registration to death due to any cause. The distribution of survival time will be estimated using the method of Kaplan-Meier (1958).
Progression-free survival time is defined as the time from registration to the earliest date of documentation of disease progression. If a patient dies without a documentation of disease progression the patient will be considered to have had disease progression at the time of their death. In the case of a patient starting treatment and then never returning for any evaluations, the patient will be censored for progression 1 day post-registration. The distribution of progression-free survival time will be estimated using the method of Kaplan-Meier (1958).

A response is defined to be either a CR or PR noted as the objective status. As response rate is a secondary endpoint, confirmation is no longer required per RECIST v1.1. The overall response rate will be estimated in the subset of patients with measureable disease by the number of responses in evaluable patients with measurable disease divided by the total number of evaluable patients with measureable disease. The appropriate confidence interval will be calculated based on the binomial distribution.

Duration of response is defined for all evaluable patients with measurable disease who have achieved a response as the date at which the patient's earliest best objective status is first noted to be either a CR or PR to the earliest date progression is documented. If a patient dies subsequent to the response without a documentation of disease progression, the patient will be considered to have had disease progression at the time of their death. In the case of a patient failing to return for evaluations before a documentation of disease progression, the patient will be censored for progression on day 1 post response or the date of last evaluation (whichever one occurs later). The distribution of duration of response will be estimated using the method of Kaplan-Meier (1958).

Translational analyses: Given the small sample size, all translational analyses are considered exploratory and no adjustment for multiplicity will be employed. One-sided p-values ≤0.10 are considered statistically significant throughout. Results will be used to design subsequent confirmatory studies (likely as translational components within the subsequent phase III study should this phase II study support the use of this combination in a phase III study).

We consider the analysis of baseline PKCι gene amplification to be the primary analysis (though still exploratory) among the

various translational anlayses. PKCι gene amplification will be evaluated at baseline using the baseline tissue specimen and explored in relation to 4-month progression-free survival and subsequently in relation to other clinical outcomes such as tumor response and adverse event incidence using two-way tables and analyzed using Fisher's exact tests. To estimate power for this primary analysis, we assume that 90% of patients (N=33) will provide a usable sample (i.e., will have gene amplification status for this tissue study). We also assume that 70% of patients (N=23) will have PKCι gene amplification (Regala et al., 2005b) and that 55% of patients overall will have 4-month PFS. The proportion of patients with 4-month PFS will be compared between patients with tumors with PKCι gene amplification to patients with tumors without PKCι gene amplification using a 1-sided $\alpha$=0.10 Fisher's exact test. We have 86% power to detect a 50% difference in the 4-month PFS rates (70% vs 20%) between gene amplified and gene not amplified groups (calculated using nQuery Advisor 6.01 with the following parameters: $\alpha$=0.10 1-sided, $\pi$1=0.70, $\pi$2=0.20, n1=23, n2=10).

PKCι protein expression (using the baseline tissue specimen) will be evaluated at baseline and explored in relation to clinical outcomes such as 4-month progression-free survival, tumor response, and adverse event incidence using two-way tables and box plots and analyzed using Fisher's exact tests or logistic regression methods, as appropriate. Further, the relationship among PKCι gene amplification and PKCι protein expression will be investigated using box plots and scatter plots and analyzed using Wilcoxon rank sum tests and Spearman correlation coefficients, as appropriate.

Adverse Events: All eligible patients that have initiated treatment will be considered evaluable for assessing adverse event rate(s). The maximum grade for each type of adverse event will be recorded for each patient, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.

16.53 Data & Safety Monitoring

16.531 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 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.

16.532 Adverse Event Stopping Rule (PHASE II ONLY): The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in

consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected

adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (ie, an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy the following:

- if 3 or more patients in the first 20 treated patients (or 10% after 20 patients have been accrued) experience a Grade 4 or higher non-hematologic adverse event.

We note that we will review Grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

**16.6** Results Reporting on ClinicalTrials.gov: At study activation, this study will have been registered within the "ClincialTrails.gov" website. The Primary and Secondary Endpoints (i.e., "Outcome Measures") along with other required information for this study will be reported on ClinicalTrials.gov. For purposes of timing of the Results Reporting, the initial estimated completion date for the Primary Endpoint of this study is 32 months after the study opens to accrual. The definition of "Primary Endpoint Completion Date" (PECD) for this study is at the time the last patient registered has been followed for at least 4 months.

**16.7** Inclusion of Women and Minorities

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

16.72 There is no information currently available regarding differential effects of this treatment regimen in subsets defined by race, gender, 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 and gender groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.73 Based on prior MCCC studies involving similar disease sites, we expect about 4% of patients will be classified as minorities by race and 40% of patients will be women. Expected sizes of racial by gender subsets are shown in the following table:

**Accrual Estimates by Gender/Ethnicity/Race for All Phase 2 and 3 Studies**

| Ethnic Category | Sex/Gender | | | |
|---|---|---|---|---|
| | Females | Males | Unknown | Total |
| Hispanic or Latino | 1 | 2 | | 3 |
| Not Hispanic or Latino | 18 | 26 | | 44 |
| **Ethnic Category: Total of all subjects*** | 19 | 28 | | **47** |
| **Racial Category** | | | | |
| American Indian or Alaska Native | 0 | 1 | | 1 |
| Asian | 0 | 0 | | 0 |
| Black or African American | 1 | 1 | | 2 |
| Native Hawaiian or other Pacific Islander | 0 | 0 | | 0 |
| White | 18 | 26 | | 44 |
| **Racial Category: Total of all subjects*** | 19 | 28 | | **47** |

**Ethnic Categories:**

**Hispanic or Latino –** a person of Cuban, Mexican, Puerto Rico, 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 Alaska 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 Pathology report 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

### 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 pathology report. Patients who consent and who have accessible disease will have tissue samples obtained by standard needle core biopsy at the time of disease progression as well. These samples will be analyzed by Dr. Alan Fields as described in Section 17.3.

Forward FFPE tissue to the following laboratory:
Laura Pacheco-Spann
Pathology Research Core
Mayo Biorepositories
4500 San Pablo Road S.
Jacksonville, FL 32224

For quality assurance purposes, biopsy tissues will be fixed immediately in 10% phosphate-buffered formalin and fixed tissues will be embedded in paraffin. Tissue processing will occur at the BAP/PRC Laboratory, which is a College of American Pathologists (CAP) certified
This laboratory follows the NCI guidelines (National Cancer Institute Best Practices for Biospecimen Resources, 2007) and develops customized Quality Assurance/Quality Control (QA/QC) policies and procedures for each study. In

this particular study, the quality of biopsy tissues will be assessed by the study pathologist (Dr. Andras Khoor) on an H&E slide. For patients who have previously undergone biopsy, existing biopsy tissue may be used.

Samples upon arrival will be accessioned into the biorepository through the Research Laboratory Information Management System (RLIMS), samples are to be forwarded to Dr. Alan Fields laboratory in Griffin Building in MCF.

**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 our board certified lung pathologist, Dr. Andras Khoor. 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 (Dr. Cherise Cortese). 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. Diagnostic Slides from Original and /or Recurrent Tissue.

## 18.0 Records and Data Collection Procedures

Submission Timetable

**Initial Material(s) -**

| **Case Report Form (CRF)** | **Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0) |
|---|---|
| On-Study Form | □2 weeks after registration |
| Baseline Adverse Event Form | |
| Pretreatment Measurement Form | |
| | |
| Baseline Research Tissue Submission Form (see Section 17.0) | |
| Baseline Concomitant Medication Form | |
| End of Active Treatment/Cancel Notification Form | Submit □2 weeks after registration if withdrawal/refusal occurs prior to beginning protocol therapy |

**Test Schedule Material(s) -**

| **CRF** | **Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0) | |
|---|---|---|
| | At each evaluation during treatment | At end of treatment |
| Evaluation/Treatment Form | X[2] | X |
| Adverse Event Form | X | X |
| Measurement Form | X[1] | X[1] |
| | | |
| Research Tissue Submission Form | X (see Section 17.0) | |
| Concomitant Medication Form | X | X |
| End of Active Treatment/Cancel | | X |

| CRF | Active-Monitoring Phase (Compliance with Test Schedule Section 4.0) | |
|---|---|---|
| | At each evaluation during treatment | At end of treatment |
| Notification Form | | |
| Notification Form – Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form | At each occurrence (see Section 10.0) | |
| ADR/AER | At each occurrence (see Section 10.0) | |

1. Submit supporting documentation of response or progression to the MCCC operations office via RAVE.
2. Complete at each evaluation during Active Treatment (see Section 4.0).

**Follow-up Material(s) -**

| CRF | Event Monitoring Phase[1] | | | | |
|---|---|---|---|---|---|
| | q. 3 months until PD[2] | At PD[2] | After PD q. 6 mos. | Death | New Primary |
| Event Monitoring Form | X | X | X | X | At each occurrence |

1. If a patient is still alive 2 years after registration, no further follow-up is required.
2. Submit supporting documentation of response or progression to the MCCC operations office via RAVE.

## 19.0 Budget

**19.1** Costs charged to patient: Routine clinical care will be the responsibility of the patient and or their insurance carrier.

**19.2** Items to be research funded: Serum gold levels; Sirolimus; Auranofin; Lipid Panel at Baseline, C2, C3, and C4; Research blood draw and tissue collection and processing.

**19.3** Other budget concerns: None

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## Appendix I: ECOG PERFORMANCE STATUS

**Grade**

| | |
|---|---|
| 0 | Fully active, able to carry on all pre-disease activities without restriction (Karnofsky 90 100). |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work (Karnofsky 70-80). |
| 2 | Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50 percent of waking hours (Karnofsky 50-60). |
| 3 | Capable of only limited self-care, confined to bed or chair 50 percent or more of waking hours (Karnofsky 30-40). |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20). |
| 5 | Dead |

## Appendix II: PATIENT'S MEDICATION DIARY

Today's date _____

Patient Name_____ Patient Study ID _____
(*initials acceptable)*

**INSTRUCTIONS TO THE PATIENT:**

1. Complete one form for each month.
2. You will take____pills each day. You may take the pill(s) one hour before or 2 hours after eating, as you prefer.
3. Record the date, the number of pills you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
3. Please bring your pill bottle and this form to your physician when you go for your next appointment.

| Date | Day | # pills and when taken | Comments | Date | Day | # pills and when taken | Comments |
|---|---|---|---|---|---|---|---|
| | 1 | | | | 17 | | |
| | 2 | | | | 18 | | |
| | 3 | | | | 19 | | |
| | 4 | | | | 20 | | |
| | 5 | | | | 21 | | |
| | 6 | | | | 22 | | |
| | 7 | | | | 23 | | |
| | 8 | | | | 24 | | |
| | 9 | | | | 25 | | |
| | 10 | | | | 26 | | |
| | 11 | | | | 27 | | |
| | 12 | | | | 28 | | |
| | 13 | | | | 29 | | |
| | 14 | | | | 30 | | |
| | 15 | | | | 31 | | |
| | 16 | | | | | | |

Patient's Signature:_____Date:
_____

**Physician's Office will complete this section:**

1. Date patient started protocol treatment_____Date patient was removed from study
   _____
2. Patient's planned daily dose_____ Total number of pills taken this month
   _____

Physician/Nurse/Data Manager's Signature _____

## Appendix III: Percent of Normal Bone Marrow Irradiated using Standard Radiation Ports*

| | Marrow Volume At Risk |
|---|---|
| Skull (not including mandible) | 12% |
| Upper limb girdle (unilateral) (humeral head, scapulae, clavicle) | 4% |
| Sternum | 2% |
| Ribs (all) | 8% |
| Ribs (hemithorax) | 4% |
| Cervical vertebrae (all) | 3% |
| Thoracic vertebrae (all) | 14% |
| Lumbar vertebrae (all) | 11% |
| Sacrum | 14% |
| Pelvis (including both innominates and both femoral heads and necks) | 26% |
| Mantle (approximate) | 25% |
| Upper para aortic nodes (approximate) | 11% |
| Inverted Y (approximate) | 45% |

*Based on Ellis RE: *Phys Med Biol* 5:255, 1961

**Appendix IV:**
Selected drugs known to be metabolized by CYP3A4

## CYP3A4

| SUBSTRATES | | INHIBITORS | | INDUCERS | |
|---|---|---|---|---|---|
| **Generic Name** | **Trade Name** | **Generic Name** | **Trade Name** | **Generic Name** | **Trade Name** |
| Anti-neoplastics: *e.g.* | | Anti-arrhythmics: *e.g.* | | Aminoglutethimide | Cytadren |
| Docetaxel | Taxotere | Amlodarone | Cordarone, Pacerone | | |
| Gefitinib | Iressa | Diltiazem | Cardizem, Dilacor XR | | |
| Irlnotecan | Camntosar | Qμ_i,11_i_<:l_i_tl | Cardioauin | | |
| Anti-virals: *e.g.* | | Anti-virals: *e.g.* | | Antibiotics: *e.g.* | |
| Amprenivir | Agenerase | Amprenavir | Agenerase | Rifabutin | Rifadin |
| Rifampin | Rifadin | Indinavir | Crixivan | Rifampin | Mycobutin |
| | | Nelfinavir | Viracept | | |
| | | Ritonavir | Norvir | | |
| Anxio\ytics: *e.g.* | | Cimetadine | Tagamet | Anticonvulsants: *e.g.* | |
| Diazepam | Valium | | | Carbamazapine | Tegretol |
| Sertraline | Zo!oft | | | Phenytoin | DHantin |
| | | | | Pentobarbital | Nembutal |
| | | | | Phenobarbital | Luminal |
| Cyc\osporine | Sandimmune | y(::IQSp_(l· :ii:i | Sandimmune | *H1mericum verforatum* (2) | St. John's Wort |
| Anti-infectives: *e.g.* | | Antibiotics: *e.g.* | | | |
| Erythromycin | Erythrocin | Ciprofloxacin | Cipro, Ciloxan | | |
| Tetracycline | Sumycin | Clarithromycin | Biaxin | | |
| | | Doxycycline | Adoxa, Periostat | | |
| | | Enoxacin | Penetrex | | |
| | | Isoniazid | Nydrazid, INH | | |
| | | Telithromycin | Ketek | | |
| Steroids: *e.g.* | | Imatinib | Gleevec | | |
| Estrogens, conjugated | Premarin | | | | |
| Estradiol | C!imara | | | | |
| Progesterone | Crinone | | | | |
| Haloperidol | Haldol | Ji. l_p r.!9£1" | Haldol | | |
| Cardiovascular agents: *e.g.* | | Diclofenac | Cataflam, Voltaren | | |
| Digitoxin | Crystodigin | | | | |
| Quinidine | G<l:r:<ii.9g _i_f!_ | | | | |
| Anti-hypertensives: *e.g.* | | Vasodi\ators: *e.g.* | | | |
| Nicardipine | Cardene | Nlcard_ipine | Cardene | | |
| Verapamil | Calan. Chronovera | yera12:amil | Calan, Chronovera | | |
| Anesthetics: *e.g.* | | Anesthetics: *e.i:* | | | |
| Ketamine | | Lidocaine | Xylocaine | | |
| Lidocaine | Xylocaine | f'.fOJJ().f()_l_ | Di2rivan | | |
| Nefazodone | Serzone | Anti-depressants: *e.g.* | | | |
| | | Nefazodone | Serzone | | |
| | | Sertraline | | | |
| Cocaine | | A ti fUrtgaiil:. *e.g.* | | | |
| | | Itraconazole | Sporanox | | |
| | | Ketoconazole | Nizoral | | |
| | | Miconazole | Lotrimin, Monistat | | |
| Ketoconazole | N""""" | Caffeine | | | |
| Sildenafil | L"Y!.fl_gra | ·o;· ;;efrUft·;_ k (fi..... | | | |
| Albuterol | \ Ventolin | | | | |
| Carbamazapine | I....I _gretol | | | | |
| Lovastatin | Mevacor | | | | |

When drugs classified as 'substrates' are co-administered with *(Study Agent).* there is the potential for higher concentrations of the 'substrate'. When *(Study Agent)* is co-administered with compounds classified as 'inhibitors', increased plasma concentrations of *(Study Agent/* is the potential outcome. The co-administration of'inducers' would potentially lower plasma *(Study Agent)* concentrations.

**Comprehensive list of drngs that may have potential interactions with CYP3A4**

**CYP3A4**

| Substrates | | | |
|---|---|---|---|
| Albuterol | Docetaxel | Ketoconazole | Quetiapine |
| Alfentanil | Doxepin | Lansoprazole | Quinidine |
| Alprazolam | Doxorubicin | Letrozole | Rabeprazole |
| Amlodiplne | Doxycycline | Levomethadyl acetate | Repaglinide |
| Amprenavir | Efavirenz | hydrochloride | Rifabutin |
| Aprepitant | Eletriptan | Levonorgestrel | Rifampin |
| Aripiprazole | Enalapril | Lidocaine | Ritonavir |
| Atazanavir | Eplerenone | Losartan | Saquinavir |
| Atorvastatin | Ergoloid mesylates | Lovastatin | Sertraline |
| Benzphetamine | Ergonovine | Medroxyprogesterone Mefloquine | Sibutramine |
| Bisoprolol | Ergotamine | Mestranol | Sildenafi! |
| Bortezomib | Erythromycin | Methadone | Simvastatin |
| Bosentan | Escitalopram | Methylergonovine | Sirolimus |
| Bromazepam | Estradiol | Methysergide | Sufentanil |
| Bromocriptine | Estrogens, conj., synthetic | Miconazole | Tacrolimus |
| Buprenorphine | Estrogens, conj., equine | Midazolam | Tamoxifen |
| Buspirone | Estrogens, conj., esterlfied | Miglustat | Tamsulosin |
| Busulfan | Estrone | Mirtazapine | Telithromycin |
| Carbamazapine | Estropipate | Modafinil | Teniposide |
| Cerivastatin | Ethinyl estradiol | Monte!ukast | Terbinafine |
| Chlordiazepoxide | Ethosuximide | Moricizine | Tetracycline |
| Chloroquine | Etoposide | Nateglinide | Theophylline |
| Chlorpheniramine | Felbamate | Nefazodone | Tiagabine |
| Cisapride | Felodipine | Nelfinavir | Ticlopidine |
| Cita\opram | Fentanyl | Nevirapine | To\terodine |
| Clarithromycin | Flurazepam | Nicardlpine | Toremifene |
| Clobazam | Flutamide | Nifedipine | Trazodone |
| Clonazepam | Fosamprenavir | Nimodipine | Triazolam |
| Clorazepate | Fu\vestrant | Nisoldipine | Trimethoprim |
| Cocaine | Gefitinib | Nitrendipine | Trimipramine |
| Colchicine | Halofantrine | Norethindrone | Troleandomycin |
| Cyclophosphamide | Haloperido\ | Norgestrel | Vardenafil |
| Cyclosporine | Ifosfamide | Ondansetron | Venlafaxine |
| Dantrolene | Imatinib | Pac!itax.e\ | Verapamil |
| Dapsone | Indinavir | Pergolide | Vinblastine |
| Delavirdine | Irinotecan | Phencyclidine | Vincristine |
| Diazepam | Isosorbide dinitrate | Pimozide | Vinorelbine |
| Digitoxin | Isosorbide mononitrate | Piog\itazone | Zolpidem |
| Dihydroergotamine | Isradipine | Primaquine | Zonisamidc |
| Diltiazem | ltraconazole | Progesterone | Zopic!one |
| Disoovramide | Ketamine | | |

**CYP3A4**

| Inhibitors | | | |
|---|---|---|---|
| Acetaminophen | Dıltıazem | Lovastatin | Progesterone |
| Acetazolamide | Disulfiram | Mefloquine | Propofol |
| Amioderone | Docetaxel | Mestranol | Propoxyphene |
| Amlodipine | Doxorubicin | Methadone | Quinidine |
| Amprenavir | Doxycycline | Methimazole | Quinine |
| Anastrozole | Drospirenone | Methoxsalen | Quinupristin |
| Aprepitant | Efavirenz | Methylprednisolone | Rabeprazole |
| Atazanavir | Enoxacin | Metronidazo!e | Risperidone |
| Atorvastatin | Entacapone | Miconazole | Ritonavir |
| Azelastine | Ergotamine | Midazolam | Saquinavir |
| Azithrornycin | Erythromycin | Mifepristone | Selegiline |
| Betamethasone | Ethinyl estradiol | Mirtazapine | Sertraline |
| Bortezomib | Etoposide | Mitoxantrone | Sildenafil |
| Bromocriptine | Felodipine | Modafinil | Sirolimus |
| Caffiene | Fentanyl | Nefazodone | Sulconazole |
| Cerivastatin | Fluconazole | Nelfinavir | Tacro!imus |
| Chloramphcnico! | Fluoxetine | Nevirapine | Tamoxifen |
| Chlorzoxazone | Fluvastatin | Nicardipine | Telithromycin |
| Cimetadine | Fluvoxamine | Nifedipine | Teniposide |
| Ciorofloxacin | Fosamnrcnavir | Nisoldioine | Testosterone |

| | | | |
|---|---|---|---|
| Cisapride | Glyburide | Nitrendipine | Tetracycline |
| Clarithromycin | Grapefruit juice | Nizatidine | Ticlopidine |
| Clemastine | I-Ialoperidol | Nortloxacin | Trany\cypromine |
| Clofazimine | Hydralazine | O!anzapine | Trazodone |
| Clotrimazole | Ifosfamide | Omeprazo\e | Troleandomycin |
| Clozapine | Imatinib | Orphenadrine | Valproic acid |
| Cocaine | Indinavir | Oxybutynin | Venlafaxine |
| Cyclophosphamide | Irbesartan | Paroxetine | Verapimil |
| Cyclosporine | lsoniazid | Pentamidine | Vinb!astine |
| Danazol | Isradapine | Pergolide | Vincristine |
| Delavirdine | Itraconazole | Phencyclidine | Vinorelbine |
| Desipramine | Ketoconazole | Pilocarpine | Zafirlukast |
| Dexmedetomidinc | Lansoprazole | Pimozide | Ziprasidone |
| Diazepam | Lidocaine | Pravastatin | |
| Diclofenac | Lomustine | Prednisolone | |
| Dihydroergotamine | Losartan | Primaquine | |

| Inducers | | | |
|---|---|---|---|
| Aminoglutethimide | Nevirapine | Phenytoin | Rifapentine |
| Carbamazapine | Oxcarbazepine | Primidone | |
| Fosphenytoin | Pentobarbital | Rifabutin | |
| St. John's wort | Phenobarbital | Rifampin | |

(Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In: Lacy CF, Annstrong LL, Goldman MP, Lance LL eds. Drug Infonnation Handbook 12TH ed. Hudson, OH; LexiComp Inc. 2004: 1619-1631.)

**Potential Drug Interactions:**

*In vitro* data indicate that sirolimus is an inhibitor for CYP2C9, CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2El, and CYP3A4. In animal studies, there were no GW786034-related effects on the activities ofCYPlA, CYP2B, CYP2E, CYP3A, and CYP4A.

*In vitro,* the most potent inhibition was seen for the isoenzyme CYP2C9. Accordingly, the following medications which are substrates for CYP2C9 are PROHIBITED in subjects receiving sirolimus (the washout period is at the discretion of the clinician based on the pharmacokinetic properties of each individual agent):

- Anticoagulants: warfarin (therapeutic doses only)
- Oral hypoglycemics: glipizide, glyburide, tolbutamide, glimepiride, nateglinide
- Ergot derivatives: dihydroergotamine, ergonovine, ergotamine, methylergonovine
- Neuroleptic: pimozide
- Erectile dysfunction agents: sildenafil, tadalafil, vardenafil
- Antiarrhythmics: bepridil, flecainide, lidocaine, mexilitine, amiodarone, quinidine, propafenone
- Immune modulators: cyclosporine, tacrolimus, sirolimus
- Miscellaneous: theophylline, quetiapine, risperidone, tacrine, cloazapine, atomoxetine

Certain medications should be used with CAUTION due to the potential for alterations in the pharmacologic effects or increased adverse events secondary to the inhibition of multiple CYP enzymes by sirolimus. These medications include (but are not limited to):

- Antidepressants: amitriptyline, buproprion, fluoxetine, fluvoxamine, imipramine

- HMG co-reductase inhibitors: atorvastatin, fluvastatin, lovastatin, simvastatin
- Benzodiazepines: alprazolam, midazolam, triazolam, clorazepate, diazepam, flurazepam
- Calcium channel blockers: diltiazem, felodipine, nifedipine, nicardipine, nimodipine, nitrendipine, verapamil, amlodipine, nisoldipine, isradipine
- Angiotensin II blockers: losartan, irbesartan
- Beta blockers: carvedilol, metoprolol, propanolol, timolol
- Anticonvulsants: phenobarbital, phenytoin, primadone, carbamazepine
- Miscellaneous: codeine, methadone, mifepristone, estrogens and progestins (including oral contraceptives)
- Oral hypoglycemics: pioglitazone, rosigitazone

*In vitro* data also suggest that sirolimus is a substrate for CYP3A4. Therefore, substances that induce or inhibit CYP3A4 may alter the pharmacologic effects of sirolimus and should be used with CAUTION. These medications include (but are not limited to):

**Inhibitors of CYP3A4:**

- Antibiotics: clarithromycin, erythromycin, troleandomycin
- HIV: anti-retrovirals (delaviridine), protease inhibitors (ritonavir, indinavir, saquinavir, nelfinavir, amprenavir, lopinavir)
- Antifungals: itraconazole, ketoconazole, voriconazole, fluconazole
- Antidepressants: nefazodone, fluvoxaine
- Calcium channel blockers: verapamil, diltiazem
- GI: cimetidine, aprepitant
- Miscellaneous: grapefruit or its juice

**Inducers of CYP3A4:**

- Glucocorticoids: cortisone (> 50 mg), hydrocortisone (> 40 mg), prednisone (>10 mg), methylprednisolone (> 8 mg), dexamethasone (> 1.5 mg)Anticonvulsants: phenytoin, carbamazepine, Phenobarbital, oxcarbazepine
- HIV: efavirenz, nevirapine
- Antibiotics: rifampin, rifabutin, rifapentine
- Miscellaneous: St. John's wort, modafinil