DF/HCC Protocol #: 19-814

TITLE: A phase 2 study of zafirlukast for the treatment of tumor-marker only relapsed ovarian cancer

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IND Sponsor: Rushad Patell, M.D.

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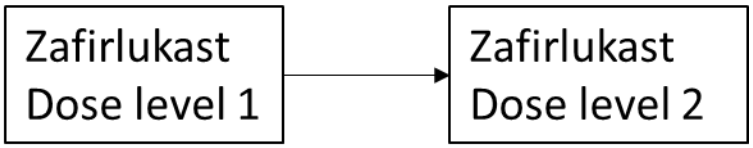
SCHEMA



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1. OBJECTIVES

1.1 Study Design

This is a single-arm Simon two-stage phase 2 clinical trial to evaluate the dual antithrombotic and antineoplastic activity of zafirlukast in tumor marker-only relapsed ovarian cancer.

1.1 Primary Objective

The primary objective is to determine efficacy of zafirlukast in terms of CA-125 response rate per Gynecologic Cancer Intergroup (GCIG) criteria.

1.2 Key Secondary Objective

Assess changes in CA-125 doubling time in the 3 months prior to enrollment and following initiation of zafirlukast

1.3 Exploratory Objectives

- Measure changes PDI and ERp57 inhibitory activity in plasma
- Measure changes in platelet-dependent thrombin generation
- Determine progression free survival (PFS)
- Assess the incidence of venous thromboembolism
- Assess incidence of major hemorrhage and clinically relevant non-major bleeding

2. BACKGROUND

2.1 Study Disease(s)

Ovarian Cancer

Epithelial ovarian cancer is the seventh most common malignancy among women with 230,000 women diagnosed annually. The rate of survival is poor, with an approximately 50% survival at 5 years following diagnosis [1]. Debulking surgery is considered front-line treatment for women with ovarian cancer with neoadjuvant and/or adjuvant chemotherapy administered for more advanced disease. Intravenous administration of carboplatin and paclitaxel is the standard first-line chemotherapy for advanced ovarian cancer. Two randomized trials demonstrated improved progression free survival with the addition of maintenance bevacizumab in high-risk stage III disease without improved overall survival [2, 3], although more recent work has called these findings into question [4]. More recently, poly ADP ribose polymerase (PARP) inhibitors have shown improved progression free survival as maintenance therapy in the first line setting for all patients, though patients with BRCA-mutated ovarian cancer gained the most benefit[5, 6]. PARP inhibitors are approved for all patients after platinum-based chemotherapy with a platinum sensitive relapse [7-9], and those patients with BRCA-mutated ovarian cancer have improved overall survival with this approach[10],

Monitoring for recurrence of ovarian cancer following initial therapy includes serial measurement of the tumor marker CA-125. Tumor marker-only relapse is defined as the absence of recurrent disease by imaging or symptoms along with a rising CA-125, i.e. CA-125 more than twice the upper limit of normal (35 U/mL) in the setting of a normal baseline CA-125 levels or CA-125 greater than twice the nadir count on two successive measurements for CA-125 values that remain above baseline. A randomized study evaluated the benefit of early versus delayed chemotherapy in patients with tumor marker-only relapse of ovarian cancer [11]. Early treatment was started within 28 days of CA-125 increase while delayed treatment was started in response to clinical or symptomatic relapse. There was no difference in overall survival between the arms. In light of these results, observation is currently standard-of-care for tumor marker-only relapsed ovarian cancer. For patients desiring systemic treatment, endocrine therapy is typically preferred given reduced toxicity compared to cytotoxic chemotherapy. This approach has been observed to result in a 17% response rate in CA-125 per GCIG criteria in patients with estrogen receptor-positive ovarian cancer [12]. However, it has not been shown to improve progression-free or overall survival compared to observation in this population.

Venous Thromboembolism and Ovarian Cancer

Venous thromboembolism (VTE) is commonly observed in cancer patients. It is a leading cause of morbidity and is associated with increased mortality in this population [13, 14]. In high-risk cancer patients especially where protocol-driven radiographic monitoring for deep vein thrombosis is implemented, the incidence of VTE within the initial few months of chemotherapy often exceeds 15% [15-17]. Ovarian cancer is among the most prothrombotic malignancies. According to Medicare discharge data, ovarian cancer was associated with the highest incidence of cancer-associated VTE [18]. Similarly, in large registries from California (528,693 adults) and the Netherlands (66,329 adults), the incidence of VTE in ovarian cancer patients exceeded essentially that of all other malignancies including pancreatic cancer and malignant glioma [19, 20]. Patients with advanced malignancies are also at an increased risk of bleeding [21]. Notably, apixaban, a direct oral anticoagulant, was recently shown to be efficacious in reducing VTE in cancer patients but were also associated with a doubling in rate of major hemorrhage [22]. Developing antithrombotics that reduce the incidence of VTE without increasing the risk of major hemorrhage would broadly impact the care of patients with advanced malignancy.

Protein Disulfide Isomerase

PDI is a member of a large family of thiol isomerase/disulfide oxidoreductases exist in humans [23]. These thiol isomerases are generally capable of oxidation reduction and isomerization reactions. Recent studies have determined that a subset of thiol isomerases, including PDI, ERp5, ERp57, and ERp72 have extracellular activity, which play important roles in both arterial and venous thrombosis as well as in a variety of cancers. PDI, ERp5, ERp57, and ERp72 are secreted

by platelets and reattach to the plasma membrane, where they function as extracellular oxidoreductases [24-27]. They are required for thrombus formation and fibrin formation [26, 28-31], as well as for platelet aggregation, dense granule secretion, fibrinogen binding, and calcium mobilization [25].

Thiol isomerases are also upregulated in many distinct cancer types, including ovarian, prostate, lung, melanoma, lymphoma, and glioma, while inhibition of PDI is cytotoxic in ovarian cancer and multiple myeloma cell lines [29, 32]. Increased levels of thiol isomerases have been positively correlated increased oncogenic transformation [33], gene transcription [34], metastasis [35], and even promoting resistance to chemotherapy [36] and radiation [37]. Similar to its role in platelets, thiol isomerases can also be secreted from tumor cells and perform functions on the cell surface [38].

In the context of thromboembolic disease, PDI is released from activated platelets and endothelial cells and is thought to modulate through oxidation, reduction, or isomerization a number of extracellular substrates, the best characterized being vitronectin [29, 39-43]. Targeting PDI activity with blocking antibodies or small molecules prevents both platelet accumulation and fibrin generation at the site of vascular injury in several distinct animal models of thrombosis [28, 30, 39, 44, 45].

Several small molecule inhibitors of PDI have been identified. In a pharmacokinetic/pharmacodynamics study conducted in healthy volunteers, isoquercetin inhibited PDI activity in plasma and reduced platelet-dependent thrombin generation in a dose and time-dependent manner [41]. In a recently completed a phase II clinical trial in advanced cancer patients demonstrating that daily isoquercetin significantly reduced coagulation markers of the thrombosis (i.e. D-Dimer and thrombin generation) and prevented the development of VTE (Figure 1). Isoquercetin was well tolerated without any attributable significant adverse events reported and no major hemorrhages observed [46].

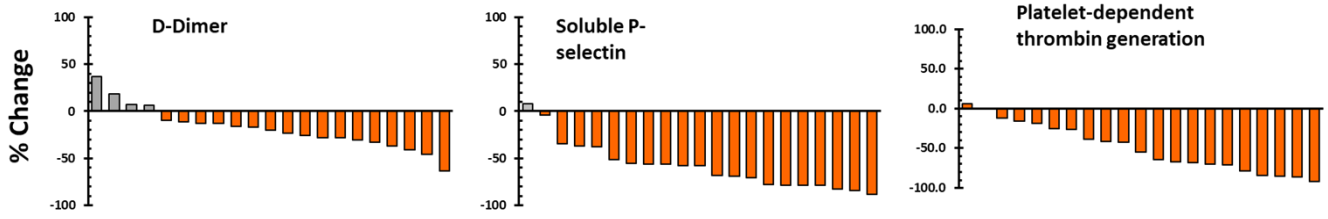

Figure 1. The PDI inhibitor, isoquercetin, reduces the hypercoagulability of advanced malignancy. Patients with advanced lung, colorectal, and pancreatic cancer were treated with isoquercetin 1000 mg daily for 2 months. Shown in figure are the waterfall plots for comparison with baseline measurements. A) D-dimer plasma concentrations decreased by a median of 20% equating to a median decrease of 206 ng/ml fibrinogen equivalent units (P<0.001). B) Median decrease of circulating soluble P-selectin was 58% (P<0.001) and C) platelet-dependent thrombin generation decreased by 55% (P=0.006).

Zafirlukast was recently identified through a small molecule screen as an inhibitor of PDI along with other thiol isomerases including ERp5, ERp57, ERp72. Similar to isoquercetin, zafirlukast reduces platelet aggregation and fibrin generation in a dose-dependent manner as shown in Figure 2.

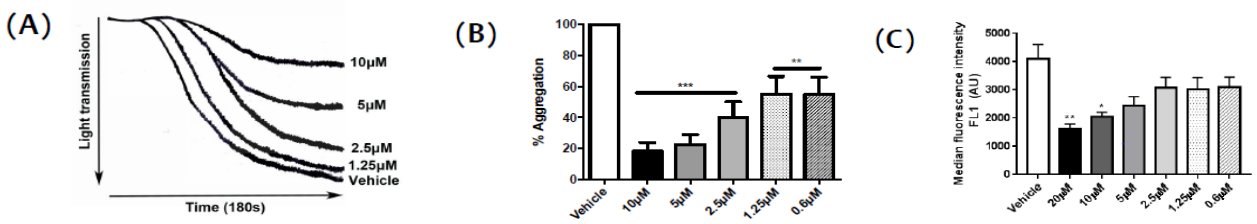

Figure 2 – (A) Washed human platelets were incubated with ZFL or vehicle for 5 minutes prior to stimulation with collagen for 180 seconds in an optical aggregometer. (B) Data were normalized to vehicle and % inhibition of aggregation (mean + SEM) calculated, n=12. (C) Fibrinogen binding was measured by flow cytometry. Platelets were incubated with vehicle or ZFL (0.6- 20μM) for 5 minutes prior to stimulation with 0.25μg/mL CRP-XL n=6. Graphs represent mean ± SEM. Data was analyzed by one-way ANOVA *p<0.05, **p<0.01, ***p<0.005.

The expression of PDI and other thiol isomerases are increased in ovarian cancer and increased PDI expression has been associated with disease progression and shortened survival [47]. Small molecule inhibitors of PDI such as propynoic acid carbomyl methyl amides (PACMA) have been shown to reduce tumor growth in human ovarian cancer mouse xenografts [48]. Zafirlukast inhibits ovarian cancer cell growth as shown in Figure 3 in a dose-dependent manner.

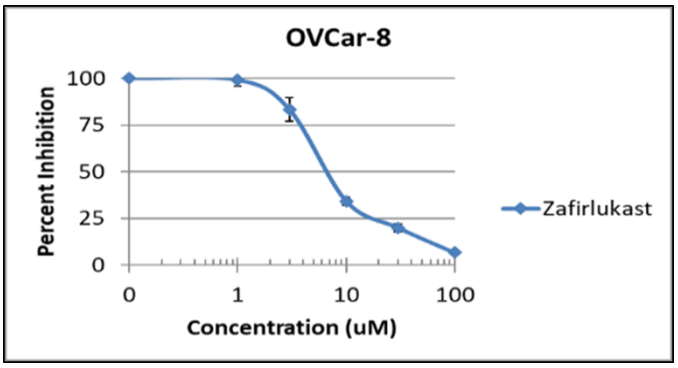

Figure 3 – The effect of Zafirlukast on cancer cell viability was determined by the Prestoblue assay. Cells were seeded at 3,000 cells/well in a 96 well plate and drug treated 24 hours later. After a 24 hour incubation, cell viability was determined per the manufacturer's instructions.

Updated results of trial in women with tumor-marker only relapsed ovarian cancer:
We enrolled a total of four women who received zafirlukast 40 mg twice daily and performed serial CA-125 measurements to assess for tumor response. None of the women demonstrated a decrease in CA-125 following the initiation of zafirlukast. However, in all 4 women the rate of rise of CA-125 was reduced following treatment with zafirulast (Figure 4). Accordingly, the mean change in CA-125 was 0.036 U/mL per day prior to treatment compared with 0.015 U/mL per day following zafirlukast (paired t-test p=0.026). This equated to a mean CA-125 doubling time of 29.8 days in the weeks prior to treatment compared with a mean doubling time of 85.69 days following treatment. There were no grade 3 or higher toxicities observed on study. As shown in Figure 4B, there was only modest inhibition of PDI activity in plasma as measured by Di-eosin-

GSSG assay. In the study subject who demonstrated the greatest PDI inhibition there was the CA-125 doubling was most pronounced. These data suggest that higher doses of zafirlukast may provide additional PDI inhibitory activity and further inhibit ovarian cancer cell growth.

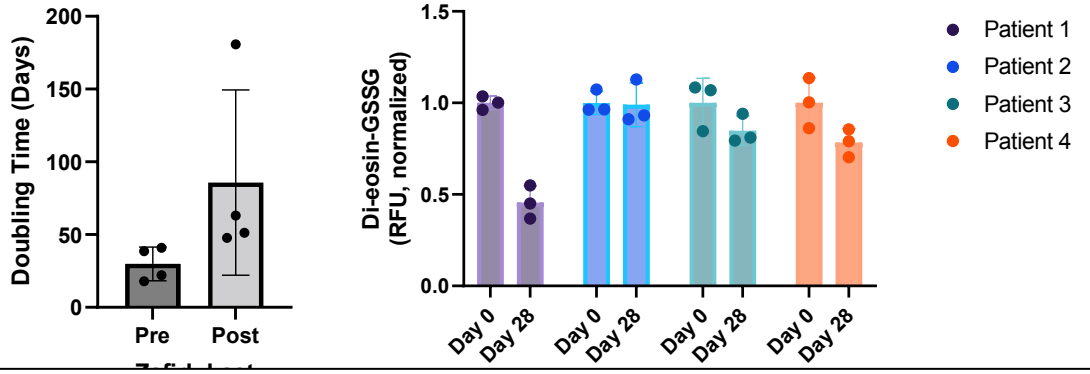

Figure 4. CA-125 doubling time following zafirlukast in women with ovarian cancer. A) Comparison of CA-125 doubling time before and after start of zafirlukast. B) Inhibition of plasma PDI activity before and 28 days following initiation of zafirlukast.

2.2 IND Agent

2.2.1 Zafirlukast

Zafirlukast (Accolate) is FDA approved for the treatment of asthma in children and adults. Its effectiveness in asthma is attributed to its activity as a competitive receptor antagonist to leukotriene D4 and E4[49].

Zafirlukast is rapidly absorbed following oral administration and 99% is bound to plasma protein in circulation. Zafirlukast is extensively metabolized in the liver. Hydroxylated metabolites are excreted in the feces and formed through cytochrome P450 2C9 pathway. In patients with biopsy-proven cirrhosis, the clearance of zafirlukast is 50-60% slower compared to healthy subjects. There is no apparent effect on pharmacokinetic profile in renal insufficiency.

While the FDA approved dose of zafirlukast is 20 mg twice daily, doses up to 80 mg twice daily appear well tolerated. In a randomized clinical study with over 360 patients to assess asthma response, observed side effects were similar in cohort receiving zafirlukast 80 mg twice daily compared to placebo for 6 weeks [50]. Serious adverse events were reported in two patients receiving zafirlukast (e.g. detached retina and exacerbation of asthma). Two patients developed transient increase in liver transaminases which resolved in one patient despite continuing therapy and another after withdrawal of treatment.

Following oral ingestion of zafirlukast 40 mg the peak plasma concentration is ~3 μM which approximates the IC50 for both inhibition of cell viability, *ex vivo* platelet inhibition and PDI inhibitory activity [51].

2.3 Rationale

The rationale to evaluate zafirluast as a dual phase 2 study in this population are several-fold. First, zafirlukast and other PDI inhibitors have demonstrated anti-tumor activity in preclinical ovarian cancer models [48]. PDI and ERp57 are both upregulated in ovarian cancer and correlate with tumor progression [47]. Women with ovarian cancer following surgery and adjuvant chemotherapy often relapse with tumor marker only (i.e. elevation of CA-125) in the absence of measurable disease by radiographic imaging. Patients are typically monitored without therapy in this setting and are a stated unmet need for a chemotherapeutic with favorable toxicity profile [52]. Finally, ovarian cancer is one of the most prothrombotic malignancies [19] and PDI inhibition has been shown to reduce the hypercoagulability associated with advanced cancer.

3. PARTICIPANT SELECTION

Laboratory tests required for eligibility must be completed within 14 days prior to the date of registration. Baseline measurements must be documented from tests within 14 days of the date of registration for protocols requiring measurable disease. Diagnostic tests, such as CT scans, must be performed within 4 weeks of the date of registration.

3.1 Eligibility Criteria

3.1.1 Participants must have histologically confirmed epithelial ovarian, fallopian tube, or primary peritoneal cancer.

3.1.2 Participants must have completed at least first-line platinum based chemotherapy and surgery with a response, in the opinion of the investigator, defined as no evidence of disease progression or rising CA-125 at any time during front-line treatment.

3.1.3 Participants must meet criteria for tumor marker-only relapse, defined as CA-125 more than twice the upper limit of normal (35 U/mL) in the setting of a normal baseline CA-125 levels or CA-125 greater than twice the nadir count on two successive measurements for CA-125 values that remain above baseline without measurable radiographic disease.

3.1.4 Minimum age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of zafirlukast in participants under 18 years of age with ovarian cancer, children are excluded from this study but will be eligible for future pediatric trials.

3.1.5 Life expectancy of greater than 4 months.

3.1.6 ECOG performance status ≤ 2 (Karnofsky ≥ 60%, see Appendix A).

3.1.7 Participants must be able to swallow tablets.

3.1.8 Participants must have adequate organ and marrow function as defined below:

- Absolute neutrophil count ≥1,000/mcL

- Platelets ≥90,000/mcL
- Total bilirubin ≤ 1.3 × institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT) ≤ 2 × institutional ULN
- Creatinine ≤ institutional ULN
  OR
- Glomerular filtration rate (GFR) ≥45 mL/min/1.73 m^2

3.1.9 The effects of zafirlukast on the developing human fetus are incompletely characterized. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men are not eligible for this study.

3.1.10 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Non-epithelial tumors (pure sarcomas) or ovarian tumors with low malignant potential (ie borderline tumors) or mucinous tumors. Mixed mullerian tumors or carcinosarcomas are allowed.

3.2.2 Participants who have had cytotoxic chemotherapy including bevacizumab or radiotherapy within 4 weeks prior to entering the study. This does not include maintenance therapy with a PARP inhibitor, such as olaparib or niraparib. (PARP inhibitor, rucaparib is not allowed to be co-administed with CYP2C9 substrates as maintenance therapy as it could increase exposure to zafirlukast).

3.2.3 Participants who have ongoing adverse effects from prior anti-cancer therapy greater than National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, v5.0) Grade 1, with the exception of Grade 2 non-hematologic toxicity such as alopecia and peripheral neuropathy.

3.2.4 Participants who are receiving any other investigational agents.

3.2.5 Participants with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to zafirlukast.

3.2.7 Currently receiving anticoagulant therapy.

3.2.8 Current daily use of aspirin (> 81 mg daily), clopidogrel (Plavix), cilostazol (Pletal), aspirin-dipyridamole (Aggrenox) (within 10 days) or considered to use regular use of higher doses of non-steroidal anti-inflammatory agents as determined by the treating physician (e.g. ibuprofen > 800 mg daily or equivalent).

3.2.9 Participants receiving any medications or substances that are inhibitors or inducers of CYP2C9 are ineligible. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently updated medical reference. As part of the enrollment/informed consent procedures, the participant will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the participant is considering a new over-the-counter medicine or herbal product.

3.2.10 Participants with uncontrolled intercurrent illness.

3.2.11 Participants with psychiatric illness/social situations that would limit compliance with study requirements.

3.2.12 Pregnant women are excluded from this study because zafirlukast is a class B agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with zafirlukast, breastfeeding should be discontinued if the mother is treated with zafirlukast.

3.3 Inclusion of Women and Minorities

The study is open to any individual who meets above eligibility criteria without discrimination based on race or gender. We do not anticipate that inclusion or exclusion criteria will negatively affect recruitment or retention of underrepresented minorities.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal

Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

1.1 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

1. TREATMENT PLAN

1.1 Treatment Regimen

Treatment will be administered on an outpatient basis every 4 weeks, with 28 consecutive days defined as a treatment cycle. Expected toxicities and potential risks as well as dose modifications for zafirlukast are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification).

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Zafirlukast	None	40 mg	Oral tablets	Twice daily	28 days

The first cohort of 4 participants will receive zafirlukast 40 mg (two 20 mg tablets) twice daily. In the absence of drug related serious adverse events (grade 3 or 4 possibly or probably related), an additional cohort of 4 patients will be enrolled at 80 mg twice daily.

Participants will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle.

1.2 Pre-Treatment Criteria

5.1.1. Cycle 1, Day 1

- Laboratory assessments recorded within 14 days of signing consent may be used to determine eligibility and does not have to be repeated (except complete blood count)
- Absolute neutrophil count > 1,000/mcL
- Platelet count > 90,000/mcL
- Participants must be able to tolerate PO tablets

5.1.2. Subsequent Cycles

- Toxicities possibly related to zafirlukast must return to grade 2 or less

1.3 Agent Administration

Zafirlukast will be supplied as oral tablets.
Cohort 1: Two tablets (total of 40 mg) will be consumed twice daily.
Cohort 2: Four tablets (total of 80 mg) will be consumed twice daily.

Dose modifications will not be made. Tablets will be dispensed from the research pharmacy on day 1 of each cycle. The tablets will be packaged as a 30-day supply (to allow for unexpected occurrences such as dropped tablet).

Tablets should be taken without food. Food decreases the bioavailability of zafirlukast by 40%. Therefore, participants will be instructed to take zafirlukast on an empty stomach, 1 hour before and 2 hours after meals. Missed doses must be taken within four hours of scheduled dose. If longer than four hours, or if dose vomited, skip dose. Subjects will keep a drug diary that will be provided to them by the study team. Subjects will be instructed to return any unused drug. The unused returns will be counted by pharmacy and destroyed on-site per policy.

1.4 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of zafirlukast with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Overall PI should be alerted if the participant is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Appendix B presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

1.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will be ongoing (+/- 7 days inclusion of windows) for up to 12 cycles or until one of the following criteria apply:

- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the study, or general or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator
- Clinical or radiographic progression of ovarian cancer
- Doubling the pre-treatment CA-125 value (confirmed at least 1 week apart)
- Grade 3 or 4 hemorrhage and/or symptomatic bleeding in a critical area or organ such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or bleeding causing a fall in hemoglobin level > 2 g/dL or bleeding leading to a transfusion of > 2 units of packed red blood cells

Participants will be removed from the protocol therapy when any of the criteria listed above applies. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Rushad Patell at 617-667-9920.

1.6 Duration of Follow Up

Following completion of treatment participants will be followed for a final 30 days post last dose visit. All AEs and SAEs will be collected during this time. After the completion of the 30 day post last dose follow up, participants may come off study. Participants removed from study treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Participants who have related unresolved AEs should be followed until resolution of the adverse event or up to 30 days post last dose, whichever occurs later. Participants removed from active treatment for reasons listed in section 1.5, will be followed for 30 days post last dose, prior to coming off study.

1.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Completed protocol defined procedures and follow-up
- Lost to follow-up
- Withdrawal of consent for data submission

Death
The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1

2. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Thrombocytopenia	**Management/Next Dose for** Zafirlukast
≤ Grade 1	No change in dose
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Do not restart

ALT/AST	**Management/Next Dose for** Zafirlukast
≤ Grade 1	No change in dose
Grade 2	Hold until within normal range. Resume at same dose level.
Grade 3	Do not restart
Grade 4	Do not restart

Bilirubin	**Management/Next Dose for** Zafirlukast
≤ Grade 1	No change in dose
Grade 2	Hold until within normal range. Resume at same dose level.
Grade 3	Do not restart.
Grade 4	Do not restart

3. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

3.1 Expected Toxicities

3.1.1 Adverse Event List(s) for Zafirlukast

Headache 12.9%
Infection 3.5%
Nausea 3.1%
Diarrhea 2.8%
Pain (generalized) 1.9%
Asthenia 1.8% 1.6%
Abdominal Pain 1.8%
Dizziness 1.6%
Myalgia 1.6%
Fever 1.6%
Back Pain 1.5%
Vomiting 1.5%
Liver function testing elevation 1.5%
Dyspepsia 1.3%

Rarely, elevations of one or more liver enzymes have occurred in patients receiving zafirlukast in controlled clinical trials. In clinical trials, most of these have been observed

at doses four times higher than the recommended dose. The following hepatic events (which have occurred predominantly in females) have been reported from post-marketing adverse event surveillance of cases of symptomatic hepatitis (with or without hyperbilirubinemia) without other attributable cause; and rarely, hyperbilirubinemia without other elevated liver function tests. In most, but not all post-marketing reports, the patient's symptoms abated and the liver enzymes returned to normal or near normal after stopping zafirlukast. In rare cases, patients have presented with fulminant hepatitis or progressed to hepatic failure, liver transplantation and death

3.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

3.3 Adverse Event Reporting

3.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

3.3.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

3.3.3 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

3.4 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

3.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

4. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational administered in this study can be found in Section 7.1.

4.1 Zafirlukast

4.1.1 Description

Zafirlukast is a synthetic, selective peptide leukotriene receptor antagonist (LTRA), with the chemical name 4-(5-cyclopentyloxycarbonylamino-1-methyl-indol-3-ylmethyl)-3-methoxy-N-o-tolylsulfonylbenzamide.

Zafirlukast is rapidly absorbed following oral administration. Peak plasma concentrations are generally achieved 3 hours after oral administration. The absolute bioavailability of zafirlukast is unknown. In two separate studies, one using a high fat and the other a high protein meal, administration of zafirlukast with food reduced the mean bioavailability by approximately 40%.

Zafirlukast is more than 99% bound to plasma proteins, predominantly albumin. The degree of binding was independent of concentration in the clinically relevant range. The apparent steady-state volume of distribution is approximately 70 L, suggesting moderate distribution into tissues. Studies in rats using radiolabeled zafirlukast indicate minimal distribution across the blood-brain barrier.

Zafirlukast is extensively metabolized. The most common metabolic products are hydroxylated metabolites which are excreted in the feces. The metabolites of zafirlukast identified in plasma are at least 90 times less potent as LTD4 receptor antagonists than zafirlukast in a standard in vitro test of activity. In vitro studies using human liver microsomes showed that the hydroxylated metabolites of zafirlukast excreted in the feces are formed through the cytochrome P450 2C9 (CYP2C9) pathway. Additional in vitro studies utilizing human liver microsomes show that zafirlukast inhibits the cytochrome P450 CYP3A4 and CYP2C9 isoenzymes at concentrations close to the clinically achieved total plasma concentrations.

The apparent oral clearance (CL/f) of zafirlukast is approximately 20 L/h. Studies in the rat and dog suggest that biliary excretion is the primary route of excretion. Following oral administration of radiolabeled zafirlukast to volunteers, urinary excretion accounts for approximately 10% of the dose and the remainder is excreted in feces. Zafirlukast is not detected in urine.

In the pivotal bioequivalence study, the mean terminal half-life of zafirlukast is approximately 10 hours in both normal adult subjects and patients with asthma. In other studies, the mean plasma half-life of zafirlukast ranged from approximately 8 to 16 hours in both normal subjects and patients with asthma. The pharmacokinetics of zafirlukast are approximately linear over the range from 5 mg to 80 mg. Steady-state plasma concentrations of zafirlukast are proportional to the dose and predictable from single-dose pharmacokinetic data. Accumulation of zafirlukast in the plasma following twice-daily dosing is approximately 45%.

4.1.2 Form

Zafirlukast occurs as a fine white to pale yellow amorphous powder. It is supplied in 20 mg tablets for oral administration. Film-coated tablet consist of croscarmellose sodium, lactose, magnesium stearate, microcrystalline cellulose, povidone, hypromellose, and titanium dioxide.

4.1.3 Storage and Stability

Store at controlled room temperature, 20-25°C (68-77°F) [see USP]. Protect from light and moisture. Dispense in the original air-tight container.

4.1.4 Compatibility

No specific compatibility issues.

4.1.5 Handling

No specific measures necessary.

4.1.6 Availability

Zafirlukast will be provided by the research pharmacy. A 30-day supply will be distributed on C1D1 and Day 1 of each cycle moving forward by the local research pharmacy.

4.1.7 Preparation

No specific measures necessary.

4.1.8 Administration

Zafirlukast will be administered orally twice daily.

4.1.9 Ordering

Zafirlukast will be purchased by research pharmacy from commercial supplier.

4.1.10 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.) Participants will record compliance with study treatment in a drug diary that will be provided to them. The drug diary will be collected and re-dispensed at each study visit.

4.1.11 Destruction and Return

At the end of the study, unused supplies of Zafirlukast should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

5. CORRELATIVE STUDIES

5.1 Laboratory Correlative Studies

The contents of the serum separator tube will be used for CA-125 level determination by the clinical laboratory. CA-125 will be performed in a clinical laboratory using a commercially available assay.

A total of 10 mL of blood will be drawn into blue top tubes (3.2% citrate) in either two 5 mL tubes or one 15 mL tube for correlative labs.

Plasma will be separated at 2100 x G for 20 minutes. **First centrifugation should be performed within 1 hour of specimen collection.** The plasma layer will be centrifuged a second time at 2100 x G x 20 minutes in a single tube. The plasma supernatant will be transferred to a clean tube leaving the 1 mL at the bottom of the tube undisturbed (to be discarded). The transferred plasma sample will be aliquoted (500 mcL) into 1.5mL Eppendorf tubes.

Tubes will be stored at -80 °C.

Correlative Studies:

- D-dimer will be performed in a research laboratory using a commercially available ELISA.
- Plasma PDI-inhibitory activity: The plasma PDI-inhibitory activity will be measured using a modified DiEGSSG assay[53]. The exposure of the

DiEGSSG probe to exogenous PDI and plasma results in increased fluorescence measured spectrophotometrically and can be used to detect the presence of PDI inhibitor activity.

Plasma samples may be sent for coagulation and PDI-based assays (e.g. platelet-dependent thrombin generation assay) to Western New England University for analysis after a DUA is executed. Samples will be de-identified and coded by study number prior to being sent for analysis.

6. **STUDY CALENDAR**

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy. All assessments must be performed prior to administration of any study medication. All study assessments and medications should be administered within +/- 7 days of the protocol-specified date, unless otherwise noted.

	Baseline	**Cycle 1[f]**				**Cycle 2[f]**		**Cycles 3-12[f]**	**Off Treatment**	**30 Day post Last Dose Follow Up/ Off Study[cd]**	**EDC Timepoints**
		Day 1	Day 8	Day 15	Day 22	Day 1	Day 15	Day 1			
Zafirlukast[B]		X				X		X			Day 1 of every Cycle
Informed consent	X										N/A
Demographics	X										Baseline
Medical history	X										Baseline
CT Scan	X[i]										Baseline
Concurrent Medications	X----------									----------X	N/A
Physical exam	X	X		X				X	X	X	Baseline
Vital signs	X	X		X				X	X	X	Baseline
Height	X										Baseline
Weight	X	X		X		X		X	X	X	Baseline
Performance status	X	X		X		X		X	X	X	Baseline, Day 1 of every Cycle, Off Treatment, Off Study
CBC w/diff,	X	X	X	X	X	X	X	X	X	X	Baseline, Cycle 1 Day 1, 8, 15 & 22, Cycle 2 Day 1 & 15, Cycle 3+ Day 1, Off Treatment and Off Study
Serum chemistry[a]	X	X[g]	X	X	X	X	X	X	X	X	Baseline, Cycle 1 Day 1, 8, 15 & 22, Cycle 2 Day 1 & 15, Cycle 3+ Day 1, Off Treatment and Off Study
CA-125 Measurement	X	X				X		X	X	X	
Adverse Event Evaluation		X----------								----------X	During all visits
Radiologic Evaluation	X	Radiologic measurements should be performed every 3 cycles[e]							X		Baseline, Every 3 Cycles during treatment, Off Treatment, each follow-up visit, Off Study. If a Radiologic Evaluation is performed during any other Cycle, it must be captured in InForm.
B-HCG	X[b]										N/A
Drug Diary Review		X				X		X	X		
Other correlative studies[h]		X				X			X		N/A

	Baseline	Cycle 1[f]	Cycle 2[f]	Cycles 3-12[f]	Off Treatment	30 Day post Last Dose Follow Up/ Off Study[cd]	EDC Timepoints
Survival		X----------X					During all visits

B: Zafirlukast 40 mg twice daily
a: Total bilirubin, BUN, Creatinine, SGOT [AST], SGPT [ALT].
b: Serum pregnancy test (women of childbearing potential).
c: 30 Days Post Last Dose: Follow up visits or other contact are required in order to identify SAWs during the 30 days (+/- 7 days) following the end of study treatment.
d: Follow-Up evaluation. After the completion of the 30 days post last dose study visit, participants may be removed from the study.
e: Radiologic measurements should be performed every 3 cycles prior to the initiation of the next cycle. Scans may be performed within a +/-7 day window.
f: Cycles are 28 days in length.
g: Laboratory assessments recorded within 14 days of signing consent may be used to determine eligibility and does not have to be repeated (except complete blood count) for C1D1 if within this window
h: Refer to section 5.1
i. CT Scan for Baseline within 4 weeks of registration

7. MEASUREMENT OF EFFECT

7.1 Antitumor Effect

Participants will be evaluated for CA-125 response every 4 weeks.

7.1.1 Definitions

Response will be defined according to GCIG criteria which requires a reduction of CA-125 of ≥ 50% relative to pre-treatment CA-125 level, maintained for at least 28 days [54].

CA-125	Non-target lesions	New lesions	Overall serological response
Response and normalized	CR	No	CR
Response	Non-PD	No	PR
Normalized but not response	Non-CR/Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

7.2 Other Response Parameters

Doubling time of CA-125 in the 3 months prior to enrollment compared to doubling time following enrollment.

Progression, defined as development of clinical symptoms deemed secondary to ovarian cancer and/or radiographically visible disease and/or a doubling in the pre-treatment CA-125 value, confirmed on successive measurements (1-3 weeks after initial measurement).[54] Zafirlukast should be continued until progression is confirmed.

8. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

8.1 Data Reporting

8.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

8.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

8.2 Data Safety Monitoring

Each participating site will conduct local data and safety monitoring in accordance with the enrolling institution's data safety monitoring plan and policies. A summary DSMC report from each site should be provided to the Study PI whenever it is generated.

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

9. STATISTICAL CONSIDERATIONS

The primary objective is to determine the rate of antineoplastic response to zafirlukast as measured by response in CA-125 concentration.

9.1 Study Design/Endpoints

Primary Endpoint: Response rate in CA-125 per GCIG criteria.

Study Design: Two dose level pilot study.

Accrual: 8 participants

9.2 Sample Size, Accrual Rate and Study Duration

In the 4 women treated with zafirlukast 40 mg twice daily, there were no responses in terms of decline of CA-125. However, However, the mean change in CA-125 was 0.036 U/mL per day prior to treatment compared with 0.015 U/mL per day following zafirlukast (paired t-test p=0.026). This equated to a mean CA-125 doubling time of 29.8 days in the weeks prior to treatment compared with a mean doubling time of 85.69 days following treatment.

A second cohort of 4 women at 80 mg twice daily will be enrolled to assess whether CA-125 doubling time is significantly prolonged compared with pre-treatment values and whether we observe PDI inhibitory activity in plasma using Di-eosin-GSSG assay.

9.3 Analysis of Primary Endpoints

CA-125 response rate will be calculated as a proportion along with a two-stage 95% confidence interval using the Atkinson and Brown procedure.

9.4 Analysis of Key Secondary Endpoint

Calculation of CA-125 doubling time before and after initiation of zafirlukast will be performed.

9.5 Analysis of Exploratory Objectives

- Changes PDI and ERp57 inhibitory activity in plasma, platelet-dependent thrombin will be performed by paired t-test analyses comparing baseline with C2D1 samples.
- Determine progression free survival (PFS)
- Assess the incidence of venous thromboembolism
- Assess incidence of major hemorrhage and clinically relevant non-major bleeding

9.6 Reporting and Exclusions

9.6.1 Evaluation of Toxicity

All participants who received a dose of study drug will be assessed for toxicity.

9.6.2 Evaluation of the Primary Efficacy Endpoint

All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible.

10. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate,

and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team

Zafirlukast interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

Zafirlukast interacts with (a) certain specific enzyme(s) in your liver.

- The enzyme in question is CYP2C9 and zafirlukast is broken down by this enzyme in order to be cleared by your liver.
- *Zafirlukast* must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Aspirin
    - Erythromycin
    - Theophylline
    - Warfarin
- *Zafirlukast* is considered an inhibitor of the enzyme, meaning that it can affect the levels of other drugs that are processed by that enzyme. This can lead to harmful side effects and/or reduce the effectiveness of those medications.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of ***CYP2C9".***
- Your prescribers should look at this web site http://medicine.iupui.edu/clinpharm/ddis/table.aspx or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.

- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is

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and he or she can be contacted at

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