

A Phase I/II Trial Of Pemetrexed (Alimta®) Combined With Sirolimus (Rapamycin, Rapamune®) In Subjects With Relapsed Or Refractory NSCLC.

Abbreviated Title: Pemetrexed/Sirolimus NSCLC

Principal Investigator: Arun Rajan, M.D.  
Medical Oncology Branch/CCR/NCI  
9000 Rockville Pike  
Bldg 10, Room 12N226  
Bethesda, MD 20892  
Telephone: 301 594-5322  
Fax +1 301 402 0172  
Email: [rajana@mail.nih.gov](mailto:rajana@mail.nih.gov)

Lead Associate Investigator: Gideon Blumenthal, M.D.  
Special Volunteer, Medical Oncology Branch/CCR/NCI  
Bldg. 10, Rm. 12N226  
10 Center Drive  
Bethesda, MD 20892  
Tel: (301) 796-5369  
Fax: 301 402-0172  
Email: [gideon.blumenthal@nih.gov](mailto:gideon.blumenthal@nih.gov)

Associate Investigators: David Schrupp, M.D., SB/CCR/NCI  
Stephen Hewitt, M.D., Ph.D.\*  
William D. Figg, Pharm.D., M.B.A., MOB, CCR/NCI\*  
Guinevere Chun, R.N., MOB, CCR/NCI \*  
Takefumi Komiya, M.D., PhD, MOB, CCR/NCI

Statistician: Seth M. Steinberg, Ph.D., OCD/CCR/NCI\*  
6116 Executive Blvd, Room 702  
Telephone: 301-496-9502  
Fax: none  
E-mail address: [steinbes@mail.nih.gov](mailto:steinbes@mail.nih.gov)

\* denotes persons who will not be making clinical decisions

Drug Name: *Sirolimus, commercial, (Rapamune®, Wyeth Pharmaceuticals; Philadelphia, PA) Pemetrexed, commercial, (Alimta®, Eli Lilly & Co.; Indianapolis, IN)*

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## PRÉCIS

### Background

- Lung cancer is the most deadly cancer due to late stage of diagnosis and intrinsic resistance to chemotherapy.
- Pemetrexed is a well tolerated FDA-approved second line chemotherapeutic agent with a 9% response rate.
- Increasing the efficacy of pemetrexed could provide clinical benefit for patients with refractory NSCLC.
- Inhibition of the PI3K/Akt/mTOR pathway may increase response to chemotherapy.
- Combining sirolimus, an mTOR inhibitor, with pemetrexed could improve patient outcomes.

### Objectives

- Determine the safety, tolerability, PKs, and MTD of the combination of sirolimus with pemetrexed in subjects with NSCLC.
- Determine the clinical response rate at the MTD of sirolimus plus pemetrexed in NSCLC subjects with activation of the Akt/mTOR pathway.
- Determine effects of sirolimus on activation of the PI3K/Akt/mTOR pathway in PBMCs and/or tumor tissues, to determine metabolic changes using PET scans, and measure PKs.

### Eligibility

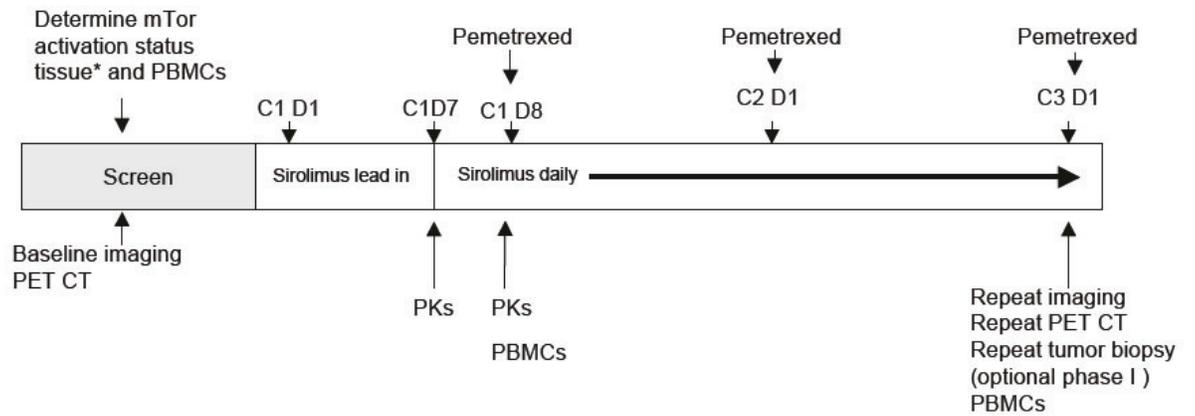
- Adults with refractory or relapsed NSCLC regardless of mTOR pathway activation are permitted to enroll in the trial.

### Design

- Phase I followed by Phase II study
- For phase I/II subjects, documentation of mTOR pathway activation is not mandatory. If accessible, tissue will be obtained at baseline and following two cycles of therapy or at time of progression, whichever occurs first. All subjects will have pathway analysis using PBMCs at baseline, day 8 and every two cycles of therapy or at time of progression, whichever occurs first. Cycle 1 is 28 days in length and all others 21 days.
- Each dose level incorporates a lead-in period of sirolimus alone that will allow for correlations of dose level, pharmacokinetics, and biologic effects.

- The phase I portion of the study has 5 dose cohorts beginning below the FDA approved doses for both agents. There are 3 dose escalations for sirolimus and 2 for pemetrexed. Up to 30 subjects may enroll in the phase I study.
- The Phase II portion will utilize the MTD from the Phase I and enroll up to 60 subjects.
- Sirolimus will be administered by mouth daily, and pemetrexed will be administered intravenously every 21 days until unacceptable toxicity or disease progression.
- Clinical imaging (CT or MRI) and a PET CT will be obtained at baseline and after two cycles of treatment. Clinical imaging will be performed every two cycles until disease progression.

### Schema



\*optional phase I; only repeated  
If baseline available and positive

Imaging and PBMCs repeated  
every 2 cycles while on study

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## **1 INTRODUCTION**

### **1.1 Study Objectives**

#### 1.1.1 Phase I objectives

1.1.1.1 The primary objectives of the phase I component of the study are to determine the safety and tolerability of the combination of pemetrexed with sirolimus in human subjects with NSCLC, and to determine the maximum tolerated dose (MTD) in this group of subjects.

1.1.1.2 The secondary objectives are to perform PKs, look for early evidence of efficacy of the combination, and to define the effects of this combination on mTOR pathway activation in PBMC and tumor tissue (optional). PET CT will be used to look for metabolic changes in tumors treated with the combination. These changes will be correlated with clinical tumor measurements as well as *in vitro* measures of mTOR pathway activation.

#### 1.1.2 Phase II objectives

1.1.2.1 The primary objective of the phase II component of the study is to determine the efficacy of the maximal tolerated dose of pemetrexed plus sirolimus in NSCLC patients. The primary outcome will be the clinical response rate.

1.1.2.2 The secondary objectives of the phase II component of the study are to determine the effects of sirolimus plus pemetrexed on mTOR pathway activation in NSCLC. A series of correlative studies will be done to measure biological and clinical effects of this combination. These studies will include analyses of tissue and blood samples, as well as correlative imaging studies using CT and PET:CT scans.

## **2 BACKGROUND & RATIONALE**

### **2.1 Clinical Burden of Lung Cancer**

Lung cancer is the leading cause of cancer death [1]. 55% of patients will present with stage IV or stage IIIB disease that is not amenable to curative resection. With platinum-based doublets given as first line treatment, the current one-year survival is 34%; moreover, 12% of patients with a good performance status are alive 2 years after diagnosis [2].

In second line treatment of NSCLC, three agents have been approved. Docetaxel was the first to be approved, and pemetrexed, a multi targeted antifolate inhibitor of dihydrofolate reductase, thymidylate synthase, and glycinamide ribonucleotide formyltransferase

(GARFT), was next to be approved. Despite the fact that docetaxel and pemetrexed have similar overall response rates (10% and 9.1% respectively) and median survival (7.6 months and 8.3 months, respectively [3-5]), pemetrexed is preferred because a favorable toxicity profile vs. docetaxel. For example, in hematological toxicities, grade 3 and 4 neutropenia occurred in only 5.3% of patients on pemetrexed vs. 40.2% in patients on docetaxel. For other toxicities, any grade alopecia occurred in only 6.4% of patients on pemetrexed vs. 37.7% of patients on docetaxel. Any grade diarrhea occurred in 12.8% of patients on pemetrexed vs. 24.3% of patients on docetaxel [3-5].

Erlotinib is the third agent approved in the second line setting and targets the epidermal growth factor receptor (EGFR). The response rate to erlotinib is also ~10%, although there may be a subset of patients who benefit from EGFR tyrosine kinase inhibitors more than the rest of the population [6]. Clearly, new approaches are needed to increase the efficacy of second line chemotherapy in NSCLC, and one possibility is to combine chemotherapy with inhibitors of signal transduction pathways such as the PI3K/Akt/mTOR pathway that promote survival and chemotherapeutic resistance.

### 2.1.1 The PI3K/Akt/mTOR pathway and lung cancer biology

Many lines of evidence show that the PI3K/Akt/mTOR pathway is important in lung cancer. First, tobacco components increase activation of the PI3K/Akt/mTOR pathway and cause a partially transformed phenotype in primary human bronchial epithelial cells [7]. Second, increased activation of Akt and mTOR correlates with phenotypic progression of murine lung lesions induced by a tobacco carcinogen, and increased activation of Akt is characteristic of human preneoplastic lung lesions [8, 9]. Third, the PI3K/Akt/mTOR pathway is constitutively active in NSCLC cells and promotes resistance to chemotherapy and radiation [10]. Fourth, pathway activation can be increased further in lung cancer cells by exposure to tobacco components or adhesion to specific extracellular matrix proteins. This confers increased resistance to traditional and targeted chemotherapies that can be reversed by sirolimus [11-13]. Fifth, activation of Akt is a poor prognostic factor for NSCLC patients [13].

### 2.1.2 Sirolimus, the prototypic inhibitor of the PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway is activated by many mechanisms relevant to cancer such as activation of receptor tyrosine kinases (RTKs) or oncogenes such as ras (Figure 1). Despite its potential importance, few inhibitors of the PI3K/Akt/mTOR pathway are available

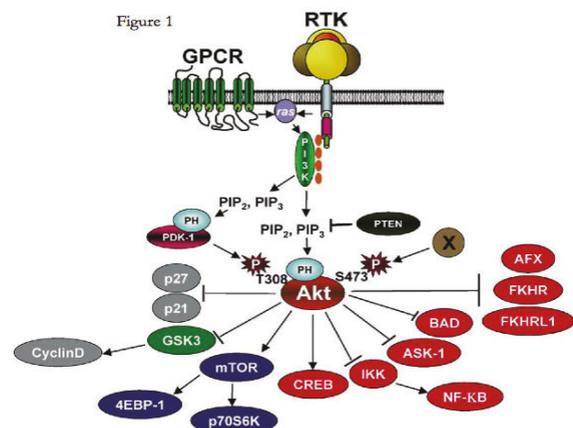


Figure 1. Activation and propagation of the PI3K/Akt/mTOR pathway.

for clinical use. The class of agents fully developed for use in patients is inhibitors of mTOR such as sirolimus. Sirolimus (Rapamycin) is FDA-approved as an immunosuppressant for renal transplant patients and is used in combination with other drugs to depress T cell function. mTOR inhibitors cause cell cycle arrest in G1 and/or apoptosis by inhibiting phosphorylation of mTOR downstream components such as 4E-BP1, S6K, and a substrate of S6K, the ribosomal protein S6 (not shown in Figure 1). The phosphorylation state of pathway components can be assessed by using phospho-specific antibodies in assays such as immunohistochemistry, immunoblotting, and flow cytometry. Importantly, mTOR inhibitors are preferentially active in cells that have mutations in PTEN or have constitutive activation of mTOR through other mechanisms such as activation of growth factor receptors, Ras, or integrins.

### 2.1.3 Preclinical Efficacy

Sirolimus is a potent cytostatic agent, while at doses above 10  $\mu\text{M}$ , it is cytotoxic to virtually all cancer cell lines in the NCI 60 cell line panel (figure 2). mTOR inhibitors can prevent tumorigenesis and inhibit tumor growth *in vivo*. mTOR inhibition prevents tumorigenesis in transgenic model of breast, prostate cells and alveolar epithelial neoplasia [14, 15] as well as in a carcinogen driven model of lung cancer [16]. mTOR inhibitors can also inhibit the growth of lung tumors in xenograft models when given individually or in combination with conventional cytotoxic chemotherapy (see below). Sirolimus can also induce apoptosis and sensitize cancer cells to induction of apoptosis by DNA-damaging agents such as cisplatin in a p53-dependent manner [17].

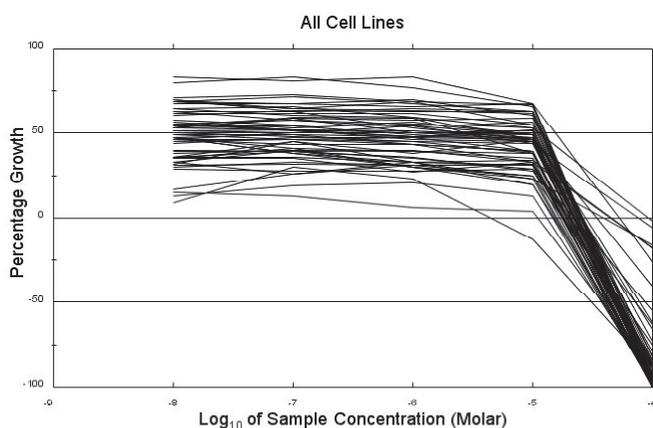


Figure 2. Composite NCI60 panel results for sirolimus. Each line represents a single cell line. Concentrations of sirolimus range from 10 nM to 100 $\mu\text{M}$ .

### 2.1.4 Clinical Efficacy

In addition to sirolimus, analogs of sirolimus, CCI-779 (Torisel, temsirolimus) and RAD-001 have under gone testing in renal cancer, breast cancer and glioblastomas [18-20]. Activity has been observed in many tumor types. Partial responses were noted in the phase I trials with CCI-779 in one patient with clear-cell renal carcinoma, one patient with breast

adenocarcinoma, one patient with non small cell lung cancer, and two patients had stable disease that lasted  $\geq 24$  weeks [21, 22]. In phase II studies, CCI-779 produced an objective response rate of 7% in advanced RCC patients with a median time to tumor progression of 5.8 months and median survival of 15.0 months [23]. Similar objective response rates were observed in a recent Phase III trial in renal cell cancer patients that led to FDA-approval of temsirolimus [24]. Galanis *et al.* [25] enrolled 65 patients with recurrent GBM on temsirolimus and observed radiographic improvement in 36% and prolonged time to progression of 2.3 months for all participants and 5.4 months for responders. The results of many other Phase II trials that demonstrate objective responses have been presented in abstract form.

mTOR inhibitors have been combined with traditional chemotherapy as well. Punt *et al.*, published a study of temsirolimus and 5FU [26]. Though some responses were seen in this trial, the results were overshadowed by the toxicities that forced early closure of the study. Pharmacokinetics studies did not indicate increased exposure to either the 5FU or temsirolimus as the source of the unexpected toxicity. Unfortunately, like most trials with mTOR inhibitors, this one did not use biomarkers to follow modulation of the pathway after therapy, so that correlations between mTOR inhibition, efficacy, and/or toxicity could not be established. While the results of Punt *et al.*, raise the possibility that pemetrexed and sirolimus might be an effective combination against tumors such as NSCLC with marked activation of the Akt/mTOR pathway, it emphasizes the need to monitor pathway inhibition during clinical trials.

### 2.1.5 Importance of Biomarkers

Biomarker analysis to confirm modulation of downstream components after mTOR inhibition has been performed in a minority studies, but has proved to be illuminating [25, 27]. In a study of temsirolimus in patients with recurrent glioblastoma by Galanis *et al.*, [25] there was a statistically significant correlation between increased mTOR activity, as defined by elevated baseline phosphorylated S6 kinase levels in IHC, and radiographic improvement. Similarly, Duran *et al.* showed that the best predictor of response of patients with neuroendocrine tumors to temsirolimus was an elevated basal level of phosphorylated mTOR S2448, and radiographic response correlated with decreases in levels of phospho-S6 [27]. These studies are consistent with preclinical data showing that mTOR must be active for mTOR inhibitors to be effective and they suggest that measuring the phosphorylation of S6 and mTOR has clinical utility. Thus, biomarker assays should be a critical component of future studies so that tumor responses or toxicity can be correlated with modulation of pathway components after mTOR inhibition therapy.

Another important application for biomarkers is studying the effect of mTOR inhibitors on feedback activation of Akt. This feedback could hypothetically negate any effect of mTOR

inhibitors by promoting propagation of the Akt signal to other downstream substrates. Two different mechanisms have been described. mTOR can exist in two types of complexes, mTORC1 and mTORC2 complexes. Several preclinical studies have shown that mTOR inhibitors preferentially decrease the proportion mTORC1 complexes and increase the proportion of mTORC2 complexes, which are comprised of mTOR bound to rictor. mTORC2 complexes can directly phosphorylate Akt at S473 and lead to its activation [28]. A separate mechanism for feedback activation has also been observed that involves IRS-1, which normally activates PI3K. S6K, a downstream substrate of mTOR, normally phosphorylates and inhibits IRS-1. Inhibition of mTOR by sirolimus decreases S6K activity, which indirectly activates IRS-1, prolongs the half-life of IRS-1, and activates PI3K [29]. Thus, the inhibition of mTOR could result in two modes of feedback activation of Akt, through direct activation of Akt by mTOR-rictor complexes, and through the indirect activation of IRS-1 by inhibition of S6K.

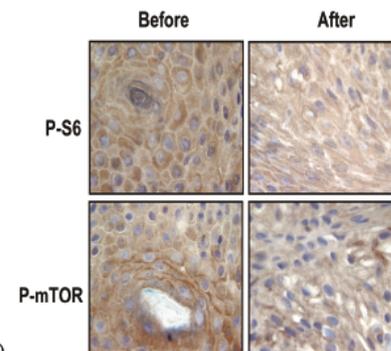
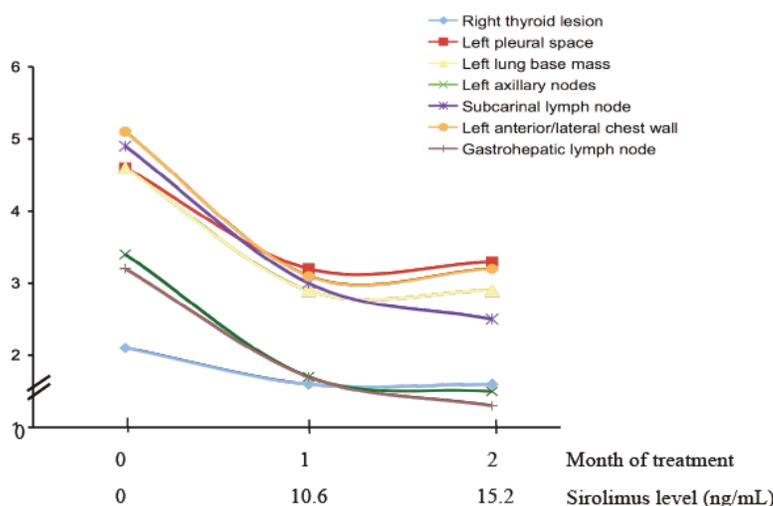
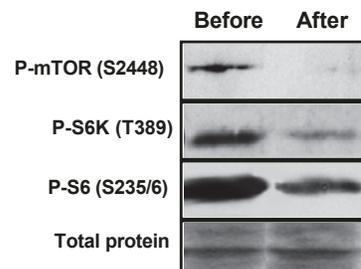
Although either feedback mechanisms could possibly counter the efficacy of an mTOR inhibitor, there is no evidence that this occurs in humans, and the clinical relevance of these mechanisms is unclear. Nonetheless, this protocol will incorporate evaluation of these biomarkers to assess whether such a feedback mechanism occurs in tissues from lung cancer patients

#### 2.1.6 Correlating biomarkers with imaging

In addition to monitoring biomarkers such as phospho-S6 or phospho-mTOR in IHC, other assays such as immunoblotting and metabolic imaging might also be useful. This has been demonstrated recently by our group in analyzing tissues from rare patients with Cowden's syndrome (CS) who were treated with sirolimus as a single agent. CS is a hamartomatous tumor syndrome characterized by benign hamartomas and trichilemmomas that occur in any organ, and patients with CS are at increased risk for breast, uterine, and thyroid cancer. Approximately 80% of CS patients have germline mutations in the PTEN gene, a tumor suppressor gene that negatively regulates activation of Akt /mTOR pathway. We have recently shown that benign and malignant tumors from CS patients have very high endogenous levels of activation of the Akt/mTOR pathway (data not shown). Due to unusual circumstances, we have had the opportunity to treat two CS patients with cancer with sirolimus. At the time sirolimus was initiated, patient #1 had metastatic uterine cancer with carcinomatous peritonitis and patient #2 had metastatic NSCLC. After one month of sirolimus, we observed inhibition of mTOR by immunoblotting in PBMCs and by immunohistochemistry in benign hamartomas (figure 3). Similar results were observed for each patient. Based upon the reports from imatinib and GIST trials using PET FDG to detect early responses to therapy [30], we obtained PET scans to assess early metabolic responses in these two patients. There was 30 to 50% decrease in the SUV uptake in both malignant and benign tissues within a month of sirolimus therapy (figure 3). This anecdotal data suggests that IHC, immunoblotting, and/or PET scans may be useful to monitor

therapy with an mTOR inhibitor.

Figure 3. Biomarkers for mTOR inhibition in clinical specimens. Inhibition of mTOR pathway components is shown after sirolimus therapy using immunoblotting (patient 1), IHC (patient 1), and PET scan results (SUVs from patient 2).



### 2.1.7 Combining pemetrexed & sirolimus

We hypothesize that because the PI3K/Akt/mTOR pathway is important in lung cancer pathogenesis, the combination of sirolimus and pemetrexed should be more effective than either agent alone. Several preclinical studies have shown that inhibition of the Akt/mTOR pathway increases the apoptotic response of cancer cell lines to 5FU [31-37] and pemetrexed [38, 39]. Moreover, anti-folate agents such as pemetrexed, 5FU, and the experimental fluoropyrimidine, S-1, are also capable of decreasing activation of the Akt/mTOR pathway as single agents. Thus, there is a rationale to test sirolimus sequentially and simultaneously with pemetrexed in lung cancer cell lines to assess additive or synergistic effects of the combination.

To assess sequence dependence and demonstrate possible synergistic effects, we combined sirolimus and pemetrexed in two human non-small cell cancer lines (figure 4A, 4B). In A549 or H157 cells, synergy was observed with combination indices <1.0 for multiple dose

levels (data not shown). There was no difference in cellular responses if sirolimus and pemetrexed were administered simultaneously or sequentially. These studies did not indicate any antagonistic effects. We did observe, however, that A549 cells were more resistant to pemetrexed as a single agent than what has been previously reported in the literature [40, 41]. Our IC<sub>50</sub> of greater than 1000  $\mu$ M for A549 cells was higher than the  $2.5 \pm 0.3$   $\mu$ M reported by Kano *et al.* [41] or the 640 nM reported by Smith *et al.* for the same cell line [40]. Because we used different assays to assess proliferation that employed different dyes, different serum concentrations, and different types of serum, we repeated the experiments exactly as performed by Kano *et al.* and Smith *et al.* [40] to confirm that the differences in IC<sub>50</sub> were due to experimental conditions. We hypothesized that the discrepancy might be principally related to the fact that Smith *et al.* and Kano *et al.* used dialyzed serum, which lacks folate and is devoid of nucleosides and bases capable of rescuing pemetrexed treated cells. (Our original experiments followed standard tissue culture protocols using undialyzed serum, which contains folate and nucleosides).

Figure 4A

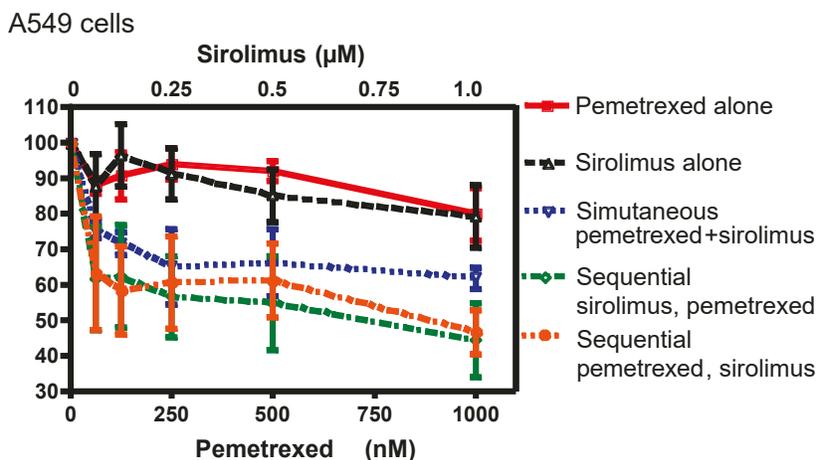
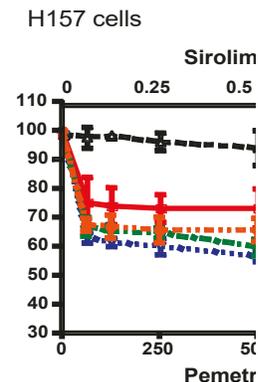


Figure 4B

Figure 4: Synergistic effects of combination lines when cultured with non-dialyzed concentrations shown. Proliferation Each condition was assessed in sext A: A459 cells B: H157 cells



We compared responsiveness of A549 cells to pemetrexed in dialyzed or non-dialyzed serum (5 %) using the SRB assay as employed by Smith *et al.* [40] (Figure 5A). The IC<sub>50</sub> in dialyzed serum was <200 nM, yet was not reached in undialyzed serum. This IC<sub>50</sub> of <200 nM in dialyzed serum is very similar to the IC<sub>50</sub> (640 nM) observed by Smith *et al.* The IC<sub>50</sub> did not significantly differ using 5% or 10% dialyzed serum (data not shown). We also tested responsiveness of A549 cells in dialyzed serum to pemetrexed using the MTT assay as employed by Kano *et al.* [41]. The IC<sub>50</sub> in these experiments was 1.8 μM, consistent with the value of 2.5 μM obtained by Kano *et al.* (data not shown).

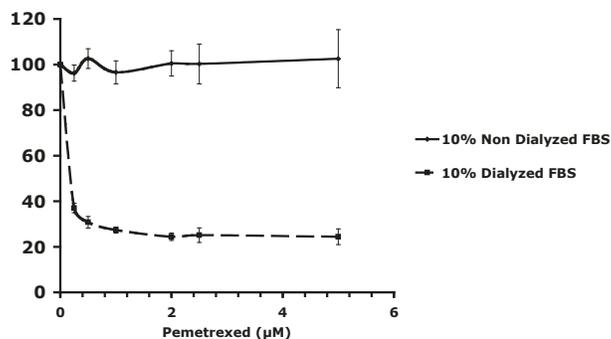


Figure 5A. Effect of dialyzed serum on response to pemetrexed. A549 cells grown in the presence of 10% dialyzed serum have an IC<sub>50</sub> of less than 1 μM. The IC<sub>50</sub> was not reached for cells grown in non dialyzed

We also repeated the combination studies shown in Figure 4 in dialyzed serum (Figure 5B, 5C). The sensitivity to pemetrexed or sirolimus as single agents was increased in dialyzed serum. The IC<sub>50</sub> of pemetrexed alone decreased about one half log with the simultaneous addition of sirolimus. In A549 cells we observed synergy with combination indices (CI) <1.0 at concentrations of pemetrexed: sirolimus of 100 nM: 1nM (CI=0.162), 300 nM: 3 nM (0.082), and 1000 nM: 10 nM (0.29), respectively. In H157 cells, we observed synergy with combination indices (CI) <1.0 at the same concentrations of pemetrexed: sirolimus 100 nM:1nM (0.209), 300 nM:3 nM (0.121), and 1000 nM:10 nM (0.17). Regarding the clinical relevance, concentrations of 1 to 10 nM of sirolimus are achievable and safe. The C<sub>max</sub> for 2 mg a day dosing per the sirolimus package insert is approximately 15.0 ± 4.9

ng/ml or approximately 11 to 22 nM (MW- 914g). Trough levels, as reported in the package insert for 2 mg a day dosing are  $8.6 \pm 4.0$  ng/ml, or approximately 5 to 14 nM. In general trough levels < 20 nM are thought to be safe. Again, the concentrations of sirolimus at which we observed synergy were 1 to 10 nM, below or within the accepted trough level range.

Figure 5B. A549 cells

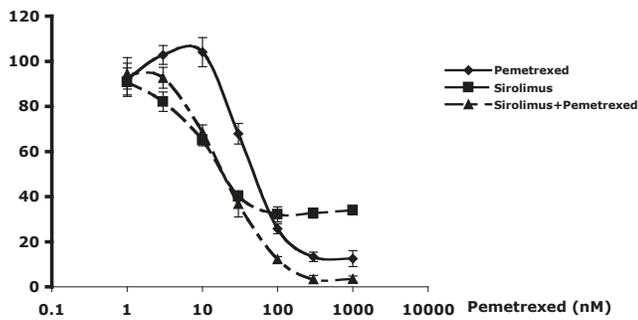
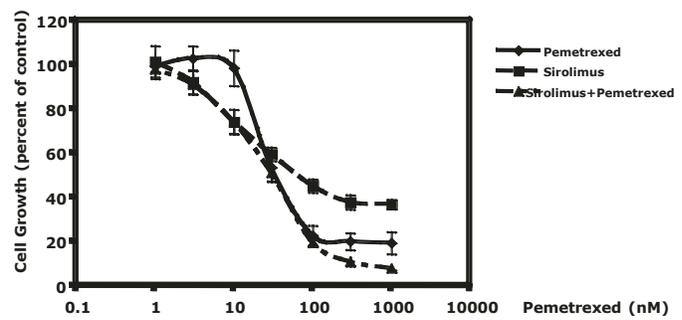


Figure 5C. H157 cells



	.01	.1	1	10	Sirolimus (nM)	.01
	.1	1	10		Sirolimus (nM)	
			.16	.08	.29	Combination Indices (CI)
	.21	.12	.17			CI

Figure 5B and 5C: Synergistic effects of combining pemetrexed and sirolimus in NSCLC cell lines when cultured in 10% dialyzed serum. A549 (5B) and H157 (5C) cells grown in the presence of pemetrexed and sirolimus with 10% dialyzed FBS show synergistic effects on growth inhibition at the three highest concentrations. Combination indices were calculated using CalcuSyn Software (Biosoft, Oxford, UK)

In summary, the discrepancy of IC50 doses observed in our *in vitro* studies and those of Smith *et al.* [40] and Kano *et al.* [41] was likely due to the presence of dialyzed serum. Given that patients treated with pemetrexed receive folate supplementation and their serum is replete with nucleosides, the use of undialyzed serum probably represents a more physiological situation. In addition, this data suggests that synergy may be most likely to be observed at higher doses ranges or dose escalation in a clinical trial.

To extend our *in vitro* studies, we performed xenograft experiments with sirolimus and pemetrexed using H157 cells in nude mice. There are no published xenograft studies using this combination of drugs. Published data does suggest, however, that down regulation of an activated Akt/mTOR pathway will enhance sensitivity of cell lines to conventional

chemotherapeutic agents [20]. In an attempt to first inactivate mTOR, we used a lead in dose of sirolimus followed by a dose of pemetrexed that has been used previously (100 mg/kg intraperitoneally administered) [42] (figure 6). This design was intended to be similar to the clinical trial design. There was no untoward toxicity experienced by the mice and the mice continued to gain weight during the study. In assessing inhibition of NSCLC tumor growth, sirolimus alone was effective. In contrast, pemetrexed alone was ineffective, which was consistent with *in vitro* assays performed with H157 cells in undialyzed serum. Together, the combination of sirolimus and pemetrexed was more effective than either agent alone in a statistically significant manner ( $p < 0.05$ ). These data are consistent with data from Teicher *et al.* who observed a 2 to 4 day delay in tumor growth (H460 cells in nude mice) after 7 or 14 days of pemetrexed alone, but up to a 28 day delay with pemetrexed in combination with paclitaxel. (Sirolimus was not used in these studies.)

Figure 6

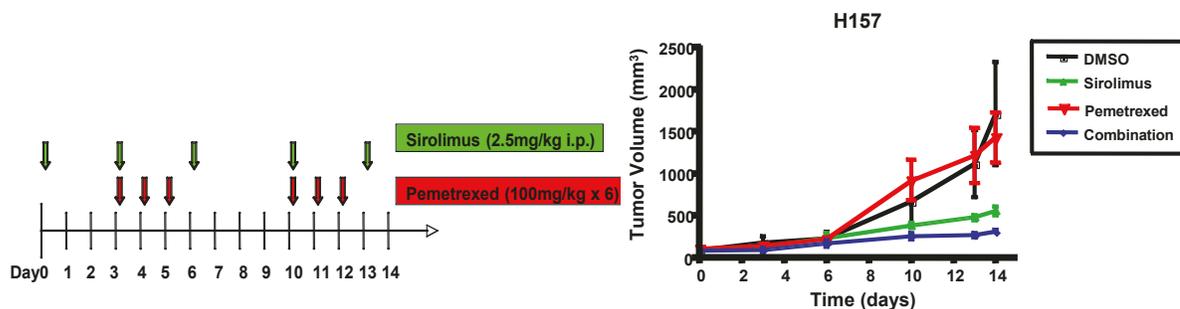


Figure 6: H157 xenograft studies. The schema for administration of sirolimus and pemetrexed is shown on the left. The effects of sirolimus and/or pemetrexed on growth of H157 tumors in nude mice are shown on the right. Combined sirolimus and pemetrexed after a sirolimus lead in had greater inhibition of tumor growth than either agent alone ( $p < 0.05$ ).

In addition to the preclinical studies [31-37] showing that inhibition of the Akt pathway enhances sensitivity to conventional chemotherapeutic agents, one clinical trial has been published that combined a sirolimus analogue with an anti-folate agent. Punt *et al.* [26] treated 28 subjects with advanced malignancies with CCI-779, followed by leucovorin, and 24 hour infusional 5FU. Cycles were repeated weekly. The dose of CCI-779 that was

chosen, 15 mg/m<sup>2</sup>, was 25% lower than the dose that was being tested in a simultaneously running phase I study. This dose was below the maximum tolerated dose and was not associated with more than grade 2 toxicity in their phase I study. The study was closed early because of toxicity. In addition to serious toxicities such as mucositis, nausea and vomiting, thrombocytopenia, and febrile neutropenia, there were two fatal bowel perforations unrelated to tumor.

Pharmacokinetic data did not suggest that CCI-779 treatment resulted in greater exposure to 5FU, nor were the sirolimus levels (CCI-779 is metabolized to sirolimus) abnormally elevated. Three subjects had confirmed partial responses of 6 to 24 months duration, otherwise 11 patients had stable disease. The median time to progression for the entire group was 3.2 months and 5.2 months for the responders (partial response and stable disease). Notably thirteen subjects had not received prior therapy for their malignancy and many subjects had tumors expected to be sensitive to 5FU. The investigators caution that future studies should consider the overlapping toxicities and not use the dose/schedule used in this trial. No biomarker analyses were employed in this study.

In summary, our preclinical data demonstrate the importance of tissue culture technique in experiments testing efficacy of pemetrexed in H157 and A549 cells. Standard techniques using non-dialyzed serum may provide a more physiological setting, but the resistance of these cell lines to pemetrexed makes determining an IC<sub>50</sub> to that agent impossible. By using dialyzed serum, we were able to demonstrate synergy and a threefold decrease in the IC<sub>50</sub> of pemetrexed when combined with sirolimus. Together our cell line and xenograft data provide a rationale to test this combination in patients with refractory NSCLC.

## 2.1.8 Sirolimus

### 2.1.8.1 Background

Sirolimus (Rapamycin: Rapamune®: Wyeth-Ayerst, PA, USA), first isolated from the soil bacteria *Streptomyces hygosopicus* in 1975, was approved by the FDA in 1999 as an immunosuppressive agent for use in preventing rejection in renal transplant recipients in combination with cyclosporine and corticosteroids. The FDA approved dose is a 6 mg loading dose on day 1 followed by 2 mg each day starting on day 2.

### 2.1.8.2 Pharmacodynamics

Sirolimus is a macrolide that acts as a signal transduction inhibitor of mTOR causing cell cycle arrest at G1 [43, 44]. It inhibits the serine-threonine kinase mammalian target of Rapamycin (mTOR) by forming a complex with the FK binding protein 12 (FKBP-12). mTOR is a member of the evolutionarily conserved phosphatidylinositide 3'-kinase (PI3K)-related family and it been shown to be involved in regulating many aspects of normal and neoplastic cell growth, including organization of the actin cytoskeleton,

membrane trafficking, protein degradation, protein kinase C (PKC) signaling, and transcription [45]. The sirolimus/FKBP-12 binding to mTOR leads to dephosphorylation and inactivation of the p70S6 kinase (S6K1) and 4E-BP1. In turn, this inhibits the translation and production of ribosomal components necessary for protein synthesis and thereby Akt activation and causes cell-cycle arrest in G1 phase [46]. This series of events effectively forms the rationale for the immunosuppressant role of sirolimus in renal transplant patients since IL-2 induced T-cell proliferation is effectively blocked.

#### 2.1.8.3 Pharmacokinetics

Mean time to peak concentration (T<sub>max</sub>) of sirolimus following oral administration is 1 hour after a single dose in healthy volunteers and 2 hours after multiple daily doses in renal transplant recipients. The bioavailability of sirolimus after administration of the tablet is approximately 27% higher than that of the oral solution. Clinical equivalence of the two formulations has been demonstrated at the 2mg dose level. A high fat meal altered the bioavailability characteristics of sirolimus and therefore the drug should be taken consistently with or without food. Sirolimus is extensively bound to plasma proteins including serum albumin (97%),  $\alpha$ 1-acid glycoprotein and lipoproteins. Sirolimus is a substrate for the cytochrome p450 IIIA4 (CYP3A4) and P-glycoprotein. Inhibitors of CYP3A4 may decrease the metabolism of sirolimus and increase sirolimus levels, while inducers of CYP3A4 may increase the metabolism of sirolimus and decrease sirolimus levels. The majority of sirolimus is excreted in the feces (91%) with only a minor amount (2.2% excreted in the urine). Mean whole blood sirolimus trough concentrations achieved steady state concentrations within 1 day after the start of dose administration following a loading dose of three times the maintenance dose. The pharmacokinetics of sirolimus administered with and without pemetrexed will be monitored during the Phase I portion of the trial. In the renal transplant setting, adequate therapeutic trough levels are between 16 to 24 ng/mL for the first year and 12 to 20 ng/mL after that.

#### 2.1.8.4 Toxicology

There are no adequate studies in pregnant women therefore effective contraception must be initiated before, during and for 12 weeks following discontinuation of therapy with sirolimus. In animal studies, sirolimus was embryo/fetal toxic resulting in mortality and reduced fetal weight but no teratogenicity.

#### 2.1.8.5 Clinical Safety Data

The incidence of adverse reactions was determined utilizing renal transplant patients, who were taking sirolimus in combination with cyclosporine and corticosteroids. Two randomized, double-blind, multicenter controlled trials were conducted [47, 48]. The first study included 558 renal transplant patients and the second 446 renal transplant patients. Patients in the sirolimus arm in both trials received Rapamune solution formulation orally in combination with cyclosporine and corticosteroids. A third study evaluated Rapamune tablet formulation in 228 renal transplant patients in combination with cyclosporine and

corticosteroids. There was no difference in toxicity between the two formulations except for an increased incidence of acne with the oral liquid formulation and an increased incidence of tremor with the tablet formulation especially in African American patients. Hyperlipidemia and hypercholesterolemia were found to be dose-related effects of sirolimus therapy. During clinical trials, renal transplant patients who began sirolimus with normal, fasting, total serum triglycerides developed hypertriglyceridemia after treatment with sirolimus 2 mg and 5 mg. Treatment of new onset hypercholesterolemia was required in 42-52% of renal transplant patients on sirolimus compared to 16% in the placebo arm and 22% in the azathioprine arm. Patients treated with sirolimus tended to develop higher creatinine and lower glomerular filtration rate compared to the placebo and azathioprine arm. During clinical trials, hypophosphatemia occurred in 15% to 23% of patients receiving sirolimus, while hypokalemia occurred in 11% to 21% of patients. A negative dose adjustment is recommended for patients with mild to moderate hepatic impairment since their steady state levels are affected by hepatic function. There is a black box warning regarding the increased susceptibility to infection and possibility of developing lymphoma secondary to immunosuppression when sirolimus is used in combination with cyclosporine and corticosteroids. Further side effects have been reported with sirolimus. CCI-779, a sirolimus analogue, was combined with 5-fluorouracil and leucovorin in a phase I study in solid tumors. Mucositis, diarrhea, nausea/vomiting, asthenia, rash, anemia, and hyperglycemia were the most common grade 2-4 toxicities [26]. When sirolimus was used with tacrolimus in liver transplant patients, there were reports of excess mortality, graft loss, and in the first 30 days following transplant, hepatic artery thrombosis was reported.

Additionally, the following symptoms were attributed to sirolimus in clinical trials: asthenia, fever, chills, malignancy, abnormal healing (including wound dehiscence and anastomotic disruption), bacterial infection, viral infection, anaphylactic/anaphylactoid reactions, hypertension, tachycardia, congestive heart failure, diarrhea, lymphoproliferative disease, lymphoma, lymphadenopathy, anemia, leukopenia, thrombocytopenia, TTP, thrombosis, hepatotoxicity, hepatic necrosis, hypercholesterolemia, hyperlipidemia, hypokalemia, edema, weight gain, Cushing's syndrome, diabetes mellitus, arthralgia, bone necrosis, insomnia, tremor, headache, interstitial lung disease (pneumonitis, BOOP, pulmonary fibrosis), acne, rash, skin cancer, dysuria, and urinary frequency. Please refer to section 8.1.5 and to FDA labeling for complete description of adverse events associated with Sirolimus.

## 2.1.9 Pemetrexed

### 2.1.9.1 Background

Pemetrexed, (Alimta), for injection, was approved by the FDA in 2004 as an anti-folate cancer agent for second line treatment of patients with locally advanced or metastatic non small cell lung cancer. It acts by disrupting folate-dependent metabolic pathways causing

decreased nucleotides synthesis, and therefore, inhibits cell growth and induces apoptosis.

#### 2.1.9.2 Pharmacodynamics

Pemetrexed acts by disrupting folate-dependent metabolic pathways. It uses both the reduced folate carrier and membrane folate binding protein transport systems to cross into the cell. Once inside, it undergoes a conversion to polyglutamate forms by polyglutamate synthetase, and those glutamate forms inhibit thymidylate synthase dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). Both of these enzymes are essential in the de novo biosynthesis of thymidine and purine nucleotides. Lack of these nucleotides inhibits cell growth and induces apoptosis. Poly-glutamated metabolites have an increased half-life and are synthesized predominantly in malignant cells.

#### 2.1.9.3 Pharmacokinetics

Pemetrexed as a single agent is 81% bound to plasma proteins. Pemetrexed is not metabolized significantly and is eliminated in the urine with 70-90% of the dose recovered unchanged within the first 24 hours following administration. The elimination half-life of Pemetrexed is 3.5 hours in patients with normal renal function (creatinine clearance of 90 mL/min). The clearance decreases as renal function decreases. Pemetrexed's total systemic exposure (AUC) and maximum plasma concentration (C<sub>max</sub>) increase proportionally with dose. Additionally, the pharmacokinetics do not change over multiple treatment cycles. Pemetrexed must be co-administered with oral folic acid and intramuscular vitamin B12, which does not affect its pharmacokinetics. No studies were conducted to determine the cytochrome P450 isoenzyme induction potential of Pemetrexed, because used as recommended, once every 21 days; it would not be expected to cause any significant enzyme induction.

#### 2.1.9.4 Toxicology

Pemetrexed was fetotoxic and teratogenic in mice at i.p. doses of 0.2 mg/kg (0.6 mg/m<sup>2</sup>). Embryotoxicity was characterized by increased embryo-fetal deaths. There are no studies of pemetrexed in pregnant women. No carcinogenicity studies have been conducted with Pemetrexed.

#### 2.1.9.5 Clinical safety data

Patients treated with Pemetrexed must take folic acid and vitamin B12. In clinical studies, there was less overall toxicity and reductions in Grade 3/4 hematologic and non-hematologic toxicities such as neutropenia, febrile neutropenia, and infection when pretreatment with folic acid and vitamin B12 was administered. Leucovorin was permitted for rescue from CTC Grade 4 leukopenia lasting >3 days, CTC Grade 4 neutropenia lasting >3 days, and immediately for CTC Grade 4 thrombocytopenia, bleeding associated with Grade 3 thrombocytopenia, or Grade 3 or 4 mucositis [4].

Pemetrexed is excreted via the kidneys and decreased renal function will result in reduced clearance and greater exposure (AUC). No dose modifications are required for creatinine clearance >45 mL/min. The incidence of CTC Grade 3/4 hypertension was the only finding demonstrating an age difference in patients treated with Pemetrexed and was greater in patients ≥65 years as compared to younger patients.

### **3 ELIGIBILITY ASSESSMENT AND ENROLLMENT**

#### **3.1 Eligibility Criteria**

##### **3.1.1 Inclusion Criteria**

3.1.1.1 Histologically documented NSCLC that is confirmed by the Laboratory of Pathology at the Clinical Center/NIH or the Laboratory of Pathology at NNMC (see section 2.2.13).

3.1.1.2 Tumor biopsy will be requested from all study subjects unless the procedure poses too great a risk. If the subject declines, he or she may still participate in the study. We will ask subjects not undergoing biopsy to provide 6 unstained slides or a tissue block of archived tissue for immunohistochemistry (IHC) evaluation. Tumors from subjects enrolling in the phase II portion of the study will be analyzed retrospectively to demonstrate mTOR activation as assessed by immunohistochemistry in a fresh biopsy. mTOR activation will be defined using distribution and intensity of staining for phosphorylation of mTOR, or its downstream substrates S6K, and S6, as defined in section 2.2.13. SOPs describing the acquisition and handling of PBMCs and tissues are outlined in appendix 10.3 and 10.4. At a minimum, a total score (sum of intensity and distribution scores, see 2.2.13) of 2 for phospho-S6 or phospho-mTOR (S2448) mTOR will be required to determine that mTOR is active. Either measurement will be sufficient to ascertain that mTOR is active. Measurement of phosphorylation of Akt, 4E-BP1, and total levels of thymidylate synthase (TS) will also be measured, but are not part of the eligibility requirements. In the event of limited tissue availability, the stains will be prioritized as follows: S6, mTOR, S6K, Akt (S473), Akt (T308), and TS. Phosphorylation of S6 correlates most closely with mTOR activity [49], while phosphorylation of mTOR at S2448 best predicts response to sirolimus [27].

3.1.1.3 Tissue from the time of original diagnosis will be adequate for enrollment on study. Optional fresh tissue biopsy must be obtained AFTER their most recent chemotherapy (including small molecule or targeted therapy) or radiation therapy. Tumors that can be biopsied percutaneously (with or without CT/ultrasound guidance) or via bronchoscopy will be considered accessible if there are no other competing risk factors such as coagulopathy, hypoxemia, unstable cardiovascular disease, uncontrolled pain, or inability to give informed consent.

3.1.1.4 Individuals with relapsed NSCLC who have received at least one standard

chemotherapeutic regimen are eligible. Patients who received adjuvant chemotherapy and then relapse or recur  $\leq 12$  months after completion of chemotherapy will be eligible. Patients who received adjuvant chemotherapy and relapse  $> 12$  months after completion of chemotherapy should receive frontline therapy for metastatic disease before enrollment, as should individuals who initially present with incurable disease that is chemotherapy naive. Individuals unwilling to receive standard front line therapy for metastatic lung cancer may enroll.

3.1.1.5 Patients must have not received any chemotherapy, biological, or radiation therapy in the 21 days prior to protocol enrollment. All previous chemo and radiation therapy induced toxicities must have resolved to grade 1 or less prior to enrollment.

3.1.1.6 Because sirolimus may affect the efficacy of hormonal birth control via CYP 3A4, study subjects of child bearing potential must be willing to use barrier birth control while receiving sirolimus therapy and for 12 weeks after discontinuation of sirolimus.

3.1.1.7 Patients must have measurable disease for the phase II portion of the study.

3.1.1.8 Age  $\geq 18$  years of age

3.1.1.9 ECOG performance score of 0-2

3.1.1.10 Expected survival of at least 3 months

3.1.1.11 Patients must have the capacity to provide informed consent and demonstrate willingness to comply with an oral regimen.

3.1.1.12 Patients must have normal organ and marrow function as defined below:

- Absolute neutrophil count  $\geq 1,500/\text{mL}$
- Platelets  $\geq 100,000/\text{mL}$
- Total bilirubin  $< 1.5 \times$  upper limit of institutional normal
- AST (SGOT)  $< 2.5 \times$  upper limit of institutional normal
- ALT (SGPT)  $< 2.5 \times$  upper limit of institutional normal
- Creatinine Estimated creatinine clearance as calculated using the MDRD equation must be  $\geq 60 \text{ ml/min/1.73m}^2$ . The formula to be used is MDRD:  $186 \times (\text{Scr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$
- Serum triglycerides  $\leq 2.5 \times$  upper limit of normal; serum cholesterol  $\leq 300 \text{ mg/dl}$  (includes subjects with familial and acquired hyperlipidemia).

3.1.1.12 Subjects on steroids must be on a stable or tapering dose of  $\leq 20 \text{ mg/day}$  of prednisone (or equivalent dose of another glucocorticoid) for at least one week prior to study entry.

### 3.1.2 Exclusion Criteria

3.1.2.1 HIV positive patients

3.1.2.2 Pregnant or lactating women

3.1.2.3 Patients who received pemetrexed previously for Phase 1 only. Patients with prior pemetrexed are eligible for Phase 2.

3.1.2.4 Patients who have had prior therapy with mTOR inhibitors such as sirolimus or its analogues within six months

3.1.2.5 Any concurrent therapy with chemotherapeutic agents or biologic agents or radiation therapy

3.1.2.6 Subjects with brain metastases may participate if the metastases are asymptomatic. Subjects are ineligible if brain metastases are symptomatic.

3.1.2.7 Patients who are on the following drugs that modulate CYP3A4 and cannot replace these medications with other equivalent medications for the period of the study: amprenavir, atazanavir, bromocriptine, cimetidine, clarithromycin, clotrimazole, cyclosporine, danazol, diltiazem, erythromycin, fluconazole, fosamprenavir, other HIV protease inhibitors, indinavir, itraconazole, ketoconazole, metoclopramide, nefazodone, nelfinavir, nifedipine, nifedipine, ritonavir, saquinavir, telithromycin, troleandomycin (TAO), verapamil, voriconazole, nevirapine, rifampicin, rifampin, rifabutin, rifapentin, phenytoin, carbamazepine, phenobarbital, and St. John's Wort. Please see appendix 10.5 (Table 5) for a list of relevant agents.

3.1.2.8 Subjects taking non steroidal anti-inflammatory agents who are unable to stop or replace the agents for the 5 days prior to and the 2 days after pemetrexed will not be eligible.

3.1.2.9 Patients who have received live vaccines in the past 21 days.

## 3.2 Research Eligibility Evaluation

### 3.2.1 History and Physical

History and physical examination including vital signs, ECOG performance status (Table 4 Section 10), narcotic use, and medication review. If examination is done within 17 days of initiation of study treatment, it may be used as baseline.

### 3.2.2 Laboratory evaluation

CBC with differential, PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, vitamin B12, folate, fasting cholesterol (HDL, LDL) and triglycerides. If tests are done within 17 days of initiation of study treatment, they may be used as baseline.

### 3.2.3 Lymphocyte subsets

If done within 17 days prior to the initiation of study treatment, this may be used as baseline.

### 3.2.4 Urinalysis

If test is done within 17 days prior to the initiation of study treatment, it may be used as baseline.

### 3.2.5 Clinical tumor measurements

Clinical tumor measurements; i.e., skin lesions, subcutaneous nodules, palpable masses, within 4 weeks for purposes of screening. If measurements are done within 17 days prior to the initiation of study treatment, they may be used as baseline.

### 3.2.6 Clinical imaging

Clinical imaging (CT or MRI) for tumor measurements: These do not need to be repeated for screening if performed within the previous 28 days. If scans are done within 28 days prior to the initiation of study treatment, they may be used as baseline.

### 3.2.7 Chest x-ray

Chest x-ray - Required only if there are no plans to obtain a chest CT. This does not need to be repeated for screening if performed within the previous 28 days. If done within 28 days prior to the initiation of study treatment, it may be used as baseline.

### 3.2.8 EKG

This does not need to be repeated for screening if performed within the previous 28 days. If done within 28 days prior to the initiation of study treatment, it may be used as baseline.

### 3.2.9 Pregnancy test

Pregnancy test: Performed within 7 days prior to the initiation of study treatment in women of childbearing potential.

### 3.2.10 BMC

BMC for storage to assess baseline mTOR pathway activation.

### 3.3 Documenting activation of the mTOR pathway

Documentation of the activation of the mTOR pathway will be done retrospectively. Activation of mTOR pathway will be assessed using immunoblotting and/or IHC. The use of commercially available antibodies against components of the mTOR pathway in immunoblotting has been used extensively in hundreds of published studies. Because documentation of activation of the Akt/mTOR pathway is an inclusion criteria in the study, the IHC will be performed in a CLIA approved laboratory. In collaboration with Dr. Stephen Hewitt, Dr. Mark Raffeld is developing assays which will meet CLIA standards by the time the phase II study begins accruing. IHC has been performed previously in paraffin-embedded, formalin-fixed tissues [13]. The methods that will be employed are as follows. Staining of slides from patients' biopsies will be compared with staining of slides from cell blocks of NSCLC cells that have high levels of pathway activation (H157) and cells that have low levels of pathway activation (H1355). Sections will be deparaffinized with xylene and rehydrated through graded alcohols into buffer. A Decloaking chamber (Biocare Medical, Walnut Creek, CA) will be used for antigen retrieval with high pH citrate buffer (Dako cytometry, Carpinteria, CA) for 30 minutes. Endogenous peroxidase will be blocked with 3% H<sub>2</sub>O<sub>2</sub> in PBS for 10 minutes, followed by washing twice in PBS. Tissues will be placed in a humidity chamber and incubated with 1.5% goat serum in PBS for 30 minutes. Slides will be incubated overnight with phospho-specific antibodies or antibodies that measure total levels of pathway components (Cell Signaling Technology, Beverly, MA) at a 1:50 dilution at 4°C. The antibodies to be employed are as follows: phospho-Akt (Ser473) (736E11) rabbit mAb (IHC Specific), phospho-Akt (Thr308) (244F9) rabbit mAb, phospho-mTOR (Ser2448) (49F9) rabbit mAb (IHC Specific), phospho-p70 S6 kinase (Thr389) (1A5) mouse mAb, and phospho-S6 ribosomal protein (Ser235/236) (91B2) rabbit mAb, and phospho 4E-BP1 (Thr37/46) (236B4) rabbit mAb (IHC Preferred). These antibodies have been widely used in immunohistochemistry and are specific (see data sheets at <http://www.cellsignal.com>). After washing three times with PBS, each series of sections will be incubated for 30 minutes with biotinylated secondary antibodies (Vector Laboratories) diluted to 1:200 by the blocking agent described above, washed three times in PBS, and then incubated for 30 minutes with avidin-biotin complex method reagent (Vectastain Elite ABC kit; Vector Laboratories). The reaction products will be washed twice with PBS, placed in 0.05 M Tris-HCl buffer (pH 7.5) for 5 minutes, and then developed in liquid 3,3'-diaminobenzidine (DAKO) for 3 minutes. After development, sections will be washed twice with distilled water, lightly counter-stained with Mayer's hematoxylin, dehydrated, cleared, and mounted with resinous mounting medium [13]. Scoring will be based on intensity and distribution, as previously described [49]. Briefly, staining will be based on the coexistence of both positive and negative cells in the same tissue sample. Signals will be considered positive when reaction products were localized in the expected cellular component. The criteria for the staining will be scored as follows: distribution score will be scored as 0 (0%), 1 (1–50%), and 2 (51–100%) to indicate the percentage of positive cells in all tumor cells present in one tissue. The intensity of the signal (intensity score) will be scored as 0 (no signal), 1 (weak), 2 (moderate), and 3

(marked). This protocol can detect a wide spectrum of mTOR activity (Figure 7).

The distribution score and intensity score will then be summed into a total score (TS) of TS0 (sum=0), TS1 (sum=2), TS2 (sum=3), and TS3 (sum=4–5). TS0 or TS1 will be regarded as negative, whereas TS2 or TS3 will be regarded as positive. Internal controls for IHC analysis will include slides made from cell blocks of lung cancer cell lines that have wide variation of pathway activation, which will assist assessment of staining intensity. Specimens with questionable signals due to insufficient tumor cells, tumor cells that are difficult to distinguish from other inflammatory or interstitial cells, or tissues with homogeneously weak signals will be excluded from further evaluation. Tissue sections with only necrotic tumor cells or tissues with high homogenous background will also be excluded. In the event of limited tissue availability, the stains will be prioritized as follows: phospho-S6, phospho-mTOR, phospho-S6K, phospho-Akt, and TS. Modulation of S6 phosphorylation correlates most closely with administration mTOR activation [49], and serine 2448 phosphorylation mTOR best predicts response to sirolimus [27]. For the phase II portion of the trial where activation of the mTOR is required for enrollment, we will require a positive score ( $TS \geq 2$ ) in either phospho-S6 or phospho serine 2448 mTOR as a minimum requirement for protocol enrollment.

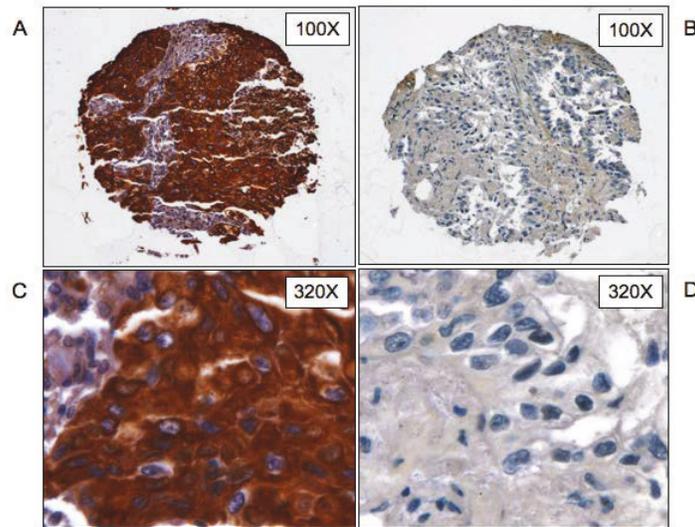


Figure 7: Immunohistochemical staining of lung carcinoma tissue to demonstrate mTOR pathway activation. Panels A and C are low and high power views, respectively, of a NSCLC specimen stained for phosphorylated S2448 mTOR with a distribution score of 2 and an intensity score of 3. Panels B and D are low and high power views, respectively, of a NSCLC specimen stained for phosphorylated S2448 mTOR with a distribution score of 2, and an intensity score of 1.

### 3.4 Patient Registration

#### 3.4.1 Patient Registration

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (<http://camp.nci.nih.gov/ccr/welcome.htm>) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

#### 3.4.2 Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (<http://camp.nci.nih.gov/ccr/welcome.htm>) main page must be completed and faxed to 301-480-0757.

#### 3.4.3 Dose Level

For dose level, contact the protocol chair or the principal investigator.

## 4 STUDY IMPLEMENTATION

### 4.1 Study Design

Treatment will be administered on an outpatient basis except when admission is required for tumor biopsy/research samples or side effects of treatment. No other investigational agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Treatment is outlined as follows:

All eligible patients will receive sirolimus orally for 7 days prior to the initiation of pemetrexed. The starting dose is 3 mg load followed by 1 mg each day continuously. Patients are to swallow the sirolimus tablets with approximately 250 ml of water each morning on an empty stomach. Tablets should not be taken with food, but patients may eat after 1 hr. On day 8, pemetrexed will be administered in an IV infusion on a 21-day cycle at an initial dose level of 375 mg/m<sup>2</sup>. As recommended by the FDA, folic acid 1mg orally every day and vitamin B12 1000 µg subcutaneously or intramuscularly every 63 days will be administered during the week preceding the first dose of pemetrexed. Folic acid will be continued for 21 days following the last dose of pemetrexed. Dexamethasone 4 mg is to be given by mouth twice daily the day before, the day of, and the day after pemetrexed

administration. Because of the effects of non steroidal anti inflammatory (NSAIDS) agents on renal blood flow, study subjects will be required to stop use of all NSAIDS beginning 5 days before and for two days after receiving pemetrexed.

For the phase I portion of the study, the doses of sirolimus and pemetrexed will be based on cohort assignment (table 1). Cohort 1 receives sirolimus at a 3 mg load, followed by 1 mg a day maintenance. The loading dose of sirolimus is administered on day 1; the maintenance dose is administered day 2 and every day thereafter. Pemetrexed starts day 8 at 375 mg/m<sup>2</sup>. The starting dose for both pemetrexed and sirolimus are below the usual FDA-approved doses. These doses were chosen because of the potential for increased toxicity with the combination as described by Punt *et al*. If two of six subjects experience a dose limiting toxicity in dose level 1, dose level -1 will be opened. If two of six subjects experience a dose limiting toxicity in dose level -1, enrollment will be suspended. If an MTD is not reached after the fifth dose level, that dose level will be used for the phase II study. No further dose escalation of either drug will be performed.

Table 1: Dose escalation scheme for SIROLIMUS and PEMETREXED

Dose Level	Sirolimus (mg)	Pemetrexed (mg/m <sup>2</sup> )
-1	1.5 mg load/0.5 mg/day	186 mg/m <sup>2</sup>
1	3 mg load/1 mg/day	375 mg/m <sup>2</sup>
2	6 mg load/2 mg /day	375 mg/m <sup>2</sup>
3	6 mg load/2 mg /day	500 mg/m <sup>2</sup>
4	10 mg load/3 mg/day	500 mg/m <sup>2</sup>
5	15 mg load/5 mg/day	500 mg/m <sup>2</sup>

Once the phase I portion of the protocol is completed, the data will be analyzed and an addendum will be made to address any modifications required and applicable to the phase II portion of the protocol. The data from the phase I part of the protocol will be analyzed and reported in a peer reviewed journal as soon as possible, regardless of the progress of the phase II portion of the protocol.

The MTD determined in the phase I portion of the study will be used for the phase II study. As many as 6 phase I subjects may enroll in the phase II study provided they met all eligibility requirements, including biopsy documenting Akt pathway activation, for the phase II study at the time of enrollment in the phase I study. Subjects who experienced toxicity while enrolled in the phase I study may enroll in the phase II study provided they recover to grade 2 neutropenia and grade 1 all other toxicities, within 21 days. In order to roll in to the phase II study, phase I subjects must have no signs or symptoms of progressive malignancy. Subjects who were receiving the dose level above the MTD in phase I will begin treatment at the MTD on day 1 of the first cycle, within the phase II

study. PKs will be repeated in the first phase II cycle for all phase I participants who were treated at the dose above the MTD. Subjects who were receiving the MTD on phase I will not have PKs repeated in the phase II study. Subjects on the phase I study who completed at least one cycle of therapy at the MTD but came off study because of progressive disease or unacceptable toxicity may be counted as a phase II subject provided they were treated for at least 3 weeks and met all the enrollment criteria for the phase II study.

There is a lead-in period for sirolimus alone in the phase II portion just like in the phase I study. Repeated cycles will be administered indefinitely provided there are no dose-limiting toxicities or clinical deterioration. During the 7<sup>th</sup> week of treatment and every two cycles thereafter, patients undergo clinical and radiographic tumor restaging.

Patients enrolled in both phases of the study will undergo a baseline imaging (CT or MRI) to establish baseline disease burden and sites of measurable disease. A PET CT will be performed prior to starting treatment on day 1 to establish baseline metabolic activity in the tumor. Patients will undergo a follow up PET CT scan if baseline was positive, defined as  $SUV \geq 2$  in known areas of tumor. There will be no further PET CT's for the rest of the study. Clinical CT or MRI will be performed for restaging process after 2 cycles or at the time of disease progression, whichever occurs first. They will undergo additional clinical imaging (CT or MRI) scans every 2 cycles as long as they remain on the protocol and until they meet off-study criteria.

mTOR activation will be studied in peripheral blood mononuclear cells (PBMCs) and tumor tissue. PBMCs will be evaluated at baseline, day 8 and after every two treatment cycles in all subjects of the phase I and phase II portions of the study. Patient consent will be obtained by the physician obtaining the biopsy, who in turn, will detail the risks of that procedure. Patients have the right to refuse any biopsy.

Patients will be monitored for compliance with sirolimus through pill count to be done at about the end of each cycle. We will use the NCI CCR Pill Count Form found on the NCI/CCR intranet, Policies/SOP section (<http://ccrintra.nci.nih.gov/>).

## 4.2 Drug Administration

### 4.2.1 Administration of Pemetrexed

Pemetrexed will be administered by intravenous infusion on day 8 of cycle 1 and day 1 of all subsequent cycles. The dose escalation scheme is outlined in Table 1. Each dose level will have a cohort of 3 to 6 subjects depending upon toxicity. There will be no inpatient dose escalation. All patients will receive standard concurrent FDA required pemetrexed therapy consisting of vitamin B12, folate, and dexamethasone in the below mentioned doses to ameliorate the side effects of pemetrexed. Vitamin B12 is to be given

subcutaneously or intra-muscularly at 1000 µg approximately every 63 days. Folic acid tablets are to be administered at 1mg orally on a daily basis and will be continued for 21 days following the last dose of pemetrexed. Both agents are to be initiated five to seven days before the first dose of pemetrexed. Dexamethasone 4 mg is to be given by mouth twice daily the day before, the day of, and the day after pemetrexed administration.

#### 4.2.2 Administration of Sirolimus

Sirolimus tablets will be administered to all eligible study subjects once daily by mouth on an empty stomach with 250 cc water beginning day 1 cycle 1. Sirolimus Oral Solution will be administered when dose reduction requires a fractional dose. The solution is drawn from the bottle with the accompanying syringe. The dose is placed in a glass or plastic cup containing at least 2 ounces (60 cc) of water or orange juice, then stirred vigorously for one minute and consumed immediately. The container is then refilled with at least 4 ounces (120 cc) of water or orange juice, stirred vigorously again, and consumed immediately. No other liquids are to be used. Only glass or plastic cups should be used. Sirolimus will be taken every day of the 28 (cycle 1 only) or 21 day cycle (because of the 7 day lead in for the first cycle, cycle 1 is 28 days in duration). Subjects may eat one hour after taking the sirolimus. The sirolimus should be taken at the same time each day, preferably in the morning around 0900 to 1000 EST to facilitate pharmacokinetics and periodic sirolimus trough levels performed at clinic visits. Sirolimus trough levels will be checked weekly in cycle 1, on day 1 of each subsequent cycle, at the time of any grade 3 toxicity, and as needed for follow up of that toxicity.

### 4.3 Treatment Modifications for Phase I

#### 4.3.1 MTD

4.3.1.1 The MTD will be based on the tolerability observed during the first 4 weeks of treatment only. Escalations of sirolimus and pemetrexed are planned in groups of three patients, with an additional three patients to be added at the first indication of Dose Limiting Toxicity (DLT). The MTD of sirolimus will be the highest dose at which less than two out of six patients experience DLT, as defined in section 3.3.2. If dose level 5 is reached and the MTD has not been established, there will be no further dose escalation.

4.3.1.2 Up to three patients may be enrolled simultaneously at each dose level specified in Table 1. These patients should be observed for DLT for at least 4 weeks from the first day of treatment before new patients are enrolled at the next higher dose level. If patients are not fully evaluable for toxicity in cycle 1, patients will be replaced.

4.3.2 The following dose escalation rules will be used:

- 4.3.2.1 Three patients are studied at the first dose level.
- 4.3.2.2 If none of these three patients experience DLT, then the dose is escalated to the next higher level in the three subsequent patients.
- 4.3.2.3 If one of the three patients experiences a DLT at a given dose level, then three more patients are accrued at the same dose. If none of these three additional patients experience a DLT, then the dose is escalated to the next level in subsequent patients. If one or more of these three additional patients experiences DLT, the MTD has been exceeded and three more patients are treated at the next lower dose (if only three patients were previously treated at that prior dose).
- 4.3.2.4 The MTD is the dose level at which 0/6 or 1/6 patients experience DLT with the next higher dose level having at least 2/6 patients experiencing DLT.

#### 4.3.3 Dose Limiting Toxicity (DLT)

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE Version 3.0) scale. If multiple toxicities are seen, the presence of DLT should be based on the most severe toxicity experienced. DLT will be defined as any of the following events occurring during the first 4 week course of treatment as is deemed to be from a possible, probable, or definite effect of the study drugs. Attributions will be made for both drugs.

- 4.3.3.1 Any grade 4 hematologic toxicity (except grade 4 lymphopenia, or grade 4 neutropenia with duration of  $\leq 7$  days) or febrile neutropenia (ANC  $< 1000$  cells/mm<sup>3</sup> and temperature  $\geq 38.5^{\circ}\text{C}$ ).
- 4.3.3.2 Grade 3 hypercholesterolemia (defined as  $> 400$  mg/dL or 10.34 mmol/L) or hypertriglyceridemia (defined as  $> 5\text{X}$  ULN) in spite of treatment with HMG-CoA reductase inhibitors.
- 4.3.3.3 Grade 3 diarrhea (defined as  $> 7$  stools per day over baseline; incontinence; hospitalization, need for IV fluids for 24 hours, severe increase in ostomy output, or interfering with ADL) that has not resolved to grade 2 diarrhea within 24 hours and grade 1 within 48 hours of starting fibercon or metamucil and diphenoxylate or loperamide.
- 4.3.3.4 Grade 3 pneumonitis (defined as symptomatic, interfering with ADL, oxygen required).
- 4.3.3.5 Grade 3 or 4 mucositis

4.3.3.6 Any other non-hematologic grade 3 and grade 4 toxicity. Grade 3 or 4 nausea and vomiting will be defined as grade 3 or grade 4 nausea and vomiting that has not resolved to grade 2 within 24 hours and grade 1 within 48 hours of starting of starting ondansetron, granisetron, emend, dexamethasone, and or ativan.

#### 4.3.4 Dose Adjustments

4.3.4.1 If a subject experiences a DLT as defined in Section 4.3.2, the treatment will be immediately stopped for a minimum of 1 week. Both drugs will be held until the patient recovers to a grade 2 neutropenia and grade 1 all other toxicities. If the patient recovers within 21 days, treatment may be resumed using the dosing guidelines in Table 2. When the patient resumes therapy, the sirolimus dose will be immediately adjusted per Table 2 guidelines. Those patients requiring fractional doses upon resumption of therapy will be placed on the commercially available oral solution of sirolimus. In patients with resting tremor or who are otherwise unable to self-administer the oral solution, the patient should take the next lowest pill form of sirolimus even if it is a greater dose reduction than that specified in table 2. Pemetrexed will be adjusted at the beginning of the next cycle.

4.3.4.2 If the subject fails to recover to a grade 2 neutropenia and grade 1 all other toxicity within 21 days, she/he will be removed from the study.

4.3.4.3 Subject will be allowed a maximum of two dose reductions before being taken off study. If subject experiences a life threatening toxicity at any time, she/he will be removed from the study.

4.3.4.4 For patients who had their dose reduced in any prior cycle for the drug related toxicity, their dose will not be re-escalated, even if there is minimal or no toxicity with the reduced dose. Patients whose dose has been reduced in prior cycles for adverse events that are subsequently not felt to be related to the drug combination may have the dose re-escalated after completion of one cycle with toxicities less than or equal to grade 1.

4.3.4.5 Patients will have sirolimus trough levels drawn on day 8±1 day, 15±1 day, 22±1 day of cycle 1 then day 1 of subsequent cycles. In addition sirolimus levels will be obtained any time the patient experiences a grade 3 or higher toxicity. Dose modification will be based upon the toxicity and not the sirolimus level.

Table 2: Dose Modifications for Pemetrexed and Sirolimus

Toxicity	Pemetrexed Dose (mg/m <sup>2</sup> )	Sirolimus (mg)
Nadir ANC <500/mm <sup>3</sup> and nadir platelets <sup>a</sup> 50,000/mm <sup>3</sup>	75% of previous dose	75% of previous dose
Nadir platelets <50,000/ mm <sup>3</sup> regardless of nadir ANC	50% of previous dose	50% of previous dose
Grade 3 or 4 non-hematologic toxicities except mucositis and pneumonitis	75% of previous dose	75% of previous dose
Any diarrhea requiring hospitalization (irrespective of grade)	75% of previous dose	75% of previous dose
Grade 3 pneumonitis	No dose reduction	50% of previous dose
Grade 3 or 4 mucositis	50% of previous dose	50% of previous dose
Grade 3 nausea, vomiting	75% of previous dose	75% of previous dose

#### 4.4 Treatment Modifications for Phase II

Based on safety and efficacy analysis from the Phase I study, the dose of sirolimus and pemetrexed used in the phase II portion of the study will be that of dose level 4 from the Phase I portion of the study (pemetrexed 500mg/m<sup>2</sup> IV every 21 days and sirolimus 10mg load on day 1 and 3 mg daily thereafter). If trough sirolimus levels exceed 15 ng/mL at any point, sirolimus will be held for 48 hours and will resume at 2 mg/po/qd.

To account for safety in the Phase II portion, if 2 of the first 6 subjects encounter a DLT as defined in the Phase I portion, we will amend the protocol to use dose level 3 (500 mg/m<sup>2</sup> IV every 21 days and sirolimus 6 mg load on day 1 and 2 mg daily thereafter) for new subject enrollments. Phase II study subjects who experience a DLT while on dose level 4 will be reduced to dose level 3. If a phase II study subject on dose level 3 experiences a DLT as defined in section 3.3.2 we will follow the same procedures outlined in section 3.3.3.1 using Table 2 for guidance. If the subject does not recover in 21 days, he/she will be taken off study. Any subject requiring more than two dose reductions will be taken off study.

#### 4.5 Pharmacokinetic Studies

Pharmacokinetic sampling will be performed during cycle 1, on days 7 and 8 to allow for comparison of steady state sirolimus pharmacokinetics in the absence and presence of pemetrexed.

On cycle 1 day 7, a single venous blood samples (5ml) will be obtained in an

ethylenediamine-tetra-acetic (EDTA) tube, at each of the following time points: immediately pre dose and at 20 min, 40 min, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours after the administration of sirolimus and will be analyzed for sirolimus levels. On cycle 1 day 8, the blood will be drawn in two EDTA tubes pre dose, then 20 min, 40 min, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 12 hours, and 24 hours after administration of sirolimus, but will be analyzed for both sirolimus and pemetrexed levels. Sirolimus ingestion and pemetrexed infusion will be administered at the same time. The time each drug was administered will be noted. Patients will be admitted to the in-patient oncology unit, if necessary, in order to obtain the 12 and 24 hour post drug administration. The samples will be drawn using the following guidelines:

PK samples should not be drawn through the drug administration line/port. Residual drug in the line or port distorts PK data obtained from specimens drawn at the same site as drug administration. PK data is collected on drug concentrations in plasma after it has traveled through the body. This is not possible if specimens are drawn at the same site as administration.

The patient name and exact draw time will be provided on all specimens. The exact draw time – not just the ideal time – for PK samples will be recorded even if they are drawn late. Also, for PK samples, cycle information will be provided on the specimen container (e.g. C1D1 - 5 hr post infusion – 12:30pm May 15, 2006). Immediately after collection, sample will be placed on ice and stored in the refrigerator. (Especially important with PK samples, as it prevents drug degradation.) Dr. Figg's lab, Gareth Peters/Kathy Compton at 102-11964 will be paged for sample pickup.

The Clinical Pharmacology Program (Dr. William D. Figg, 5A-01, 301-402-3622) will coordinate rapid acquisition and processing of blood samples. HPLC-MS analytical method will be used to quantify sirolimus and pemetrexed whole blood concentrations. The pharmacokinetic characteristics of sirolimus and its interactions with pemetrexed will be evaluated using the WinNonLin pharmacokinetic software (Pharisight, Mountain View, CA). The maximum plasma concentration, time to maximum concentration, area under the curve extrapolated to infinity, and apparent terminal half-life will be calculated by non-compartmental analysis. Pharmacodynamic analysis will attempt to correlate any correlation in sirolimus concentration with disease response and/or toxicities.

All samples will be barcoded, with data entered and stored in the Patient Sample Data Management System (PSDMS) utilized by the Clinical Pharmacology Program (CPP). This is a secure program, with access to the PSDMS limited to defined CPP personnel, who are issued individual user accounts. The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without PSDM System access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer

location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.). In addition to the barcode, the sample label contains an abbreviation for the clinical study, along with an abbreviation for the sample type, followed by the sample and aliquot number, i.e. ETT-PL-0001-001. As such, these labels are coded and the sample number does not specifically denote the patient or patient ID.

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Samples will be stored until requested by a researcher on the protocol. All requests are monitored and tracked in the PSDM System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the PSDMS. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

#### **4.6 Periodic evaluations during treatment**

(See Table 3, section 10)

##### **4.6.1 Cycle 1 Day 1**

4.6.1.1 History and Physical examination (including vital signs, ECOG performance status (Table 4 Section 10), narcotic use, medication review). If performed within 17 days,

the baseline History and Physical is acceptable.

4.6.1.2 Adverse events. If performed within 17 days, the baseline Adverse Events is acceptable.

4.6.1.3 Laboratory evaluation: CBC with differential, PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, fasting cholesterol (HDL, LDL) and triglycerides, vitamin B12 and folate. If performed within 17 days, the baseline laboratory results are acceptable.

4.6.1.4 Lymphocyte subsets. If performed within 17 days, the baseline lymphocyte subsets are acceptable.

4.6.1.5 EKG: within 28 days prior to initiation of treatment

4.6.1.6 CXR: within 28 days prior to initiation of treatment, only required if there are no plans to perform chest CT.

4.6.1.7 Clinical tumor measurements, i.e. skin lesions, subcutaneous nodules, palpable masses within 17 days of treatment

4.6.1.8 Clinical imaging (CT or MRI) for tumor measurement: within 28 days prior to initiation of treatment

4.6.1.9 Pregnancy test for women of childbearing potential within 72 hours of starting study drug.

4.6.1.10 PET CT: within 28 days prior to initiation of treatment. Inability to accomplish study will not disqualify subject

4.6.1.11 PBMC for mTOR pathway activation within 17 days prior to starting study drug.

4.6.2 Day 1 all subsequent cycles

These studies may be performed up to 72 hours prior to day 1 of subsequent cycles unless otherwise specified.

4.6.2.1 History and Physical examination (including vital signs, ECOG performance status (Table 4 Section 10), narcotic use, medication review and adverse events).

4.6.2.2 Adverse events

- 4.6.2.3 Clinical tumor measurements, i.e. skin lesions, subcutaneous nodules, palpable masses
- 4.6.2.4 Laboratory evaluation: CBC with differential, PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, PT, APTT, fasting cholesterol (HDL, LDL) and triglycerides.
- 4.6.2.5 Lymphocyte subsets
- 4.6.2.6 Imaging (CT or MRI); within 72 hours of day 1 cycle 3 and every 2 cycles thereafter
- 4.6.2.7 PET CT: within 72 hours of day 1 cycle 3 only, provided baseline scan was positive (maximum 2 PET CT scans).
- 4.6.2.8 Tissue for follow up on mTOR pathway activation – optional for phase I subjects, required for phase II - within 7 days of day 1 cycle 3 only. If subject progresses before cycle 3, then tissue will be obtained at time of progression.
- 4.6.2.9 PBMC for mTOR pathway activation – all subjects phase I and phase II within 7 days of day 1 cycle 3 and every 2 cycles thereafter. If subject progresses before cycle 3, then PBMC will be studied at the time of progression.
- 4.6.2.10 Pregnancy test for women of childbearing potential within 72 hours
- 4.6.2.11 Trough sirolimus levels within 72 hours
- 4.6.3 Day 8 evaluations will be performed in cycle 1 for every patient.  
The following will be included:
  - 4.6.3.1 History and physical examination (including vital signs, ECOG performance status (Table 4 Section 10), narcotic use, and medication review)
  - 4.6.3.2 Adverse events
  - 4.6.3.3 Laboratory evaluation: CBC with differential, PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, fasting cholesterol (HDL, LDL) and triglycerides
  - 4.6.3.4 Pharmacokinetics for sirolimus and pemetrexed

4.6.3.5 PBMC for mTOR pathway activation, day 8 cycle 1 only

4.6.3.6 Trough sirolimus level

4.6.4 Day 15 evaluation will be performed in cycle 1 for every patient.

4.6.4.1 Adverse events

4.6.4.2 CBC with differential

4.6.4.3 Trough sirolimus level

4.6.5 Day 22 evaluation will be performed in cycle 1 only (cycle 1 is 28 days all other cycles are 21 days) for every patient

4.6.5.1 Adverse events

4.6.5.2 CBC with differential

4.6.5.3 Trough sirolimus level

4.6.6 Unexpected evaluations

If a dose adjustment is made in any cycle following cycle 1, evaluations will be performed on day 8 and or day 15 depending upon when in the cycle the dose adjustment is made. Subjects will always have at least 2 weekly follow up evaluations following dose modification. If medically indicated, more frequent evaluations will take place. If, following dose modification, the cycle is completed without further adverse events, weekly visits may be omitted in subsequent cycles. The weekly visits will include the following if indicated:

4.6.6.1 History and physical examination (including vital signs, ECOG performance status (Table 4 Section 10), narcotic use, and medication review)

4.6.6.2 Adverse events

4.6.6.3 CBC with differential

4.6.6.4 Laboratory evaluation: PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, fasting cholesterol (HDL, LDL) and triglycerides

#### 4.6.6.5 Trough sirolimus level

### 4.7 Off Study Evaluations

#### 4.7.1 History and Physical

The history and physical examination (including vital signs, ECOG performance status, adverse events, narcotic use, and medication review) will be performed at the off study visit.

#### 4.7.2 Follow-up PET CT and tissue for mTOR pathway determination

If the subject comes off study after cycle 1 but prior to cycle 3, then off study evaluation will include follow up PET CT (if baseline was positive) and tissue for mTOR pathway determination (optional for phase I subjects).

#### 4.7.3 CBC with differential

#### 4.7.4 Laboratory evaluation

Laboratory evaluation: PT, APTT, electrolytes, BUN, creatinine, albumin, calcium, magnesium, phosphorus, LDH, SGOT, SGPT, total bilirubin, alkaline phosphatase, amylase, lipase, fasting cholesterol (HDL, LDL) and triglycerides

### 4.8 Concurrent Therapies

As recommended by the FDA for the amelioration of toxicity associated with the administration of pemetrexed, all patient will receive folic acid, vitamin B12, and dexamethasone. Vitamin B12 is to be given subcutaneously or intra-muscular at 1000 µg approximately every 63 days. Folic acid tablets are to be administered at 1mg orally on a daily basis. Both agents are to begin five to seven days before the initial dose of pemetrexed. Dexamethasone 4 mg is to be given by mouth twice daily the day before, the day of, and the day after pemetrexed administration. Because of the effects of non steroidal anti inflammatory (NSAIDS) agents on renal blood flow, study subjects will be required to stop use of all NSAIDS beginning 5 days before and for two days after receiving pemetrexed.

### 4.9 Correlative Studies

#### 4.9.1 Measurement of mTOR pathway activation in PBMCs and tumor tissue

A total of 16 ml of blood will be collected from each patient in blue/black speckled (CPT) tubes at baseline (within 17 days) and day 8 of cycle 1 and every 2 cycles (within 72 hours) thereafter to study inhibition of the pathway by sirolimus in peripheral blood mononuclear

cells (PBMC) and to correlate pathway inhibition with sirolimus levels. Activation of mTOR, S6K1, 4E-BP1, S6, and Akt will be assessed by immunoblotting of PBMC lysates from each patient. Paired specimens will be run simultaneously and band intensity will be quantified using densitometry. This technique has been successfully employed in other protocols using sirolimus analogues [50]. Additionally, fixed tumor biopsies will be assessed for activation of p-S6K, p-S6, p-4E-BP1, pS473-Akt, and TS levels using immunohistochemical techniques [13]. Internal controls for IHC analysis will include slides made from cell blocks of lung cancer cell lines that have wide variation of pathway activation, which will assist assessment of staining intensity. Scoring of tumor biopsies will incorporate distribution and intensity of staining, and will be scored as being positive or negative as previously described [49].

#### 4.9.2 Tissue Biopsy

Any residual samples will be stored permanently in the laboratory of Dr. Giuseppe Giaccone. The IRB will be updated in the continuing review regarding the status of the specimen and any use beyond this protocol. We will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher.

#### 4.9.3 Stored Tissue

Blood and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations), the principal investigator or the protocol chairperson must amend the protocol and obtain informed consent from all research subjects.

#### 4.10 Surgical Guidelines

If the primary tissue is not appropriate because of intervening therapy, if the tissue provided does not confirm the NSCLC diagnosis, or is deemed inadequate to test for mTOR pathway activation, biopsies will be obtained by the NNMC or NIH Clinical Center staff via the least invasive and least risky method. A separate informed consent will be obtained by the physician performing the procedure.

#### 4.11 Radiation Therapy Guidelines

There is no radiation therapy component in this study.

#### **4.12 Off Study Criteria**

##### **4.12.1 Progression of disease (as defined in 6.2)**

4.12.1.1 Subjects must be followed with the same types of clinical imaging (CT or MRI) that were used for baseline tumor measurements.

4.12.1.2 Subjects will be removed from study 4 weeks after disease progression is documented.

4.12.2 The patient may withdraw from the study at any time for any reason if he/she wishes.

4.12.3 Subjects may be removed for medical or psychiatric illness, which in the investigator's judgment renders the patient incapable of further therapy.

4.12.4 Subjects may be removed for non-compliance with the oral regimen as judged by missing  $\geq 7$  days of sirolimus within one cycle, unless this was secondary to resulting toxicities and coordinated with the principal investigator and/or study chairperson.

4.12.5 All reasons for discontinuation of treatment must be documented in the medical record and Central Registration Office must be notified.

##### **4.13 Post Study Evaluation (Follow-up)**

Patients will be followed for 4 weeks after the patients are taken off therapy or until death, whichever occurs first. No studies are required. The follow-up can be performed by the patient's primary oncologist. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

## **5 SUPPORTIVE CARE**

### **5.1 Medical Care**

All cancer related care will be provided during and following protocol administration for cancer related complication including any treatments for drug-related complications such as tumor lysis syndrome, development of organ dysfunction (e.g., renal dysfunction), infections/ fever and neutropenia, blood product support and cytokine support. Care will be taken to avoid agents that are strong CYP3a4 inhibitors.

### **5.2 Management of hypersensitivity reactions**

Hypersensitivity reactions will be managed with slowing or stopping the infusion and/or diphenhydramine with or without dexamethasone. Patients experiencing life-threatening hypersensitivity reaction to pemetrexed in the setting of steroid pre-medication will not be rechallenged with pemetrexed.

### 5.3 **Febrile neutropenia**

Febrile neutropenia is potentially life threatening and requires hospitalization and urgent broad-spectrum antibiotics.

### 5.4 **Symptomatic Anemia**

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

### 5.5 **Thrombocytopenia**

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm<sup>3</sup>. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count of >50,000/mm<sup>3</sup>.

### 5.6 **Central Venous Access**

Placement of central venous access will be at the discretion of protocol chair and/or the primary investigator. Possible lines include mediport implantable devices, HICKMAN or GROSHONG catheters.

### 5.7 **Nutritional Support and Psychosocial support**

During cancer treatments it is sometimes difficult for patients to maintain good nutrition. If it is deemed necessary, or it could benefit the patient, the patient will be referred for a nutritional consult. Patients who are having emotional difficulty coping with their disease and/or their treatment will be referred to a social worker for evaluation and support.

### 5.8 **Anti-emetics**

Anti-emetics will be prescribed by the treating physician as necessary and as used traditionally in cancer patients.

### 5.9 **Hypercholesterolemia and hypertriglyceridemia**

Patients will be given HMG-CoA reductase inhibitors as anti-hyperlipidemic medications according to National Cholesterol Education Program (Adult Treatment panel III) (NCEP ATIII) guidelines, if their cholesterol and triglycerides levels require (<http://www.nhlbi.nih.gov/guidelines/cholesterol/atglance.pdf>).

Pravastatin and rosuvastatin are the preferred agents since they are not metabolized by CYP3A4.

### 5.10 **Monitoring for lymphoma**

To address the increased incidence of lymphoma in patients on sirolimus when it was used in combination with cyclosporine and corticosteroids, all patients on this study will be

monitored with physical exam every scheduled visit for any new lymph node enlargement and with complete blood count every cycle for any abnormality in cell differential. Lymphadenopathy that develops after enrollment that persists for 4 wk will be biopsied.

## **6 DATA COLLECTION AND EVALUATION**

### **6.1 Data Collection**

The NCI investigators will be responsible for the collection, maintenance, security and quality control of all study data. Meetings chaired by the principal investigator and/or protocol chairperson will be held on a biweekly basis to review the study data for quality, completeness, and interim analysis (see section 5.9). Data will be captured on the NCI C3D database. Research records will be maintained to include but not limited to the following:

- Signed, dated consent form.
- Completed eligibility checklist
- Source documents verifying eligibility criteria such as path reports and pathway activation
  - Interim monitoring test results
  - PK collection forms
  - Response evaluations
  - Off study summary

### **6.2 Response Criteria**

#### **6.2.1 Criteria Determination**

For the purposes of this study, patients should be reevaluated for response after about 6 weeks (or 2 cycles, except for the first two cycles which are 7 weeks). In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks following initial documentation of objective response, (which refers to complete response, or partial response as defined in section 5.2). Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [51]. Changes in only the largest diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term “evaluable,” in reference to measurability, will not be used because it does not provide additional meaning or accuracy.

#### **6.2.2 Definitions of Disease State**

##### **6.2.2.1 Measurable disease**

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

#### 6.2.2.2 Evaluation of Measurable Disease

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

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. Tumor lesions that have been biopsied previously might or might not be considered measurable.

#### 6.2.2.3 Non-measurable disease

All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

#### 6.2.2.4 Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

#### 6.2.2.5 Non-target lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

#### 6.2.2.6 Clinical lesions

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

#### 6.2.2.7 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of

target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

#### 6.2.2.8 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of “non-target” lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail and the progression status should be confirmed at a later time by the principal investigator or the study chairperson.

#### 6.2.2.9 Evaluation of best overall response:

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

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Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR

PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration.” Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

### 6.3 Confirmatory Measurement/Duration of Response

#### 6.3.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed four weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry and at a minimum interval of eight weeks (see section 6.2.2).

#### 6.3.2 Duration of overall response

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

#### 6.3.3 Duration of overall CR

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

#### 6.3.4 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression

are met, taking as reference the smallest measurements recorded since the treatment started.

#### 6.4 **Progression-Free Survival**

Progression free survival will be determined from the date treatment began until radiographic evidence of progressive disease is noted.

#### 6.5 **Response Review**

Patients with measurable disease will be assessed by standard criteria. The purpose of tumor measurements will be to assess benefit to the patients from treatment and to determine appropriateness for continuing on study. For the purposes of this study, patients should be re-evaluated every cycle and undergo imaging studies every two cycles. Following documentation of an objective response, confirmatory scans will also be obtained four weeks after. The response will be evaluated by Dr Peter Choyke from the NCI at the Clinical Center.

#### 6.6 **Toxicity Criteria**

This study will utilize the CTCAEv3 (<http://ctep.cancer.gov/forms/CTCAEv3.pdf>) for toxicity and adverse event reporting.

#### 6.7 **Statistical Section**

##### 6.7.1 Statistical considerations for Phase I portion of the trial

6.7.1.1 Baseline characteristics will be summarized across all enrolled patients. An accounting will be done of all patients registered in the study. The number of patients who died or withdrew before treatment began will be specified. Patients who did not meet all eligibility criteria will be described. Patients who were evaluated separately due to first-cycle non-drug-related death or cessation of first-cycle therapy prior to DLT will be characterized. Patients not fully evaluable for toxicity in cycle 1 will be replaced.

6.7.1.2 Treatment administration will be described for all cycles. Doses administered, dose modifications or delays, and duration of therapy will be evaluated.

6.7.1.3 Safety variables will be summarized at each dose level using descriptive statistics. Adverse events that occur will be reported for each dose level and described in terms of incidence and severity. Laboratory data will be presented by dose level at each observation time. Values outside of normal limits will be identified and their frequency calculated. Parameters will be described based on the CTCAE version 3.0 severity grading. Distribution by CTC severity grade (when applicable) and clinical relevance will be given.

- 6.7.1.4 A descriptive analysis of evidence of anti-tumor activity at each dose level, focusing on the MTD, will be provided based on clinical, radiographic, and biologic assessments of efficacy.
- 6.7.1.5 Bioanalytical and Pharmacokinetic Analysis: The whole blood concentration of sirolimus and pemetrexed will be quantified by LCMS/MS method.
- 6.7.1.6 Whole blood concentration of sirolimus will be evaluated and analysis will determine concentration ranges that may be used for allowable dose adjustments in phase II study.
- 6.7.1.7 The anticipated rate of accrual for the phase I portion of the trial will be approximately 1 subject/month. Depending on the dose level identified as the MTD, up to 30 subjects maybe required for this portion of the trial (5 levels with a maximum of 6 patients per level). If dose level 5 is reached and the MTD has not been established, dose level 5 will be used as the dose for subjects enrolling on the phase II portion of the study.
- 6.7.2 Statistical considerations for phase II portion of the trial
- 6.7.2.1 The primary objective of this study is to determine if the combination of sirolimus and pemetrexed, at the MTD for the combination, on a standard schedule, is able to result in an enhanced clinical response rate compared to that of pemetrexed alone.
- 6.7.2.2 Based on a prior published study, the response rate (CR+PR) to Pemetrexed in a population similar to that for this study is approximately 9%. It would be desirable to determine if the combination could be associated with a clinical response rate consistent with a 15% improvement, to 24%.
- 6.7.2.3 Prior to Amendment G, the study was planned to be conducted using a phase II optimal design [52] as follows. The study was to have been designed to determine if the combination of agents is able to be associated with a response rate (CR+PR) that can rule out 9% ( $p_0=0.09$ ) in favor of a 24% response rate ( $p_1=0.24$ ). Using  $\alpha=0.10$  (10% probability of accepting a poor therapy) and  $\beta=0.10$  (10% probability of rejecting a good therapy), 17 evaluable patients were to be enrolled onto the trial. If 0 to 1 of the 17 patients experiences a response, then enrollment was to be terminated. A pause in the accrual of patients may have been required until it could have been determined that there were adequate patients with a response to justify accrual to the second stage.

This pause in accrual may have been up to 13 weeks in duration following enrollment of the 17<sup>th</sup> subject if 2 responses had not been previously been documented. If 2 or more of

the first 17 evaluable patients enrolled would have had a response, then accrual would have continued until a total of 46 evaluable patients would have been enrolled. If 2 to 6 of the 46 were to have a response, then this would have been considered inadequate for further investigation. If 7 or more of 46 patients were to have had a response, then this would have indicated that this strategy provided a new approach that may be worthy of further consideration as it is consistent with 24%. Under the null hypothesis (9% response rate), the probability of early termination is 54%.

6.7.2.4 For patients who are pemetrexed-naïve, the study will be conducted using a phase II optimal design [52]. The study will be designed to determine if the combination of agents is able to be associated with a response rate (CR+PR) that can rule out 9% ( $p_0=0.09$ ) in favor of a 34% response rate ( $p_1=0.34$ ). Using  $\alpha=0.10$  (10% probability of accepting a poor therapy) and  $\beta=0.10$  (10% probability of rejecting a good therapy), 7 evaluable patients will be enrolled onto the trial. If 0 of the 7 patients experience a response, then enrollment will be terminated. A pause in the accrual of patients may be required until it can be determined that there are adequate patients with a response to justify accrual to the second stage. This pause in accrual may be up to 13 weeks in duration following enrollment of the 7th subject if 1 response has not previously been documented. If 1 or more of the first 7 evaluable patients enrolled have a response, then accrual will continue until a total of 20 evaluable patients have been enrolled. If 1 to 3 of the 20 patients has a response, then this will be considered inadequate for further investigation. If 4 or more of 20 patients have a response, then this will indicate that this strategy provides a new approach that may be worthy of further consideration as it is consistent with 34%. Under the null hypothesis (9% response rate), the probability of early termination is 52%.

6.7.2.5 For patients who had prior pemetrexed, the study will also be conducted using a phase II optimal design [52]. In this stratum, the study will be designed to determine if the combination of agents is able to be associated with a response rate (CR+PR) that can rule out 5% ( $p_0=0.05$ ) in favor of a 20% response rate ( $p_1=0.20$ ). Using  $\alpha=0.10$  (10% probability of accepting a poor therapy) and  $\beta=0.10$  (10% probability of rejecting a good therapy), 12 evaluable patients will be enrolled onto the trial. If 0 of the 12 patients experience a response, then enrollment will be terminated. A pause in the accrual of patients may be required until it can be determined that there are adequate patients with a response to justify accrual to the second stage. This pause in accrual may be up to 13 weeks in duration following enrollment of the 12th subject if 1 response has not previously been documented. If 1 or more of the first 12 evaluable patients enrolled have a response, then accrual will continue until a total of 37 evaluable patients have been enrolled. If 1 to 3 of the 37 patients has a response, then this will be considered inadequate for further investigation. If 4 or more of 37 patients have a response, then this will indicate that this strategy provides a new approach that may be worthy of

further consideration as it is consistent with 20%. Under the null hypothesis (5% response rate), the probability of early termination is 54%.

6.7.2.6 Twenty-two patients were enrolled on this protocol in the phase I portion. The phase II portion for both strata requires up to 57 evaluable patients. We will allow up to 60 patients to be accrued to the phase II portion to allow for the possibility of a small number of in-evaluable patients. Thus, the accrual ceiling for Phase II will be set at 22+60 for a total of 82 patients.

### 6.7.3 Secondary statistical Analysis of biological data

#### 6.7.3.1 Tumor Tissue Assays

A variety of markers will be evaluated at baseline as well as after the conclusion of treatment. Changes from baseline will be determined in either absolute or relative terms as appropriate, and evaluated for statistical significance, as well as to determine if the changes or the actual values at a time point are associated with clinical response. Paired comparisons with baseline will be done using a paired t-test or Wilcoxon signed rank test as appropriate, and the changes will be compared between responders (CR +PR) and non-responders (PD) using a two sample t-test (adequate numbers of subjects with normally distributed data in both groups) or a Wilcoxon rank sum test, if enough patients respond for this to be appropriate. Changes in PET scan SUVs will also be compared in a similar fashion with respect to any association with clinical response.

6.7.3.2 In all such cases, these secondary analyses will be considered exploratory and not formally adjusted for multiple comparisons. However, to ensure proper interpretation in the context of a potentially large number of explorations being performed, only p-values <0.01 will be interpretable as being associated with statistical significance.

6.7.3.3 The phase I portion of the study will enroll 12 to 30 patients depending upon the number of dose levels needed to reach the MTD, and therefore will require anywhere from 12 to 30 months to complete accrual. A review of the literature reveals a median prevalence rate for mTOR pathway activation of approximately 60% [13]. In the past 6 months we have received 13 lung cancer referrals for a phase I study. About half of these potential study subjects are ineligible for a number of reasons (other than mTOR pathway activation). Using our current accrual rate and the prevalence rate of mTOR activation from the literature, we would predict an accrual rate of 7 – 8 subjects/year (60%[50%(13)/6mos]). Up to 6 patients treated in the phase I study may enroll in the phase II study. As we are a young group we hope that through increased awareness and recruiting efforts we will be able to accrue at a faster rate in the near future.

## **7 HUMAN SUBJECTS PROTECTION**

### **7.1 Rationale for Subject Selection**

This study will be open to all individual with relapsed or refractory NSCLC regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. This study will be recruited through internal referral, our local physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer). Patients should realize that we are hopeful that they may gain benefit from this study, but there is no objective evidence to support our optimism at this time. Patients must have failed or be unable or unwilling to be treated with front line therapy (platinum or non platinum containing regimens) of proven efficacy for NSCLC. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

### **7.2 Inclusion of Women and Minorities**

Both men and women and members of all races and ethnic groups are eligible for this trial. Every effort will be made to recruit women and minorities in this study.

### **7.3 Justification for Exclusions**

Due to lack of knowledge of the effects of sirolimus and pemetrexed on the fetus or on infants, as well as the possibility of teratogenic effects, pregnant and nursing women will be excluded from this trial. Some animal studies have shown fetal malformations and fetal toxicity.

HIV-positive patients receiving anti-retroviral therapy are excluded from this study due to pharmacokinetic interactions between anti-retroviral medications such as protease inhibitors, which are CYP3A4 inhibitors, and sirolimus. HIV positive patients not receiving antiretroviral therapy are excluded due to the possibility that sirolimus may worsen their condition secondary to the immunosuppression of sirolimus and the likelihood that their underlying condition may obscure the attribution of adverse events with respect to sirolimus. Appropriate studies should be undertaken in this subgroup of patients in the future. Patients with unstable or serious medical or psychiatric conditions are excluded due

to the possibility that the underlying condition may obscure the attribution of adverse events with respect to sirolimus and pemetrexed.

#### **7.4 Participation of Children**

Patients under the age of 18 will be excluded from study because of the relative infrequency of NSCLC in this group. The effect of sirolimus and pemetrexed in children may be investigated in studies that exclusively enroll children.

#### **7.5 Evaluation of Benefits and Risks/Discomforts**

The potential benefit to a patient who enters study is a reduction in the bulk of his/her tumor, which may or may not have a favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects that are listed in the pharmaceutical section and the consent document. Subjects will receive an effective radiation dose of 3.7 rem from the PET CT scans. Subjects who undergo a CT directed biopsy will receive an additional 0.1 rem per biopsy procedure. For subjects who complete both PET scans and undergo two CT directed biopsies, the total effective dose of radiation will be 3.9 rem which falls within the 5 rem/year allowed by the radiation safety committee. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis as described earlier.

#### **7.6 Risk/Benefits Analysis**

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the National Naval Medical Center in Bethesda and Clinical Research Center at NIH in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

#### **7.7 Consent and Assent Process and Documentation**

An associate or principal investigator or the protocol chairperson on the trial will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient at a subsequent visit. The original copy of the signed informed consent will be placed in the patient's medical record and a copy will be stored in the Research Record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on

study.

## **8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN**

### **8.1 Definitions**

#### **8.1.1 Adverse Event**

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

#### **8.1.2 Suspected adverse reaction**

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

### 8.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. “Unexpected”, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

### 8.1.4 Serious

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

### 8.1.5 Disability

A substantial disruption of a person’s ability to conduct normal life functions.

### 8.1.6 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

### 8.1.7 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved study procedures in a research protocol that **does not** have a major impact on the subject’s rights, safety or well-

being, or the completeness, accuracy and reliability of the study data.

#### 8.1.8 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the IRB-approved study procedures in a research protocol that **does** have a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

#### 8.1.9 Unanticipated Problem

Any incident, experience, or outcome that:  
Is unexpected in terms of nature, severity, or frequency in relation to  
(a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, AND  
(b) the characteristics of the subject population being studied; AND  
Is related or possibly related to participation in the research; AND  
Places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

## 8.2 NCI-IRB Reporting

### 8.2.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research.
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

### 8.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

For reporting of adverse events at time of continuing review, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol since the previous continuing review and in aggregate. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk:benefit determination. The summary report is based on the following guidance: any

unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-IRB:

- All Grade 2 unexpected events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

### 8.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

## 8.3 Data and Safety Monitoring Plan

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

Data will be monitored regularly by the principal investigator and/or the study chairperson in order to identify significant toxicity trends. This will be done in biweekly meetings that review the treatment course of all the patients on the protocol at that point in time.

All new significant findings that may affect the patient's willingness to continue in the study will be shared with patients. All patients receiving study agents will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, CNS observations, physical examination findings, and spontaneous reports of adverse events reported to the investigator by patients. All toxicities encountered during the study will be evaluated according to the Common Terminology Criteria Adverse Events version 3.0 and recorded prior to each course of therapy. Life-threatening toxicities should be reported immediately to the Principal Investigator or study chairperson and the Institutional

## Review Board (IRB).

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations and violations will be immediately reported to the IRB using iRIS. These reports will be reviewed, entered into the protocol database, circulated and reviewed at the next scheduled IRB meeting, and then filed in the IRB protocol file. For confidentiality reasons, PIs should remove names, addresses and social security numbers from any supplemental information submitted with the AE/SAE form. Medical record numbers and/or study ID numbers are the only identifiers that should be used. Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released without patient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act.

## 9 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

### 9.1 Sirolimus

#### 9.1.1 Product Description

9.1.1.1 Other Names: Sirolimus, Rapamycin, Rapamune®

9.1.1.2 Classification: Immunosuppressive agent.

9.1.1.3 Mechanism of Action: Sirolimus binds to the FK Binding Protein-12 (FKBP-12). This complex binds to and inhibits the activation of the mammalian regulatory kinase, Target Of Rapamycin (mTOR). This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.

9.1.1.4 Molecular Weight: 914.2

#### 9.1.2 Storage Requirements

Sirolimus tablets should be stored at USP Controlled Room Temperature (68° to 77°F). Cartons should be used to protect blister cards and strips from light. Dispense tablets in a tight, light-resistant container as defined in the USP. Sirolimus (Rapamune) Oral Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days for the bottles). An amber syringe and cap are provided for dosing and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately. Rapamune Oral Solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

#### 9.1.3 Stability

Sirolimus tablets are stable through the manufacturer's expiration date imprinted on the product container.

#### 9.1.4 Administration

Sirolimus is to be administered orally consistently without food, at a dose and schedule defined by the protocol dose escalation schema. Study patients are not to ingest grapefruit, grapefruit juice or grapefruit juice containing products while on sirolimus therapy.

#### 9.1.5 Toxicity

Refer to FDA labeling for complete description of adverse events associated with Sirolimus. The most frequent adverse effects of sirolimus include peripheral edema, lymphocele, hyperlipidemia, hypercholesterolemia, tachycardia, venous thromboembolism, gastrointestinal disturbances, stomatitis, epistaxis, acne, rash, bone necrosis, arthralgia, hypokalaemia, and pyelonephritis. Please see the FDA labeling for complete description of adverse events associated with sirolimus.

9.1.5.1 Body as a Whole: abdomen enlarged, abscess, ascites, cellulitis, chills, face edema, flu syndrome, generalized edema, hernia, Herpes zoster infection, lymphocele, malaise, pelvic pain, peritonitis, sepsis, asthenia, fever, chills, malignancy, delayed wound healing has been reported in transplant patients with bronchial anastomotic dehiscence has occurred in lung transplant and in some cases fatal , bacterial infection, viral infection, anaphylactic/anaphylactoid reactions.

- 9.1.5.2 Cardiovascular: atrial fibrillation, hemorrhage, hypervolemia, hypotension, palpitation, postural hypotension, peripheral vascular disorder, syncope, thrombophlebitis, thrombosis, vasodilatation, venous thromboembolism, hypertension, tachycardia, congestive heart failure.
- 9.1.5.3 Digestive: anorexia, dysphagia, eructation, esophagitis, flatulence, gastritis, gastroenteritis, gingivitis, gum hyperplasia, ileus, liver function tests abnormal, mouth ulceration, oral moniliasis, stomatitis, diarrhea.
- 9.1.5.4 Heme and Lymphatic: ecchymosis, leukocytosis, lymphadenopathy, polycythemia, thrombotic thrombocytopenic purpura (hemolytic-uremic syndrome), lymphoproliferative disease, lymphoma, lymphadenopathy, anemia, leukopenia, thrombocytopenia, especially at higher doses, TTP.
- 9.1.5.5 Hepatic: hepatotoxicity, hepatic necrosis
- 9.1.5.6 Metabolic: acidosis, alkaline phosphatase increased, BUN increased, creatine phosphokinase increased, dehydration, healing abnormal, hypercalcemia, hyperglycemia, hyperphosphatemia, hypocalcemia, hypoglycemia, hypomagnesemia, hyponatremia, lactic dehydrogenase increased, AST/SGOT increased, ALT/SGPT increased, weight loss, hypercholesterolemia, hyperlipidemia, hypokalemia, edema, weight gain, Cushing's syndrome, diabetes mellitus, glycosuria.
- 9.1.5.7 Musculoskeletal: arthralgia, bone necrosis arthrosis, leg cramps, myalgia, osteoporosis, and tetany.
- 9.1.5.8 Nervous: anxiety, confusion, depression, dizziness, emotional lability, hypertonia, hypesthesia, hypotonia, insomnia, neuropathy, paresthesia, somnolence insomnia, tremor, and headache.
- 9.1.5.9 Respiratory: interstitial lung disease (pneumonitis, BOOP, pulmonary fibrosis) asthma, atelectasis, bronchitis, cough increased, epistaxis, hypoxia, lung edema, pleural effusion, pneumonia, rhinitis, and sinusitis.
- 9.1.5.10 Skin: acne, rash, skin cancer fungal dermatitis, hirsutism, pruritus, skin hypertrophy, skin ulcer, sweating.
- 9.1.5.11 Urinary: dysuria, urinary frequency albuminuria, bladder pain, hematuria, hydronephrosis, impotence, kidney pain, kidney tubular necrosis, nocturia, oliguria, pyelonephritis, pyuria, scrotal edema, testis disorder, toxic nephropathy, urinary frequency, urinary incontinence, urinary retention

9.1.5.12 Special Senses: abnormal vision, cataract, conjunctivitis, deafness, ear pain, otitis media, tinnitus.

9.1.5.13 Serious Toxicities include: Cases of interstitial lung disease (including pneumonitis, and infrequently bronchiolitis obliterans organizing pneumonia [BOOP] and pulmonary fibrosis), some fatal, with no identified infectious etiology have occurred in patients receiving immunosuppressive regimens including sirolimus. In some cases, the interstitial lung disease has resolved upon discontinuation or dose reduction of sirolimus. Alveolar hemorrhage has also been reported. Hepatotoxicity has been reported, including fatal hepatic necrosis with elevated sirolimus trough concentrations. Abnormal healing following transplant surgery has been reported, including fascial dehiscence and anastomotic disruption (e.g., wound, vascular, airway, ureteral, biliary).

9.1.5.14 Less frequently occurring adverse events included: mycobacterial infections, Epstein-Barr virus infections, and pancreatitis.

#### 9.1.6 Drug interactions

9.1.6.1 Sirolimus is a substrate for P-glycoprotein and gut and liver CYP3A4. The co-administration of a potent inhibitor or inducer of CYP3A4 may respectively increase or decrease sirolimus AUC. Inhibitors of CYP3A4 include (but are not limited to): amprenavir, atazanavir, bromocriptine, cimetidine, clarithromycin, clotrimazole, cyclosporine, danazol, diltiazem, erythromycin, fluconazole, fosamprenavir, other HIV protease inhibitors, indinavir, itraconazole, ketoconazole, metoclopramide, nefazodone, nelfinavir, nifedipine, nifedipine, ritonavir, saquinavir, telithromycin, troleandomycin (TAO), verapamil, and voriconazole. Inducers of CYP3A4 include: nevirapine, rifampicin, rifampin, rifabutin, rifapentin, phenytoin, carbamazepine, phenobarbital and St. John's Wort.

9.1.6.2 The use of live vaccines should be avoided in patients receiving sirolimus.

9.1.6.3 Grapefruit juice inhibits CYP3A4. Patients should not ingest grapefruit juice while on sirolimus therapy.

9.1.6.4 Angioneurotic edema-type reactions have been observed when sirolimus and an ACE inhibitor are given concurrently.

#### 9.1.7 Agent Ordering/Availability

9.1.7.1 Agent Ordering: Sirolimus will be procured via commercial mechanisms.

9.1.7.2 Availability: Sirolimus is available commercially as 1mg mg white triangular shaped tablets and 2 mg yellow to beige tablets, supplied in bottles of 100 tablets and cartons of 100 tablets (10 blister cards of 10 tablets each). Sirolimus is also available commercially as an oral solution, supplied at a concentration of 1 mg/mL in cartons which contain a 2 oz (60 mL fill) amber glass bottle. In addition to the bottles, each carton is supplied with an oral syringe adapter for fitting into the neck of the bottle, sufficient disposable amber oral syringes and caps for daily dosing, and a carrying case.

## 9.2 **Pemetrexed**

### 9.2.1 Product Description

9.2.1.1 Other Names: Alimta®

9.2.1.2 Classification: Antifolate antineoplastic.

9.2.1.3 Mechanism of Action: Pemetrexed inhibits the folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides, thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase.

9.2.1.4 Molecular Weight: 597.49

### 9.2.2 Storage Requirements

Pemetrexed for injection should be stored at 25°C with excursions permitted to 15-30°C (USP Controlled Room Temperature).

### 9.2.3 Stability

Reconstituted and infusion solutions of pemetrexed are stable for up to 24 hours following initial reconstitution, when stored refrigerated at 2-8°C (36-46°F), or at USP Controlled Room Temperature under ambient lighting.

### 9.2.4 Preparation

Infusion solutions of pemetrexed are to be prepared aseptically, following precautions recommended for the preparation of antineoplastic agents which limit exposure of the preparer to the drug. Each 500-mg vial of pemetrexed for injection is to be reconstituted with 20ml of 0.9% Sodium Chloride Injection (preservative free) to yield a solution containing 25mg/ml pemetrexed per ml. The desired dose of pemetrexed is to be further

diluted to 100ml with 0.9% Sodium Chloride Injection (preservative free). Unused pemetrexed is to be discarded.

#### 9.2.5 Administration

Pemetrexed is to be administered as an intravenous infusion over 10 minutes. Patients receiving pemetrexed must receive premedication with a corticosteroid, folic acid, and cyanocobalamin. Dexamethasone 4mg shall be taken twice daily for 3 days beginning the day prior to pemetrexed. Folic acid 1mg shall be taken by mouth every day, with least 5 daily doses taken during the 7-day period preceding the first dose of pemetrexed. Folic acid dosing shall continue during the full course of therapy and for 21 days after the last dose of pemetrexed. The patient shall receive one intramuscular injection of vitamin B12 1000mcg during the week preceding the first dose of pemetrexed, and day 1 of all subsequent cycles.

#### 9.2.6 Toxicity

Refer to the FDA-approved package insert provided with the drug for a complete description of adverse events associated with Pemetrexed.

9.2.6.1 Body as a Whole: Fatigue, fever, allergic reaction, and hypersensitivity.

9.2.6.2 Cardiovascular: Hypertension, edema, thromboembolism, and cardiac ischemia.

9.2.6.3 Digestive: Nausea, vomiting, stomatitis, constipation, diarrhea, anorexia.

9.2.6.4 Heme and Lymphatic: Myelosuppression.

9.2.6.5 Hepatic: Elevated hepatic enzymes.

9.2.6.6 Musculoskeletal: arthralgia, myalgia,

9.2.6.7 Nervous: Sensory Neuropathy, depression/mood alteration.

9.2.6.8 Renal: Decreased creatinine clearance.

9.2.6.9 Respiratory: Dyspnea

9.2.6.10 Skin: Rash, desquamation, alopecia

#### 9.2.7 Drug interactions

9.2.7.1 Daily ibuprofen doses of 400 mg qid reduce pemetrexed clearance by 20% and increase pemetrexed AUC by 20% in patients with normal renal function. Patients will be counseled to avoid all NSAIDS for 5 days prior to pemetrexed and 2 days afterward. Longer acting NSAIDS will be avoided for 5 days prior and 2 days afterward

9.2.7.2 Aspirin 325mg qid does not affect pemetrexed pharmacokinetics

### 9.2.8 Agent Ordering/Availability

9.2.8.1 Availability: Pemetrexed for injection is available commercially as a sterile lyophilized powder for reconstitution containing 500mg pemetrexed per vial.

9.2.8.2 Agent Ordering: Pemetrexed will be procured via commercial mechanisms.

### 9.3 **Dexamethasone**

Dexamethasone will be obtained from commercial sources. The FDA approved package insert provides information on the storage and administration of the agent. Possible side effects include euphoria or mood changes, headache, insomnia, edema, hypertension, glucose intolerance, acne, may be observed with dexamethasone given intermittently as in this protocol. Other side effects associated with longer chronic administration of dexamethasone include adrenal insufficiency, muscle weakness, Cushingoid state, and osteoporosis. This medication should be taken with food.

### 9.4 **Folic acid**

Folic acid will be obtained from commercial sources. The FDA approved package insert provides information on the storage and administration of the agent. Side effects from folic acid are not common. Allergic reaction manifested by rash, itching, swelling, dizziness, trouble breathing have all been reported.

### 9.5 **Cyanocobalamin**

Cyanocobalamin will be obtained from commercial sources. The FDA approved package insert provides information on the storage and administration of the agent. Side effects are generally rare but the following have been reported: Dermatologic: itching, rash, transitory exanthema, and urticaria have been reported. Gastrointestinal: diarrhea has been reported. Hematologic: peripheral vascular thrombosis has been reported. Treatment of vitamin B12 deficiency can unmask polycythemia vera, which is characterized by an increase in blood volume and the number of red blood cells. There may also be pain or redness associated with the injection.

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**11 APPENDICES**

**11.1 Appendix A: Table 3: Study Calendar**

Evaluation	Baseline	Cycle 1 Day 1	Cycle 1 Day 7	Cycle 1 Day 8	Day 14 & 21	C2 D1	C3 D1	Day 1 Every cycle	Every two cycles	Off study
Informed consent	X									
Medical History/ Demographics	X <sup>a</sup>									
Inclusion/Exclusion Criteria	X <sup>a</sup>									
PE (VS, Ht, Wt, Performance status, narcotic use, meds)	X <sup>a</sup>	X	X			X <sup>k, l</sup>	X <sup>k, l</sup>	X <sup>k, l</sup>		X
Adverse Events	X	X	X	X	X	X	X	X		X
EKG	X <sup>b</sup>									
CXR	X <sup>b</sup>									
Hematology profile CBC with differential <sup>n</sup>	X <sup>a</sup>		X		X	X <sup>k, l</sup>	X <sup>k, l</sup>	X <sup>k, l</sup>		X
Biochemical Profile <sup>c</sup>	X <sup>a</sup>		X			X <sup>k, l</sup>	X <sup>k, l</sup>	X <sup>k, l</sup>		X
Coagulation Profile (PT, APTT)	X <sup>a</sup>		X			X <sup>k, l</sup>	X <sup>k, l</sup>	X <sup>k, l</sup>		X
Lipid Profile (fasting chol panel &	X <sup>a</sup>		X			X <sup>k, l</sup>	X <sup>k, l</sup>	X <sup>k, l</sup>		X

Evaluation	Baseline	Cycle 1 Day 1	Cycle 1 Day 7	Cycle 1 Day 8	Day 14 & 21	C2 D1	C3 D1	Day1 Every cycle	Every two cycles	Off study
triglycerides)										
Vitamin Profile (B12 and folate)	X <sup>a</sup>					X <sup>k,l</sup>	X <sup>k,l</sup>	X <sup>k,l</sup>		
Urinalysis	X <sup>a</sup>		X <sup>k</sup>			X <sup>k,l</sup>	X <sup>k,l</sup>	X <sup>k</sup>		
pregnacy test	X <sup>a</sup>					X <sup>k,l</sup>	X <sup>k,l</sup>	X <sup>k,l</sup>		
Lymphocyte subsets	X <sup>a</sup>					X <sup>k,l</sup>	X <sup>k,l</sup>	X <sup>k,l</sup>		
Trough sirolimus levels			X		X	X <sup>k,l</sup>	X <sup>k,l</sup>	X <sup>k,l</sup>		
CT scans: Chest Abd Pelvis	X <sup>b</sup>						X <sup>k,m</sup>		X <sup>k</sup>	
PET/ CT scan	X <sup>h</sup>						X <sup>k</sup>			
Akt/mTOR pathway activation Tumor (T) or PBMC (P)	X T, P, P <sub>d</sub>		X P				X T, P <sub>d</sub>		X P	
Pharmacokinetics			X <sup>l</sup>	X <sup>l</sup>						
Start daily sirolimus <sub>e</sub>		X								
Start Pemetrexed infusions Q 21 days				X		X	X	X		

Evaluation	Baseline	Cycle 1 Day 1	Cycle 1 Day 7	Cycle 1 Day 8	Day 14 & 21	C2 D1	C3 D1	Day1 Every cycle	Every two cycles	Off study
Start vitamin B12 folate <sup>f</sup>	X									
Start folate <sup>g</sup>	X									

a = if done within 17 days of initiation of therapy may count as baseline (pregnancy test within 7 days)

b = if done within 28 days of initiation of therapy may count as baseline

c = includes Mg, Na, K, Cl, HCO<sub>3</sub>, Ca, PO<sub>4</sub>, Cr, BUN, Glu, AST, ALT, T bili, Alk phos, alb, T protein, LDH, amylase, lipase

d = Akt activation in tumor determines eligibility for phase II subjects (testing required)

e = administered once a day every day of the cycle on an empty stomach

f = 1000µg first dose within 7 days before pemetrexed initiated then q 63 days

g = 1mg starting within 7 days before pemetrexed initiated, then administered continuously

h = must be performed within 28 days of day 1 cycle 1

i = for sirolimus pre dose, 20min, 40min, 1h, 2, 3h, 4h, 6h, 12h, 24h AFTER SIROLIMUS

j = for both sirolimus and pemetrexed, pre dose 20min, 40min, 1h, 2, 3h, 4h, 6h, 12h, 24h  
AFTER PEMETREXED

k = within 72 hours of the start of each cycle

l = repeated for toxicity follow up on day 7 or day 14 of any cycle in which dose reduction took place on day 1

m = after two cycles of treatment or at time of progression, whichever ever occurs first

n = CBC twice weekly for subjects with Grade 3 or 4 neutropenia, until resolution

11.2 Appendix B: Table 4: Performance Status Criteria

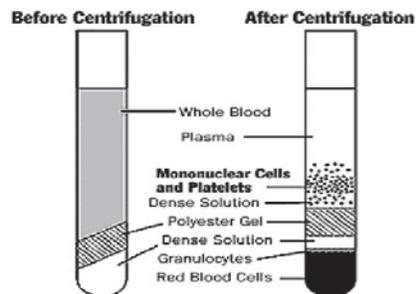
ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or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

### 11.3 Appendix C: Standard Operating Procedure for Isolating PBMCs

The following protocol will be used to isolate PBMCs

Using Vacutainer CPT collection tube (BD), with sodium heparin

Note: This tube contains a Ficoll gradient that will separate cell layers upon centrifugation. The cell layers are as follows..



Collect blood in CPT tube. Spin tube down at ROOM TEMP (18-25°C) @ 3000 rpm (6<sup>th</sup> floor centrifuge) for 30 min.

To calculate rpm: Measure radius of centrifuge/rotor. Desired RCF (relative centrifugal force) is ~ 1500-1800. Enter these values on BD website and it will calculate for you what the corresponding rpm is.

<http://www.bd.com/vacutainer/products/molecular/heparin/procedure.asp>

PBMC layer will be a cloudy layer (~0.5-1 inches tall) right above the polyester gel plug. The height and density of the layer will vary depending on the WBC count of each patient.

If plasma is desired- remove the TOP few mls of plasma carefully, and store at -80C in several eppendorf tubes.

To isolate PBMCs- Remove the remaining plasma (including the PBMC layer) and place in a 15 ml conical tube. Fill the tube to the top with 1X PBS. Be careful not to aspirate any of the polyester gel, as this will pellet with the PBMCs.

Pellet PBMCs by spinning at 1500-2000 rpm for 5-10 minutes.

Aspirate PBS from pellet. Lyse in 2X LSB with protease inhibitors. Store @ -80°C.

#### 11.4 **Appendix D: Standard Operating Procedure for Tumor Tissue Acquisition and Storage**

After OR specimen is obtained, it should be divided in half. One sample is sent to Pathology for routine histopathologic examination, the other is for research.

One half will be fixed in formalin and processed for immunohistochemistry. Plastic jar containing 10mL formalin (Sigma) will be provided.

Processing for IHC includes paraffin-embedding of tissue specimen, and subsequent sectioning onto charged glass slides. Approximately 20 sections will be obtained for each specimen, and the remainder saved in paraffin-embedded blocks. Histoserv® (Germantown, MD) will perform these processes.

Blocks and slides will be stored at NNMC, Building 8, 4<sup>th</sup> floor, Room 4151 in cabinets marked “clinical specimens”. Samples will have numbers corresponding to protocol and patient ID #.

One half will be flash-frozen in a bath of dry ice and 100% ethanol, and transported back to NNMC to be stored at -135°C.

Sample will remain frozen until it is processed for immunoblotting. Processing includes thawing sample and immersing in 2X LSB (lysis buffer), and using a standard Tissue Tearor ® (tissue homogenizer) to make a lysate.

Lysates obtained from each sample will be aliquoted and stored in Eppendorf microfuge tubes (1.5mL), labeled with protocol and patient ID #.

Lysates will be stored at NNMC, Building 8, 6<sup>th</sup> floor, freezer room (at end of hall, no room #), in freezer # 4 (open-top freezer, -135°C), box will be labeled with protocol #.

### 11.5 Appendix E: Table 5: Drugs with potential CYP3A4 interactions

#### CYP3A4 Substrates

Albuterol	Dihydroergotami ne	Isradipine	Quinidine
Alfentanil	Diltiazem	Itraconazole	Rabeprazole
Alprazolam	Disopyramide	Ketamine	Ranolazine
Amiodarone	Docetaxel	Ketoconazole	Repaglinide
Amlodipine	Doxepin	Lansoprazole	Rifabutin
Amprenavir	Doxorubicin	Letrozole	Ritonavir
Aprepitant	Doxycycline	Levonorgestrel	Salmeterol
Aripiprazole	Efavirenz	Lidocaine	Saquinavir
Atazanavir	Eletriptan	Losartan	Sibutramine
Atorvastatin	Enalapril	Lovastatin	Sildenafil
Benzphetamine	Eplerenone	Medroxyprogester one Mefloquine	Simvastatin
Bisoprolol	Ergoloid mesylates	Mestranol	Sirolimus
Bortezomib	Ergonovine	Methadone	Spiramycin
Bosentan	Ergotamine	Methylergonovine	Sufentanil
Bromazepam	Erythromycin	Methysergide	Sunitinib
Bromocriptine	Escitalopram	Miconazole	Tacrolimus
Budesonide	Estradiol	Midazolam	Tamoxifen
Buprenorphine	Estrogens, conj., synthetic	Miglustat	Tamsulosin
Buspirone	Estrogens, conj., equine	Mirtazapine	Telithromycin
Busulfan	Estrogens, conj., esterified	Modafinil	Teniposide
Carbamazepine	Estrone	Montelukast	Tetracycline
Cerivastatin	Estropipate	Moricizine	Theophylline
Chlordiazepoxide	Ethinyl estradiol	Nateglinide	Tiagabine
Chloroquine	Ethosuximide	Nefazodone	Ticlopidine
Chlorpheniramine	Etoposide	Nelfinavir	Tipranavir
Cilostazol	Exemestane	Nevirapine	Tolterodine
Cisapride	Felbamate	Nicardipine	Toremifene
Citalopram	Felodipine	Nifedipine	Trazodone
Clarithromycin	Fentanyl	Nimodipine	Triazolam
Clobazam	Flurazepam	Nisoldipine	Trimethoprim
Clonazepam	Flutamide	Norethindrone	Trimipramine
Clorazepate	Fluticasone	Norgestrel	Troleandomycin
Cocaine	Fosamprenavir	Ondansetron	Vardenafil
Colchicine		Paclitaxel	
Conivaptan		Pergolide	
Cyclophosphamide		Phencyclidine	
		Pimozide	

de Cyclosporine Dantrolene Dapsone Dasatinib (1) Delavirdine Diazepam	Gefitinib Haloperidol Ifosfamide Imatinib Indinavir Irinotecan Isosorbide Isosorbide dinitrate Isosorbide mononitrate	Pipotiazine Primaquine Progesterone Quetiapine	Venlafaxine Verapamil Vinblastine Vincristine Vinorelbine Zolpidem Zonisamide Zopiclone
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