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Local Protocol #:14-114

Title: Randomized, placebo-controlled, double-blind phase II/III trial of oral isoquercetin to prevent venous thromboembolic events in cancer patients.

Short Title: Cancer associated thrombosis and isoquercetin (CAT IQ)

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Agent(s): Isoquercetin (IND# 121404) – Quercegen Pharma

Support Given By: NHLBI and Quercegen Pharma

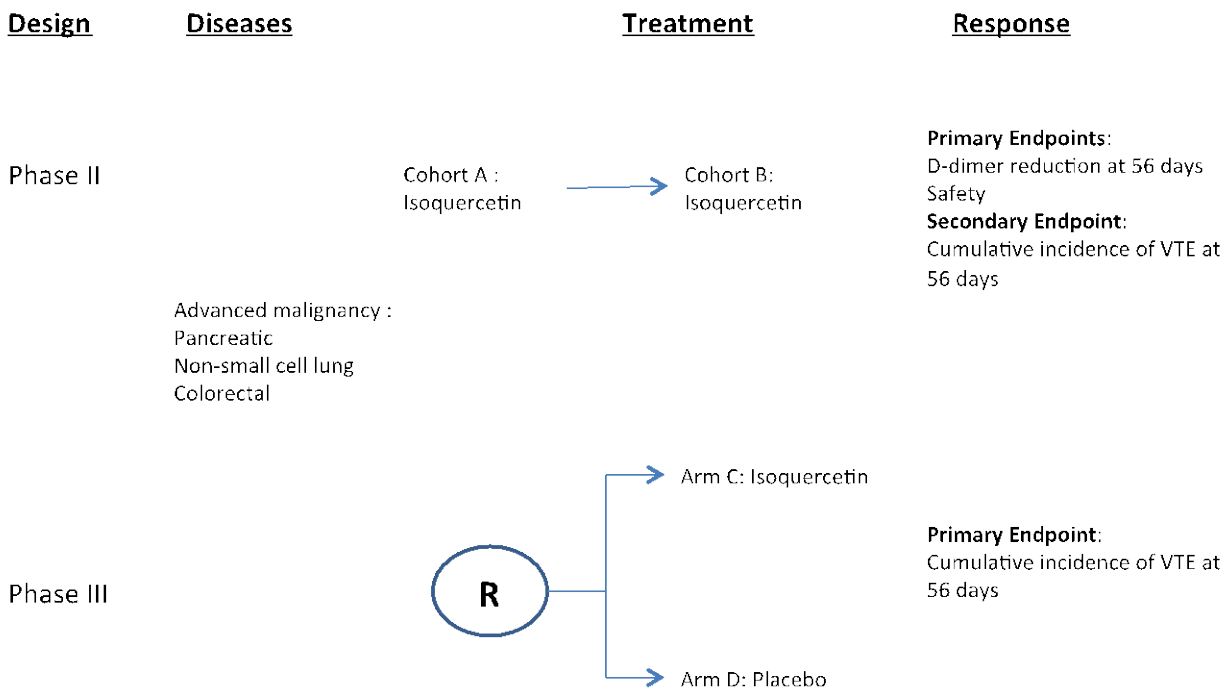

Figure 1. Protocol Schema

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1. OBJECTIVES

1.1 Study Design

The goal of this randomized, placebo controlled, phase II/III trial is to evaluate isoquercetin as a novel anticoagulant in patients with advanced cancer (pancreatic, non-small cell lung cancer, and colorectal cancer). The primary goal of the phase II study is to assess the relative reduction in D-dimer levels following isoquercetin administration and evaluate safety. Based on efficacy and safety data, a dose of isoquercetin will be selected for continued enrollment into a phase III trial in order to evaluate the effectiveness of isoquercetin in preventing VTE in cancer patients.

1.2 Primary Objectives

- The phase II primary objective is to evaluate the efficacy of isoquercetin to reduce D-dimer values
- The phase III primary objective is to assess the effectiveness of oral isoquercetin to prevent venous thromboembolic events in cancer patients

1.3 Secondary Objectives

- To investigate the cumulative incidence of VTE according to tissue factor bearing microparticle status (and isoquercetin randomization).To evaluate the cumulative incidence of major hemorrhage between the groups of patients treated with isoquercetin versus placebo.
- To assess overall survival according to randomization and tissue factor bearing microparticle status
- To investigate the cumulative incidence of total or symptomatic VTE in all treatment arms (and according to tissue factor bearing microparticle status)
- To study the influence of isoquercetin on TFMP levels and change in TFMP levels over time.
- To evaluate the cumulative incidence of VTE in patients with elevated TFMP as well as very elevated d-dimer (>1500 mcg/L) overall and according to randomization.
- To assess for correlation between complete blood count, ECOG, histology, stage, D-dimer, and levels of tissue factor bearing microparticles
- To investigate the influence of chemotherapy, blood counts, growth factors, ECOG performance status on tissue factor bearing microparticle levels as well as VTE risk.
- Influence of VTE and isoquercetin on Quality of Life (QOL)
- To investigate the association between isoquercetin plasma concentrations, inhibition of protein disulfide isomerase activity, and reduction in D-dimer.
- To evaluate the efficacy of isoquercetin to reduce D-dimer or VTE in cancer patients based on levels of circulating tissue factor bearing microparticles or Khorana risk model
- To compare VTE rates at completion of phase II with historical rates of VTE in similar population

2 BACKGROUND

2.1 Study Agent(s) - Isoquercetin

Isoquercetin is naturally occurring flavonol present in a number of fruits and vegetables such as berries, apples, and onions. Isoquercetin is the 3-0 glucoside form of the flavonol quercetin which is designated as GRAS (generally recognized as safe) by the Food and Drug Administration.[1] Recent studies have demonstrated potent antithrombotic activities of quercetin and related flavonoids in vitro and in animal models through inhibition of extracellular protein disulfide isomerase (PDI).[2] The goal of this trial is to evaluate the anticoagulant and antithrombotic activity of isoquercetin in patients with advanced malignancy.

Mechanism of action:
Extracellular disulfide isomerization has been explored as a regulatory pathway in platelet activation and fibrin generation. Protein disulfide isomerase is a thiol isomerase that is secreted by platelets and endothelial cells following activation and localizes to the membrane surface.[3-5] The hemostatic targets of extracellular PDI continue to be elucidated and may include tissue factor and platelet integrins such as αIIβIIIa. The Furie laboratory recently demonstrated the critical role of extracellular PDI in regulating platelet thrombus formation and fibrin deposition in vivo.[6] Employing intravital fluorescence microscopy to monitor a laser-induced vascular injury in mice, it was observed that inhibition of PDI with either bacitracin, a non-specific thiol isomerase inhibitor, or a specific and inhibitory monoclonal anti-PDI antibody completely blocked both platelet thrombus formation and fibrin generation in vivo.

Following the observation that inhibitory antibodies against PDI block platelet thrombus and fibrin generation in vivo, a high throughput screen identified several related flavonoids (quercetin-3-rutinoside and isoquercetin) as potent inhibitors of PDI.[2] The Kd of quercetin-3-rutinoside for PDI in vitro is 2.8 μM as determined by surface plasmon resonance (SPR). PDI inhibition appears to be specific without evidence of significant inhibition of other thiol isomerases such as ERp57, ERp5, ERp72, or thioredoxin.

We recently conducted a pharmacokinetic/pharmacodynamic study to assess the PDI-inhibitory activity of isoquercetin in humans. Following an oral dose of isoquercetin (1000 mg capsules), the median peak concentration was 4.45 μM with a half-life of 8.2 hours. PDI inhibitory activity of plasma was measured using the fluorescent substrate dieosin glutathione disulfide (di-E-GSSG) to liberate a self-quenching N-terminal GSSG residue following cleavage of a disulfide bond. [7-9] In all study subjects, we detected significant PDI-inhibitory activity in plasma following the oral administration of isoquercetin 1000 mg capsule.[10]

Quercetin as an antithrombotic agent

The antithrombotic potential of flavonoids first garnered attention in the 1990's following the publication of several large epidemiologic studies. In the Zupthen Elderly Study, the group of individuals who consumed over 30 mg of flavonoids daily experienced a nearly 70% reduction in mortality from myocardial infarction (adjusted RR 0.32, 95% CI 0.15-0.71) compared with those individuals who consumed less than 20 mg daily.[11] A prospective study of nearly 35,000 postmenopausal women demonstrated that flavonoid consumption reduced both cardiovascular and overall mortality[12] which was also confirmed in a meta-analysis of 7 cohort studies.[13] Flavonoid consumption has also been linked with a decreased incidence of nonfatal and fatal stroke.[14,15] Attempts have been made to identify which thrombotic risk factors are modulated with dietary flavonoid consumption but endpoints such as blood pressure, high and low-density lipoprotein concentrations, and flow-mediated dilatation have yielded inconsistent results. Flavonoids such as isoquercetin are known to inhibit platelet function in vitro.[16-19] The ingestion of either onion soup or quercetin -4'-O-β-D-glucoside (the predominant quercetin found in onions) inhibited ex vivo platelet aggregation.[20,21] However there has never been a late stage clinical study on the use of isoquercetin to specifically prevent thrombotic events in humans.

[redacted]

[redacted]

Selection of dose:
The inhibitory IC50 of PDI activity in vitro for isoquercetin metabolites ranges between 3-10uM.[2] Following the oral administration of isoquercetin (1000 mg) in healthy volunteers, the peak concentration of isoquercetin metabolites in healthy volunteers was 4.45μM and was associated with 67% inhibition of PDI activity in plasma at 4 hours.[10] Isoquercetin is widely available as a health food supplement and small clinical studies have not demonstrated toxicity. In a pilot trial conducted in 16 patients with mild cognitive impairment, isoquercetin 350 mg and ascorbic acid 400 mg taken for 6 months demonstrated a positive trend in cognitive function without any reported side effects (Investigators Brochure). In another study designed to evaluate the effect of isoquercetin and quercetin, thirteen individuals took isoquercetin 400 mg isoquercetin in conjunction with quercetin 1000 mg daily for two weeks without toxicity.[22] Based on in vitro, animal, and human data as well as safety profile of isoquercetin (and parent compound quercetin), the planned sequential dosing cohorts for the clinical phase II trial are 500mg and1000 mg once daily.

Absorption, Metabolism, and Bioavailability
A number of clinical studies have investigated the metabolic fate of quercetin and its glucosides. Isoquercetin is better absorbed than quercetin aglycone, possibly due to increased stability at low pH.[23] The pharmacokinetic study we conducted in healthy adults (isoquercetin 1000 mg capsules) AUC was 1714 and T ½ of 8.23 hours. Isoquercetin undergoes deglycosylation by an extracellular β-glycosidase on the brush border of enterocytes yielding the aglycone.[24,25] Although the absorption of quercetin and quercetin glucosides differ, all forms of quercetin are metabolized both in the enterocyte and liver and circulate as similar glucuronated, sulfated, and methylated conjugates.[26-28] These conjugates have demonstrated biologic activity [2,29,30].

Distribution

Isoquercetin and its metabolites possess high affinity for serum albumin.[31] Quercetin metabolites did not accumulate in tissue following four weeks of daily administration to pigs. Only the organs involved in metabolism and excretion such as the small intestine, liver, and kidney, contained higher concentrations than plasma[32]. The elimination of absorbed quercetin follows a biphasic profile, likely due to enterohepatic recirculation following elimination of quercetin metabolites in bile.[31,33]

Excretion

Following metabolism in the liver and/or degradation in the intestine, quercetin is excreted as exhaled CO_2 with smaller amounts found in feces and urine.[25,34]

2.2 Study Disease and correlative studies background

The prothrombotic state associated with malignant disease has been a recognized entity since the 1800's. Almost 1 in 5 cases of venous thromboembolism (VTE) are thought to be related to cancer, and thrombosis is the second leading cause of mortality in cancer populations.[35] The risk of thrombosis attributed to the malignant state is not uniform across all histological diagnoses. There is a 10-fold greater rate of VTE among patients suffering from ovarian, brain, and pancreatic cancers compared to those with other histologies.[36] Randomized clinical trials have demonstrated a small absolute risk reduction in VTE with primary thromboprophylaxis of low molecular weight heparins. The absolute risk of VTE in these unselected cohorts is low (3-5%) and thus the benefit of daily injection and potential risk for bleeding has tempered enthusiasm for non-targeted thromboprophylaxis approaches. Different models and biomarkers are under investigation to identify those patients who are highest risk of developing thrombosis and thus would benefit the most from primary thromboprophylaxis. Khorana and colleagues developed and validated a scoring model that identified higher risk patients based on elevated platelet count, white blood cell count, body-mass index, and anemia.[37]

Pathologic changes in microparticle populations contribute to the hypercoagulability associated with various disease states, including malignancy. Microparticles are vesicular structures measuring less than 1 μm in size and are derived from a number of cells within the vascular compartment including leukocytes, platelets, red blood cells, and endothelial cells.[38] Some microparticles are considered procoagulant due to the exposure of negatively charged phospholipids on the external membrane leaflet for the support of thrombin generation.[39,40] Subpopulations of microparticles also express tissue factor, the in vivo initiator of blood coagulation.[41,42] Circulating tissue factor-bearing microparticles augment thrombus formation in animal models[43] and tumor-derived microparticles accumulate at the site of vascular injury.[44] Our group previously demonstrated that tissue factor bearing microparticles are elevated in cancer patients diagnosed with VTE and appear to be derived from the underlying tumor.[45] The association between tissue factor bearing microparticles and VTE has been described by several groups as well, including in a mouse model of vascular injury.[44,46] Notably, in our study the 1 year cumulative incidence of thrombosis in cancer patients with increased tissue factor bearing microparticles was 34.8% versus 0% in those without detectable tissue factor bearing microparticles.[45] We recently completed a randomized phase II trial of low molecular weight heparin to prevent thrombosis in cancer patients with elevated tissue factor bearing microparticles.[47] The primary endpoint was the cumulative incidence of VTE at 60 days, as defined by symptomatic VTE or asymptomatic proximal DVT diagnosed by protocol-mandated bilateral lower extremity ultrasound. The overall cumulative incidence of thrombosis across the study was 11.3% at 60 days. The cumulative incidence of thrombosis in the high tissue factor bearing microparticle patients was 27% (N=11) compared with 5% (N=23) in those treated with low molecular weight heparin (P=0.06).

2.3 Rationale

Despite the high incidence and morbidity associated with VTE in cancer patients, primary thromboprophylaxis is not routinely recommended by oncologists largely due to a modest absolute benefit in unselected cancer cohorts and the need for patients to self-administer a daily injection. We previously observed a cumulative incidence of VTE of 11.3% at 60 days in patients with advanced non-small lung cancer, pancreatic cancer, and colorectal cancer.[47]

Protein disulfide isomerase released by platelets and endothelial cells regulates both platelet accumulation and fibrin generation in animal models. Quercetin and related glucosides were recently discovered to be potent inhibitors of extracellular PDI activity in vitro and in vivo.[2] Antithrombotic properties of quercetin have been described in humans and we observed that the oral administration of isoquercetin results in PDI-inhibitory activity in human plasma. Fortuitously, isoquercetin is widely available flavonoid nutritional supplement. Based on the safety profile and evident antithrombotic properties of isoquercetin, we aim to evaluate isoquercetin as an anticoagulant in cancer patients in a combined phase II/III clinical trial.

3 PARTICIPANT SELECTION

Laboratory tests required for eligibility must be completed within 15 days prior to study entry

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in phase 2 and 3 of the study:

3.1.1 Participants must have histologically confirmed malignancy that is metastatic or currently unresectable.
Eligible malignancies include:

- Adenocarcinoma of the pancreas (currently unresectable or metastatic)
- Colorectal (stage IV)
- Non-small cell lung cancer (currently unresectable stage III or stage IV)

3.1.2 Receiving or scheduled to receive first or second line chemotherapy (within 30 days of registration)

3.1.3 Minimum age 18 years. Because limited dosing or adverse event data are currently available on the use of isoquercetin in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric isoquercetin trials.

3.1.4 Life expectancy of greater than 4 months.

3.1.5 ECOG performance status $\leq$2 (see Appendix B).

3.1.6 Patient must be able to swallow capsules (phase III only)

3.1.7 Participants must have preserved organ and marrow function as defined below:

Absolute neutrophil count $\geq$ 1,000/mcL

Platelets $\geq$ 90,000/mcL

PT and PTT $\leq$ 1.5 x institutional upper limit of normal

Total bilirubin < 2.0 mg/dl

AST (SGOT)/ALT (SGPT) $\leq$ 2.5 X institutional upper limit of normal

Creatinine < 2.0 mg/dl

3.1.8 The effects of isoquercetin on the developing human fetus are unknown. For this reason, women of child-bearing potential and men 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 participating in this study, she should inform her treating physician immediately.

3.1.9 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 phase 2 or 3 of the study.

3.2.1 Participants may not be concurrently receiving any other study agents.

3.2.2 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.

3.2.3 Prior history of documented venous thromboembolic event within the last 2 years (excluding central line associated events whereby patients completed anticoagulation).

3.2.4 Active bleeding or high risk for bleeding (e.g. known acute gastrointestinal ulcer)

3.2.5 History of significant hemorrhage (requiring hospitalization or transfusion) outside of a surgical setting within the last 24 months

3.2.6 Familial bleeding diathesis

3.2.7 Known diagnosis of disseminated intravascular coagulation (DIC)

3.2.8 Currently receiving anticoagulant therapy

3.2.9 Current daily use of aspirin (>81mg 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).

3.2.10 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.11 Known intolerance of niacin or ascorbic acid (including known G6PD deficiency)

3.2.12 Pregnant women are excluded from this study because isoquercetin is a PDI inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with isoquercetin, breastfeeding should be discontinued if the mother is treated with isoquercetin. These potential risks may also apply to other agents used in this study.

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

The study is open to any individual who has an advanced malignancy 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 and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment 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 QACT protocol specific eligibility checklist. .

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study may be cancelled. . Notify the QACT Registrar of registration cancellations as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Participating Institutions

Eligible participants will be entered on study centrally at BIDMC by the Study Coordinator. All sites should call the Study Coordinator at 617-667-1939 to verify treatment availability and eligibility.

Following registration, participants should begin protocol treatment within 72 hours or as soon as possible. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. The Study Coordinator should be notified of participant status changes as soon as possible.

4.4 Registration Process for Other Participating Institutions

To register a participant, the following documents should be completed by the research nurse or data manager and faxed [redacted] to the Study Team:

- Copy of all eligibility and screening documents
- Signed participant consent form

- HIPAA authorization form (if applicable)
- Eligibility Checklist

The research nurse or data manager at the participating site will then call [redacted] or e-mail [redacted] the Study Coordinator to verify eligibility. To complete the registration process, the Coordinator will follow DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) and register the participant on the protocol. The coordinator will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site. The coordinator will also call the research nurse or data manager at the participating site and verbally confirm registration

5 TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for isoquercetin are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification).

Phase	Agent*	Isoquercetin Dose	Route	Schedule	Cycle
Phase II	Isoquercetin (IQC-950AN) 250mg	Cohort A: 500 mg or Cohort B: 1000 mg	Oral capsules	Cohort A: 2 capsules once daily Cohort B: 4 capsules once daily	28 days
Phase III	Isoquercetin (IQC-950AN) or Placebo	Cohort C: TBD or Cohort D: Placebo	Oral capsules	Once daily (TBD)	28 days

All capsules (placebo and isoquercetin) include ascorbic acid 62 mg and niacin 5 mg.

Phase II
Cohort A: Patients will receive isoquercetin 500 mg once daily (2 capsules). Continued enrollment into the dosing cohort will be based on the safety evaluation of the initial 5 patients. If there are $\geq$1 grade 3 or 4 toxicity (possibly, probably or definitely related to isoquercetin), then decision to enroll an additional cohort of 25 patients will be based on DSMC recommendations. If there are $\geq$3 grade 3 or 4 adverse events (possibly, probably or definitely related to isoquercetin), decision to proceed with enrollment into cohort B will be based on recommendations of the DSMC.

Cohort B: Patients will receive isoquercetin 1000 mg once daily (4 capsules). Similar to cohort A, continued enrollment will be based on the absence of attributable significant toxicities.

For both cohorts A and B, lower extremity ultrasound will be performed at end of treatment visit. Baseline D-dimer and correlative labs will be drawn at Day 1 and at end of treatment visit. Patients will be followed for survival after completion of end of treatment visit.

Phase III
Following the completion of the phase II portion, enrolled patients will be randomized 1:1 to Arm C (isoquercetin) or Arm D (placebo). The dose for Arm C will be determined after evaluation of the Phase II portion of the trial. The protocol will be amended when the decision is made whether to proceed to Phase III and what dose to use for Arm C. The study will be double-blinded to treatment arm. Lower extremity ultrasound will be performed at end of treatment. Baseline D-dimer and correlative labs will be drawn at Day 1 and at end of treatment. Patients will be followed for survival after completion of end of treatment assessment.

At BIDMC, optional blood draw will be performed at time 0 and 4 hours following the first dose of study drug. Non-collection of the optional blood draw will be permitted if patients ultimately decide not to stay for the 4 hour collection.

NOTE: all capsules also have ascorbic acid and niacin to improve stability and bioavailability of isoquercetin.

5.1 Pre-treatment Criteria

5.1.1 Cycle 1, Day 1 (see Appendix C for C1D1 checklist)

- Laboratory assessments recorded within 15 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 /μl
- Patients must be able to tolerate PO capsules (phase III only)

5.1.2 Cycle 2, Day 1 (Day 29) (see Appendix C for C2 D1 checklist)

- Adequate platelet count (see section 6.3)
- Non-heme toxicities possibly related to isoquercetin must return to grade 2 or less

5.2 Agent Administration

5.2.1 Isoquercetin or placebo

Depending on assigned Cohort during Phase II or randomization in Phase III each capsules will contain 250 mg isoquercetin versus placebo. Isoquercetin will be supplied as oral capsules. Capsules will be consumed once daily (2 or 4 capsules depending on cohort designation). Dose modifications will not be

made. Capsules will be dispensed from the research pharmacy on C1D1 and again on C2D1. The capsules will be packaged as a 30 day supply (to allow for unexpected occurrences such as dropped capsule).

Capsules may be swallowed with or without food. The capsules can be opened and the contents mixed with a small portion of soft acidic food such as apple sauce, puree of carrots, or pumpkin puree (Phase II only). 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.

5.3 Duration of Therapy

Duration of therapy will be 56 days (+/- 7 days inclusion of windows) or until one of the following criteria applies:

- 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.
- 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/L or bleeding leading to a transfusion of > 2 units of packed red blood cells.
- Proximal deep vein thrombosis or pulmonary embolism
- Condition requiring therapeutic anticoagulation
- Prophylactic heparin or low molecular weight heparin for greater than 7 consecutive days

5.4 Duration of Follow Up

Following completion of treatment, only the cause and date of death will be recorded. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Participants removed from active treatment will be followed for survival.

5.5 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.3 applies. The reason for study removal 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, [redacted] at [redacted].

6 EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP latest Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

A number of clinical trials have shown minimal to no adverse effects from oral quercetin (the parent compound of isoquercetin). In several studies conducted to investigate various potential effects of quercetin on immune function, risk factors for heart disease, or exercise-induced oxidative stress among others, the ingestion of quercetin by healthy subjects (males and females, group sizes ranging from 11 to 63; mean ages for treatment and control groups ranged from 20 to 46 years) at a daily dose of 600 to 1000 mg/day for periods of 1 to 6 weeks was not associated with any reports of adverse effects[48-52] A randomized, double-blind, placebo-controlled 12-week study was conducted in which subjects (male and female, ages 18 to 85 years) were randomized to consume a placebo (n = 335) or 500 (n=334) or 1,000 (n=333) mg of quercetin daily. There were no significant toxicities or metabolic changes between the groups.[53]

Intravenous administration of quercetin can be associated with nephrotoxicity. Doses up to 11.4 mg/kg body weight (equivalent of 694 mg in a 60 kg patient) were associated with transient flushing and mild pain at the injection site[54]. At the higher dose levels of up to 54.1 mg/kg body weight (equivalent to 10,000 mg orally), grade 1-4 nephrotoxicity was described, as well as dyspnea and emesis.

Despite the potential antithrombotic activity of quercetin and flavonoids, hemorrhage has not been described as a toxicity in clinical studies.

The supplied capsules contain ascorbic acid (62 mg) and niacin (5 mg) to prevent oxidation and improve absorption of isoquercetin. The recommended daily allowance of niacin is between 14 and 16 mg. Higher doses of niacin (between 300 mg and 6000 mg daily) are prescribed for hyperlipidemia and can be associated with flushing as well as other side effects such as hepatotoxicity. At physiologic dosing, side effects attributable to niacin would be unexpected. Similarly, ascorbic acid supplementation is not associated with significant toxicity. There are case reports of hemolytic anemia in patients with G6PD deficiency receiving extreme doses (several grams) of ascorbic acid.

6.1.1 Adverse Event Lists(s) for Isoquercetin (with ascorbic acid and niacin)

Mild hypotension (decrease in systolic blood pressure of less than 10mmHg)
Mild nausea or diarrhea
Hemolysis (rare)
Hemorrhage
Headache

6.2 Toxicity Management

Hemorrhage

- **No modification:** CTCAE grade 1

- **HOLD:** CTCAE grade 2 hemorrhage

Hold isoquercetin until resolution of grade 2 hemorrhage. The maximum number of consecutive days that isoquercetin can be held is 10 days before the participant must be taken off study. A participant may only hold isoquercetin for one ten day period. If a second grade 2 hemorrhage develops the participant must be taken off study.

- **Discontinue:** CTCAE grade 3-4 hemorrhage or otherwise meets criteria for major hemorrhage such as requiring transfusion 2 units PRBC, postoperative interventional radiology, endoscopic or operative intervention or life

threatening consequences including spontaneous bleeding at critical site (intracranial, pericardial, retroperitoneal, intraocular, intraspinal)

In cases of suspected isoquercetin toxicity (non-hematologic only):
•Grade 2: if toxicity is tolerable to patient, maintain at same dose. If toxicity is intolerable to the patient, then interrupt quercetin until recovery to grade <1
•Grade 3 : interrupt until grade <1
•Grade 4 : discontinue isoquercetin

6.3 Dose Modifications/Delays

For non-hematologic toxicity, see section 6.2 for dose delays.

For thrombocytopenia: Grade 3 (platelet count <50,000 /mcl). Hold study drug until grade 2. The maximum amount of time that the study drug can be held without withdrawing the participant from the study is 10 days.

7 DRUG FORMULATION AND ADMINISTRATION

7.1 Isoquercetin

7.1.1 Description

Isoquercetin, a glucoside form of quercetin, is a naturally occurring flavonol belonging to a broad group of pigmented substances of plant origin known as flavonoids. Flavonoids are a group of polyphenols characterized by a phenyl benzo(γ)pyrone-derived structure, consisting of 2 benzene rings, linked by a heterocyclic pyran or pyrone ring. The chemical name is: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one. The molecular formula is $C_{21}H_{20}O_{12}$ and a molar mass of 464.38 g/mol. It is also known as quercetin-3-O-glucoside CAS Number is 482-35-9.

Isoquercetin undergoes deglycosylation by an extracellular β-glycosidase on the brush border of enterocytes yielding the aglycone.[24,25] Although the absorption of the related quercetin compounds may differ, all forms of quercetin are metabolized in the enterocyte and liver and circulate as similar glucuronated, sulfated, and methylated conjugates.[26-28] These conjugates have demonstrated biologic activity[2,29,30]. The pharmacokinetic study we conducted in healthy adults (isoquercetin 500mg chews), resulted in a median peak concentration of 3.5 μM and T ½ of 9 hours. Following metabolism in the liver and/or degradation in the intestine, quercetin is principally excreted as exhaled CO_2 with much smaller amounts found in feces and urine.[25,34]

There is limited data regarding cytochrome interactions and isoquercetin (see Investigators Brochure for additional details). The data is inconsistent data regarding the interaction of quercetin and metabolism of drugs by cytochrome CYP3A4. The AUC of nifedipine was not altered when co-administered with quercetin nor was the bioavailability of the saquinavir affected (both metabolized by CYP3A4). However, there is some evidence that cyclosporin levels can increase following quercetin administration.[1] Pharmacokinetic profiles of drugs metabolized by other cytochromes (e.g. CYP2C9, CYP2C8) such as warfarin and rosiglitazone do not appear to be influenced by quercetin co-administration. Quercetin does not affect P-gp-mediated efflux as reflected by lack of effect on digoxin pharmacokinetics.[1]

7.1.2 **Form**

Isoquercetin occurs as a greenish-yellow, crystalline solid that is purified from plant sources (>99.5% purity) formulated into immediate release HPMC dark green capsules (size 0).

Both placebo and isoquercetin capsules contain the following: ascorbic acid (62mg), niacin (5mg), magnesium stearate (5mg), colloidal silicon dioxide. The capsules are manufactured and supplied by Pharmavize N.V. Isoquercetin capsules will contain 250 mg isoquercetin.

7.1.3 **Storage and Stability**

The isoquercetin and placebo capsules are packaged into high density polyethelene bottles and should be kept at room temperature. Stability studies have demonstrated that isoquercetin capsules are stable for at least 2 months and longer term stability studies are ongoing (packaging will be updated accordingly).

7.1.4 **Compatibility**

No specific compatibility issues

7.1.5 **Handling**

No specific measures necessary

7.1.6 **Availability**

Isoquercetin is commercially available but will be supplied free-of-charge from Pharmavize (as will placebo) as a 30 day supply distributed on C1D1 and C2D1 by the local research pharmacy.

7.1.7 **Preparation**

No specific preparation required.

7.1.8 **Administration**

Subjects will consume either 2 or 4 capsules daily (depending on cohort allocation). Capsules should be separated by approximately 24 hours.

7.1.9 **Ordering**

For shipment of isoquercetin, participating sites will contact:

[redacted]

Isoquercetin will be sent directly to the participating hospital pharmacy for disbursement.

7.1.10 **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.

7.1.11 **Destruction and Return**

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

8 CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacodynamic and Correlative Studies

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

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 1ml at the bottom of the tube undisturbed (to be discarded). The transferred plasma sample will be aliquoted (500µl) into 1.5ml eppendorf tubes.

Tubes may be stored locally at -80°C and batch shipped on dry ice to CAT IQ Study Team [redacted]

(BIDMC only) Optional blood draws include an additional 10ml drawn into citrated tubes for platelet aggregometry at time 0 and 4 hours. Tubes will be kept at room temperature for analysis in the Flaumenhaft laboratory. (see below)

Correlative Studies:

- Tissue factor bearing microparticles (TFMP) will be measured by impedance based flow cytometry in triplicate using internal positive controls. A determination of "high" TFMP is based on concentration greater than two-thirds of samples previously collected from patients with advanced cancer (highest tercile of cancer standards). Plasma samples will be stored at -80oC for up to 5 years following the completion of the study. Additional testing for thrombosis risk factors or laboratory markers of coagulation activation may be performed on these stored samples. (**See Appendix E for specimen collection and shipping report)**

- Plasma PDI-inhibitory activity: The plasma PDI-inhibitory activity will be measured using a modified DiEGSSG assay.[55] 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.

- D-dimer will be performed in a research laboratory using a commercially available ELISA (Roche Diagnostics).

- Coagulation substrates of PDI. The physiological basis for anticoagulant activity of PDI inhibition continues to be investigated. Proposed mechanisms include altered activity of coagulation factors or inhibitors such as tissue factor or antithrombin. The functional activity of proposed coagulation substrates will be assessed on stored plasma (pending additional funding).

- Platelet aggregometry (BIDMC only) will be performed by standard methodology to assess effect of PDI inhibition on platelet activity at baseline and 4 hours after study drug administration.

9 STUDY CALENDAR

Baseline evaluations are to be conducted within 15 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 $\pm$ 7 days of the protocol-specified date, unless otherwise noted.

Event/Procedure	Screening/ Pre-treatment visit	Day 1 (C1D1)	Day 29 (C2D1)	Day 56 or End of Study Treatment	Survival Assessment
Day Range	-15 to 0		± 2 days	± 5 days	Q6 months
History Assessments	X				
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Medical History	X		X	X	
ECOG Performance Status Score	X		X	X	
Concomitant medication review	X		X	X	
Treatment allocation[6]	X				
Dispense study drug		X	X		
Drug Diary Collection[3]			X	X	
Safety Assessments					
Physical examination including vital signs	X		X	X	
Height and Weight	X				
AE/SAE assessment	X		X	X	
Assessment of signs and symptoms of VTE and bleeding	X		X	X	
Laboratory Tests					
Complete blood count with diff	X	X	X	X	
PT/PTT	X				
Creatinine	X		X		
AST/ALT	X		X		
Total bilirubin	X				
Urine HCG[4]	X				
Correlative plasma samples (all sites)[1]		X		X	
Correlative plasma samples (BIDMC only)		X[5]		X	
Imaging					
Bilateral lower extremity ultrasound				X	
Quality of Life Survey[2]		X		X	

[1]Correlative lab instructions (TFMP) provided in Appendix E
[2]QOL survey provided in Appendix D
[3]Drug Diary
[4]If applicable
[5]Optional correlative labs to be drawn at time 0 and 4 hours (see section 7.1)
[6]Phase II treatment allocation is by sequential dosing cohort, Phase III allocation is by randomization

10 MEASUREMENT OF EFFECT

10.1 Venous Thromboembolism (VTE)

Cumulative incidence of venous thromboembolic events at 56 days (+/- 7 day windows) is the primary endpoint. ***The composite endpoint includes any symptomatic proximal or distal lower extremity DVT, symptomatic pulmonary embolism or fatal PE diagnosed by autopsy, asymptomatic proximal DVT diagnosed by screening compression ultrasound***. All other venous or arterial events will be recorded and analyzed as secondary endpoints.

Patients presenting with symptoms compatible with DVT and/or PE will undergo radiographic imaging with either compression ultrasound, VQ lung scan, or spiral CT. Confirmed episodes of VTE will be managed by the treating physician per standard of practice.

Presence of any of the following will be considered diagnostic for a VTE:

- New non-compressibility of lower extremity deep venous segments by compression ultrasound. Distal lower extremity thrombus (lower than popliteal vein) qualifies for primary VTE endpoint only if symptomatic. All proximal thrombi qualify as primary study endpoint if identified by screening ultrasound or are symptomatic.
- Intraluminal defects in two or more views on pulmonary angiography
- Sudden contrast cut-off of one or more vessels greater than 2.5 mm in diameter on a pulmonary angiogram
- A high probability VQ lung scan showing one or more segmental perfusion defects with corresponding normal ventilation (mismatch defect)
- Abnormal spiral CT showing thrombus in pulmonary vessels (subsegmental or larger)

Note: intrabdominal thrombus related to tumor compression is not considered a VTE endpoint

Other VTE

Those VTE that do not qualify for the primary endpoint such as asymptomatic distal extremity deep vein thrombosis, asymptomatic pulmonary emboli diagnosed by CT, central line-associated thrombus will be recorded and analysed as secondary endpoints.

Response Review

An independent adjudication of VTE qualifying for the primary outcome will be performed. The review committee will be comprised of two independent hematologists and a radiologist. The committee will review the medical records and radiology report for all VTE that qualify for the primary VTE endpoint. In cases where committee feels that the radiology reports or medical records are equivocal then the primary radiology images will be reviewed by an independent radiologist for adjudication.

10.2 Major Hemorrhage

Adhering to published guidelines, the criteria of major hemorrhage in non-surgical patients is:[56]

- Fatal bleeding, 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 $\geq$2 g/L or
- Bleeding leading to a transfusion of $\geq$ 2 units of packed red blood cells.

10.3 D-dimer

D-dimer will be measured at Beth Israel Deaconess Medical Center at baseline and end of treatment . Relative change in D-dimer will be assessed for each arm.

10.4 TFMP levels and thrombotic risk

Absolute TFMP levels will be measured at baseline and 56 days. A correlation between TFMP and thrombotic risk will be investigated according to randomization arm (see Section 13.2).

10.5 Quality of Life

Quality of Life (QOL) will be assesses using EORTC QLQ-30 which is an internationally validated and widely utilized questionnaire (Appendix D).[57] Scoring is based on responses to 30 questions dealing with functional quality (such as cognitive, physical, and emotional) as well as symptom burden (such as fatigue, nausea, and sleep). The questionnaire will be completed at baseline visit and at the end of study assessment. For non-English speaking participants, the questionnaire will be completed with a translator.

10.6 Overall Survival

Will be recorded every 6 months.

10.7 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for VTE and D-dimer endpoints. Only those participants who undergo an evaluation for VTE after day 1 (either diagnosed with VTE) or completed at least 1 cycle of treatment (along with requisite off treatment evaluation) will be considered evaluable for VTE and D-dimer endpoints.

11 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalizations will not be considered SAEs unless they meet the criteria specified in the table in Section 11.3.2: DF/HCC Reportable AEs. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do

not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for: routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures elective or pre-planned treatment for a pre-existing condition that did not worsen. Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission respite care.

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.4 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.5 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list or when it is not included in the informed consent document as a potential risk.

11.1.6 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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

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

11.3 Adverse Events: List and Reporting Requirements

11.3.1 Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI any serious adverse event (SAE) 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.

For multi-institution studies where a DF/HCC investigator is serving as the Overall Principal Investigator, each participating institution **must** abide by the reporting requirements set by the DF/HCC. This applies to any medical event equivalent to an unexpected grade 2 or 3 with a possible, probable or definite attribution, unexpected grade 4 toxicities, and grade 5 (death) regardless of study phase or attribution.

11.3.2 DF/HCC Expedited Reporting Guidelines

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

Other investigative sites will report SAEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to the Overall PI within the timeframes detailed in the table below.

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days[#]	5 calendar days	24 business hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days[#]	5 calendar days	24 business hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 24 business hours of learning of the event.					

The Overall PI will submit SAE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

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11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 10.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 1 business day of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. In the case of a medical emergency which may require unblinding, contact the Overall Principal Investigator. Report serious adverse events by telephone, email or facsimile to:

[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

[redacted]

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

11.6 Reporting to the Food and Drug Administration (FDA)

The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.7 Reporting to National Heart Lung and Blood Institute

The DF/HCC Overall Principal Investigator will report unanticipated problems or unexpected serious adverse events (fatal, life threatening or serious) that may be related to the study protocol to the NHLBI Program Officer within 7 days. If the event or problem is unexpected, and possibly/probably/or definitely related to the study drug and suggests greater risk of harm to the study participant(s) than was previously known or recognized will be reported within 30 calendar days.

11.8 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.9 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating

investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12 DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation

Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

During the phase II portion of the study, 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 clinical specialists with experience in oncology and who have no direct relationship with the study. 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 or more often if required to review toxicity and accrual data. 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 with 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.

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this trial during the Phase III portion of the study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed with the Principal Investigator, statistician and study team members. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. A DSMB review will also be triggered if the number of toxicity events (possible or definite grade 3 or 4 toxicity or major hemorrhage) prompts an automatic enrollment hold within either cohort or prior the next dosing cohort. Information to be provided to the DSMB may include: participant accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The

purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion. Refer to section 5 of Data Safety and Monitoring Plan for additional information regarding monitoring plan.

13 REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each

research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.4 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.5 Multi-center Guidelines

This protocol will adhere to the policies and requirements of the Dana-Farber/Harvard Cancer Center. The specific responsibilities of the DF/HCC Overall Principal Investigator (or Protocol Chair), Coordinating Center, and Participating Institutions are presented in the Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (see Appendix A).

- The DF/HCC Overall Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports to all participating institutions for submission to their individual IRBs for action as required.

- Mechanisms will be in place to ensure quality assurance, protocol compliance, and adverse event reporting at each site.

- Except in very unusual circumstances, each participating institution will order the agent(s) directly from the supplier. A participating site may order the agent(s) only after the initial IRB approval for the site has been forwarded to the Coordinating Center.

14 STATISTICAL CONSIDERATIONS

The aim of this combined phase II/phase III trial is to evaluate isoquercetin as an effective anticoagulant in cancer patients.

14.1 Study Design/Endpoints

14.1.1 The primary endpoint of the **Phase II trial** is to investigate whether isoquercetin significantly reduces d-dimer values in cancer patients. D-dimer is a readily available biomarker used clinically in the prediction and diagnosis of VTE. In cancer cohorts, the D-dimer has modest specificity for predicting symptomatic thrombosis.[58] However, D-dimer remains the most validated surrogate marker of thrombin generation in vivo and symptomatic VTE.

14.1.1.1 **Study Design**: The trial is a sequential cohort study of isoquercetin 500 mg and 1000 mg daily.

14.1.1.2 **Randomization**: Patients will not be randomized.

14.1.1.3 **Accrual:** Anticipated accrual is 10 patients per month across all study sites.

14.1.1.4 **Sample Size Justification:** Plasma D-dimer levels typically decrease at least 20% following the initiation of anticoagulation.[59] The sample size is based on a paired t-test of D-dimer concentrations measured on day 1 and end of treatment, to be performed separately for each arm. In the recently completed MicroTEC study, the median baseline D-dimer concentration for all cancer patients enrolled was 815 ug/L.[47] Based on an estimated standard deviation of 0.3 for the ratio of the D-dimer levels at day 56 over the D-dimer levels at day 1, a total of 26 subjects are needed per arm for a 90% power to declare the reduction significant if the true concentration at end of treatment is reduced by 20% in each isoquercetin arm (one-sided alpha 0.05). Estimated death or dropout of 5% prior to 56 days, the sample size will be increased to a total of total 30 per arm (one-sided alpha 0.05). With 30 eligible patients per dose level, there is greater than 60% power to detect an unacceptable attributable grade 3 or 4 rate of 15% at 56 days using a one-sided 10% level exact binomial test in each dose level. (see table below)

14.1.1.5 **Decision to proceed with enrollment into Cohort B**

A sequential two-stage design will be employed in the Phase II portion of this trial, within each dose level, to evaluate the adverse events of major toxicity (probable or possibly related grade 3 or 4 adverse events including hemorrhage) at end of treatment for potential early termination of the dose in the case of excessive toxicity. After 5 patients have finished 56 days of therapy, the study will be placed on hold and toxicities evaluated. If more than one major toxicity is observed within the first 5 patients enrolled in Cohort A, the cohort will be suspended and detailed toxicity data reviewed for possible closure of the study to further accrual based on DSMC review. The probability of stopping accrual after the first 5 patients based on the true but unknown toxicity event for major hemorrhages is shown in the table below. If the study criteria are met to reopen the second stage, accrual will begin after the first 5 patients have finished study treatment and all toxicity data have been reviewed. With 5 eligible patients in the first stage, the probabilities of observing at least one toxicity with corresponding true rate of 1% and 5% are, respectively, 5% and 23%. With 30 eligible patients the respective probabilities of one or more events are 26% and 79%. The maximum width of a 95% confidence interval on any given toxicity, unadjusted for the two-stage design is 36%. Adjusting for the two-stage design, the maximum 95% confidence interval width would be 50%. If there are greater than 3 major toxicities in the 30 patients in Cohort A, then the cohort will be suspended and detailed toxicity data reviewed for possible closure of the study to further accrual based on DSMC recommendations. If the true but unknown toxicity event for major hemorrhages is 5% and 15%, the probability of proceeding to Cohort B after the evaluation of the 30 patients in Cohort A is 92.5% and 30.8%, respectively. A similar two-stage design will be employed for patients enrolled in Cohort B. If there are more than 1 major toxicities in the first 5 patients or three or more

major toxicities in the 30 patients in Cohort B, then we will only consider proceeding to the phase III portion of the study with isoquercetin 500 mg daily.

True attributable toxicity	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45
Probability of stopping early after 5 patients	0.023	0.081	0.165	0.263	0.367	0.472	0.572	0.663	0.744
Probability of stopping at the end of the cohort A	0.075	0.373	0.692	0.883	0.964	0.991	0.998	0.999	0.999

14.1.1.6 **Primary endpoint analysis:** D-dimer concentrations will be compared for each patient at day 0 and end of treatment by a paired-t test analysis. Analysis will be performed on an intention to treat basis for patients who undergo randomization and completed the baseline and end of treatment D-dimer assessments.

14.1.1.7 **Determination of continuation with phase III trial and selection of dose.** Following completion of the phase II study, decision will be made by principal investigators whether to proceed with enrollment into the phase III trial. The decision to continue will be principally guided by whether either isoquercetin arm reached the primary endpoint definition. If both isoquercetin arms meet the primary endpoint then selection of dose will be based on degree of reduction in D-dimer levels in each arm relative to baseline and toxicity profile with special attention to major hemorrhage episodes. If neither isoquercetin arm results in significant reduction in d-dimer values relative to baseline, then consideration to advance to phase III will be made based on overall VTE rate in the combined isoquercetin arms. D-dimer is a recognized biomarker of the prothrombotic state but is known to lack specificity in cancer cohorts[58]. Because it is possible that isoquercetin is an effective antithrombotic without altering plasma D-dimer values especially considering potential antiplatelet activity, we will also analyze the cumulative incidence of VTE collectively for the isoquercetin treated patients. A true cumulative incidence VTE rate of less than 10% would be considered promising in the combined isoquercetin arms, whereas a true cumulative incidence rate of 25% would not be worthy of further study. Treatment is considered promising among the 60 patients treated with isoquercetin when nine or fewer VTE are observed. This design has at least a 92% chance of concluding the treatment is effective when the true cumulative incidence

rate is 10%, and less than a 5% chance of concluding the treatment is effective when the true cumulative incidence rate is 25% or more (Fisher exact test). If determination to proceed to phase III is based on promising VTE rates, then the higher dose level of isoquercetin will be utilized.

14.1.2 The primary aim of the **phase III trial** is determine whether isoquercetin reduces the cumulative incidence of symptomatic or proximal VTE in cancer patients at 56 days.

14.1.2.1 **Study design:** The trial is a randomized, placebo-controlled, double-blinded phase III trial. Study subjects are randomized to placebo or isoquercetin. The structure of the study matches the phase II study, the central differences being that VTE is the primary endpoint and a single isoquercetin arm is compared to placebo.

14.1.2.2 **Randomization:** Central randomization (1:1) will be performed by QACT Office at DF/HCC and stratified by cancer diagnosis and institutions.

14.1.2.3 **Accrual:** Anticipated accrual is 20 patients per month across all sites.

14.1.2.4 **Sample size justification:**

The overall incidence of thrombosis in cancer populations varies between 2-40% based on histology, stage, chemotherapeutics, additional risk factors and mode of surveillance (imaging or symptomatic events). The overall cumulative incidence of VTE in recently conducted MicroTEC study with similar eligibility criteria and study design was 11.3%.The estimate of isoquercetin benefit is based on data from primary thromboprophylaxis trials that typically achieve VTE rates of 4% or less.[60,61] Based on the estimated VTE rates of 11% in the placebo and 4% isoquercetin arms, a total of 281 isoquercetin-treated subjects and 281 placebo-treated subjects would be required to reject the null hypothesis that the failure rates for experimental and control subjects are equal with probability (power) 0.85. The 1-sided type I error probability associated with this test of this null hypothesis is 0.025. In order to adjust for death or dropout of 5% prior to 56 days, the sample size is increased by an additional 28 patients. We plan an interim analysis for efficacy or futility after 50% of patients have been randomized that will be guided by O'Brien-Fleming boundaries. This will require an additional increase in sample size of 5% or 28 patients. Therefore, we plan to enroll a total of 618 patients. A total of 30 patients enrolled in the phase II trial will be included in the total accrual target and final VTE endpoint analysis.

14.1.3 Primary endpoint analysis will be performed on an intention to treat basis following randomization for all patients who undergo baseline assessment for the presence of VTE and at least one dose of study medication. The cumulative incidence of VTE

will be assessed by competing risk analysis and differences between arms will be evaluated by the Gray test.[62,63]

14.2 Secondary endpoint analysis

- Cumulative incidence of VTE in cancer patients with or without tissue factor bearing microparticles will be assessed for isoquercetin or placebo arms by a competing risk analysis and differences evaluated by the Gray test. We previously identified elevated tissue factor bearing microparticles as a significant risk factor for developing thrombosis with a cumulative incidence of 27-38%.[45] We also will evaluate whether isoquercetin reduces the cumulative incidence of symptomatic or proximal VTE in cancer patients with elevated tissue factor bearing microparticles. Based on our previous data, we anticipate that approximately 45% of patients enrolled will have elevated tissue factor bearing microparticles (N=278) and half will be randomized to placebo or isoquercetin. Based on estimated VTE rates of 20% in the placebo and 7% in the isoquercetin arms for individuals with high tissue factor bearing microparticles, the probability (power) to reject the null hypothesis is 0.84. The 1-sided type I error probability associated with this test of the null hypothesis is 0.025 (Fisher exact test).
- The cumulative incidence of major hemorrhage between the groups of patients treated with isoquercetin versus those without. Analysis will include time to first major bleed, overall incidence of bleeding and analysis of bleeding events over time.
- Overall survival according to randomization and tissue factor bearing microparticle status will be analyzed by Kaplan Meier and significance testing by log-rank analysis
- Cumulative incidence of total or symptomatic VTE at 2 months in all treatment arms (and according to tissue factor bearing microparticle status)
- Influence of isoquercetin on TFMP levels and change in TFMP levels over time. This will be analyzed by continuous measurement of difference using ANOVA approach.
- Cumulative incidence of VTE in patients with elevated TFMP and very elevated d-dimer (>1500 mcg/L) overall and according to randomization.
- Correlation between complete blood count, d-dimer, ECOG, histology, stage, and TFMP levels will be determined by assessing the correlation coefficient by standard methods (Pearson or Spearman's coefficients).
- Linear regression modelling will be performed to assess the influence of chemotherapy, blood counts, growth factors, ECOG performance status on TFMP levels.
- Fine and Graey regression modelling will be performed to assess the influence of chemotherapy, blood counts, growth factors, ECOG performance status on VTE risk.
- Quality of Life (QOL) will be assesses using EORTC QLQ-30 version 3 which is an internationally validated and widely utilized questionnaire.[57] Scoring is based on responses to 30 questions dealing with functional quality and symptom burden. The questionnaire will be completed at baseline visit and end of treatment assessments. All are reported on a range 0-100; a high score represents a high level of functioning and QOL. We will analyze the data using analysis of variance techniques if the QoL has constant variance across groups (based on VTE rates and treatment groups). If not, we will use the Kruskal-Wallis test based on ranked QoL scores. If the questionnaire developers provide guidelines for scoring in the presence of missing data, we will

follow those guidelines. Alternatively, we will tally the number of questionnaires for which a particular data item is missing.

- Correlation between isoquercetin dose, plasma PDI inhibitory activity, and reduction in D-dimer or VTE incidence
- Cumulative incidence of VTE according to Khorana score[37]
- At completion of phase II portion, we will compare the cumulative incidence of VTE of all 60 patients enrolled with similar historical population. As described above the overall cumulative incidence of VTE with similar inclusion criteria and design was 11%. We hypothesize the cumulative incidence of VTE in the phase II cohort will be 4%. Based on these assumptions, the power for this comparison is 80% (N=60 with a 1-sided alpha of 0.1 comparing 4% to historical 11% cumulative incidence rate using a Fisher exact test.)

14.3 Reporting and Exclusions

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

14.3.2 **Evaluation of response.** 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. For the phase II study, primary endpoint analysis will be performed on an intention to treat basis for patients who undergo randomization and completed both the baseline and day 56 blood draw. For the phase III study, primary endpoint analysis will be performed on an intention to treat basis following randomization for all patients who undergo baseline assessment for the presence of VTE.

15 PUBLICATION PLAN

The primary investigators hold primary responsibility for publication of study results without third party approval. Phase II and Phase III results may be presented and/or published separately. The results will be made public within 24 months of the end of data collection. Planned report will be in a peer-reviewed journal and an initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes will be made no later than three (3) years after the end of data collection.

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17 APPENDICES

APPENDIX A

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Children's Hospital Boston (CHB), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA, OBA etc.). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Coordinating Center: The entity (i.e. Lead Institution) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Quality Assurance Office for Clinical Trials: A unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The DF/HCC Sponsor, Dr. Zwicker will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (i.e. FDA, OBA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with FDA (investigator-held IND trials) or OBA (gene therapy trials), as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.

- Maintain FDA or OBA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).

2.3 DF/HCC Quality Assurance Office for Clinical Trials (QACT)

In addition to the Coordinating Center, the DF/HCC QACT provides the following support services to assist the DF/HCC Sponsor:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and QACT approval required)

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2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder in accordance with DF/HCC requirements.
- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event (SAE) reports to local IRB and directly to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Secure and store investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.

- For protocols using investigational agents, the Participating Institution will order their own investigational agents regardless of the supplier (i.e. National Cancer Institute (NCI), pharmaceutical company). [This is the preferred method of ordering investigational agent. Some pharmaceutical companies may require the Coordinating Center to order study drug for external sites. Contact pharmacy to confirm this is feasible. Revise as necessary.]

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Site.

Participating sites are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to international trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution's IRB
- Participating IRB's approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPPA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB and if applicable NCI/CTEP, will provide a consent template, which covered entities (Participating Institutions) must use.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health

information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

Refer to Section 4.3 and 4.4 of the protocol for instructions on patient registrations.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

Randomization can only occur during QACT's normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Time.
Individual research pharmacies will not be blinded to randomization allocation. The participant, investigator, and research team will be blinded to randomization allocation.

3.7.2 Initiation of Therapy

Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any exceptions to this policy.

3.7.3 Eligibility Exceptions

The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.4 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of

the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

3.8 DF/HCC Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and correspondence, and correspondence with the Coordinating Center.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe derivations from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB

approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

The participating Institutions will review and submit to their IRB according to their institutional policies and procedures. .

3.10.1 Guidelines for Reporting Serious Adverse Events

Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol section 10.2.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.10.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.11 Data Management

The DF/HCC QACT develops a set of either paper or electronic case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users.

3.11.1 Data Forms Review

When data forms arrive at the DF/HCC QACT, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

Incomplete or Questionable Data
If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms
If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of four times a year.

4.0 REQUISITIONING INVESTIGATIONAL DRUG

The ordering of investigational agent is specified in the protocol. Refer to section 6.4.9 for ordering information.

Participating Institutions should order their own agent regardless of the supplier (i.e., NCI or a pharmaceutical company.)

If the agent is investigational, ensure that the pharmacy will be able to receive and store the agent according to state and federal requirements. The local IRB should be kept informed of who will supply the agent (i.e., NCI or a pharmaceutical company) so that any regulatory responsibilities can be met in a timely fashion.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the QACT provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

At each Participating Institution, the first patient enrolled and then every tenth patient enrolled will be monitored for protocol compliance by the Coordinating Center. The Participating Institutions will be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur before the clinical phase of the protocol begins, continue during protocol performance and through study completion. Additional monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants , informed consent procedures, adverse events and all associated documentation, study drug administration/treatment, regulatory files, protocol deviations, pharmacy records, response assessments, and data management.

Participating institutions will be required to participate in monthly Coordinating Center initiated teleconferences. “Newsletters” highlighting overall protocol progress and important announcements will be distributed regularly.

Virtual Monitoring will be an ongoing process. Prior to enrolling patients, participating sites will be given templates for suggested binder organization as well as treatment checklists (See Appendix C) in order to ensure all required data is captured. Source documentation will be reviewed prior to enrollment of participants. All required correlative plasma samples will be tracked using template provided (see Appendix E). All sites will use a data entry system which provides a missing data report monthly for Coordinating Centers review. Participating Institutions will provide Coordinating Center with Delegation of Authority logs within 60 days of opening at a participating site and then annually prior to the continuing review report. Participating Institutions will also provide Coordinating Center with Drug accountability logs every six months. Participating Institutions will be required to forward de-identified copies of participants’ medical record and source documents to the Coordinating Center to aid in source document verification.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center and if applicable, QACT Data Analysts assigned to the protocol, will perform the ongoing protocol data compliance monitoring.

Additional monitoring activities may occur at the discretion of the DF/HCC Sponsor if incidents on non-compliance are discovered.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

5.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each participating institution. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination. As per the protocol feasibility questionnaires, all external sites are expected to enroll at least 2-5 patients a year.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Sponsored Trials

At a minimum, two on-site audits at each participating institution will be scheduled by the QACT assuming the enrollment threshold for an audit has been met. A site will be eligible to be audited assuming at least three participants have received protocol treatment at that site. The site would be eligible for a second on-site audit once an additional 5-10 subjects have been treated at the site. Approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited. Participating Institutions may be subject to additional audits if incidents of non-compliance are found.

6.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates (internal) and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 DF/HCC Sponsor and Coordinating Center

The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Sub-Standard Performance

The DF/HCC Sponsor, DFCI IRB, is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation.

Appendix B: 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 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:

Cycle 1, Day 1 (Day 1)

Patient ID and Initials: _____ Hospital:_____

☐ Complete blood count with diff

☐ Platelet count > 90,000/µl

☐ Absolute neutrophil count >1,000/mcL

☐ Correlative plasma sample

☐ Correlative plasma sample at 0 and 4 hours (BIDMC only, optional)

☐ Quality of life survey

☐ Dispense study drug

☐ Dispense drug diary

Investigator signature: _____
Date: _____

Cycle 2, Day 1 (Day 29)

Patient ID and Initials: _____ Hospital:_____

☐ Medical history, ECOG and concomitant medication review

☐ Physical exam including vital signs

☐ AE/SAE assessment

☐ Assessment of signs and symptoms of VTE and bleeding

☐ Complete blood count with differential, Creatinine, AST/ALT

☐ Absence of grade 3 thrombocytopenia (platelet count > 50,000/μl)

☐ Non-heme toxicities possibly related to isoquercetin must return to grade 2 or less

☐ Collect signed drug diary and provide a new one

Investigator signature: _____
Date: _____

Day 56 or End of Study treatment

Patient ID and Initials: _____ Hospital: _____

☐ Medical history, ECOG and concomitant medication review

☐ Physical exam including vital signs

☐ AE/SAE assessment

☐ Assessment of signs and symptoms of VTE and bleeding

☐ Complete blood count with differential

☐ Correlative plasma sample

☐ Bilateral lower extremity ultrasound

☐ Quality of Life Survey completed

☐ Collect signed drug diary

Investigator signature: _____
Date: _____

APPENDIX D: QUALITY OF LIFE QUESTIONAIRE (EORTC QLQ-C30 v3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:
Your birthdate (Day, Month, Year):
Today's date (Day, Month, Year):

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor Excellent

[redacted]

Sample (Plasma) Shipment Report
14-114: Randomized, placebo-controlled, double-blind phase II/III trial of oral isoquercetin to prevent venous thromboembolic events in cancer patients.

Medical Center: _____

Patient ID and Initials: _____

Collection Date: ____/____/_____

Time of draw: ☐ ☐ : ☐ ☐ **AM/PM** **Number Samples drawn:** _____
Time of spin: ☐ ☐ : ☐ ☐ **AM/PM** **Number Samples sent:** _____
Number Samples stored: _____

Select the Appropriate Visit:
Screening/Pre-treatment visit
Day 56 (± 5 days) **or End of Study treatment**

Completed By:

Comments:

NOTE: Please see attached study calendar and a detailed instruction about each sample day (visit).

Contact Study Coordinator at Medical Center: _____
Phone: _____ **Email:** _____

This complete form and samples should be mailed to the above address. Samples should be shipped Monday-Thursday only to ensure samples will still be viable upon receipt.
Please email the study team at BIDMC the day the shipment is sent out. Thank you!

[redacted]

Mixing IQC-950AN capsule contents with food

Quercegen Pharma. 2015-10-29 rev 1

The contents of IQC-950AN capsules can be mixed with a small amount of food to aid in patient compliance. Based on the dissolution profile of the capsules we can expect that capsule contents are released in the stomach, and the stability of IQC-950AN is expected to be good in the stomach. The capsule contents are light yellow colored and the powder is lightly acidic with an otherwise neutral to slightly bitter taste. The capsules can be opened and the contents mixed with a small portion of soft food. The capsules are "locking", but not sealed, so they can be opened with some twisting and pulling over the food however it is recommended that the opening and emptying be performed for the subject as the capsules are tightly filled and assuring full emptying is important.

The contents are not strong-tasting so it is not necessary to make extra efforts to mask the taste. It is recommended to mix the capsule contents with an already acidic soft-food as this will blend well with the taste. It is not recommended to disperse in a liquid because of low solubility and possibility of powder sticking to container walls.

Since the capsule contents are yellow colored, it is recommended to use a yellowish soft-food which is readily available such as puree of carrots or pumpkin puree, or a darker soft food such as prune puree. However, for dosing consistency a single option or fewer options are recommended.

Suggested instructions;

A. *Carefully twist off the top portion of the capsule over the soft-food you plan to use. Rolling the capsules helps with emptying.*

B. *Sprinkle the contents of the capsule on a small amount of room temperature acidic food, we recommend puree of carrots or pumpkin puree. It is important to verify complete transfer.*

C. *Swallow the capsule contents in the soft food mixture right away, and follow with enough water to make sure the contents of the capsules are swallowed completely. Do not store the capsule content mixed with food for later use.*

Related Information on Stability in Aqueous Suspensions

It is recommended to use the IQC-950AN capsule content with a lightly acidic soft-food and cool or cold temperatures soon after preparation.

Standard Dissolution Testing of the IQC-950AN capsules shows that the contents are released in the stomach (see GMP release documents). It is also known that isoquercetin (the active component of IQC-950AN) is stable during incubation with gastric content preparations (Chang *et al*., 2005; Zuo *et al.,* 2006).

The stability of the parent quercetin molecule is both pH and temperature dependent, with acidic conditions and lower temperatures assuring the stability of quercetin suspensions. In an acidic aqueous suspension (pH 2.7) and refrigerator temperature (4 °C) quercetin remained stable for the duration of the storage period (96 hours) while greater than 80% of quercetin was recovered in the pH 7 system. At room temperature, the stability of quercetin was reduced, such that 50% and 20% of quercetin degraded in the first 25 hours at pH 7 and 2.7, respectively (Moon *et al*., 2008) .

With the proposed conditions there should not be a significant difference between the use of filled capsules or the use of capsule contents consumed within a soft-food.

References

Chang, Q., Zuo, Z., Chow, M.S.S., Ho, W.K.K., (2005) Difference in absorption of the two structurally similar flavonoid glycosides, hyperoside and isoquercitrin, in rats. Eur. J. Pharm. Biopharm. 59, 549–555.

Moon YJ, Wang L, DiCenzo R, Morris ME. (2008) Quercetin pharmacokinetics in humans. Biopharm Drug Dispos. May;29(4):205-17.

Zuo, Z., Zhang, L., Zhou, L.M., Chang, Q., Chow, M., (2006) Intestinal absorption of hawthorn flavonoids – in vitro, in situ and in vivo correlations. Life Sci. 79, 2455–2462.

Study Participant Self-Administration Instructions

The study staff will explain how to take the study drug(s) *Isoquercetin capsules,* but these are points to remember:

1) Each capsule will be consumed once daily

2) Capsules may be swallowed with or without food. The capsules can be opened and the contents mixed with a small portion of soft acidic food such as apple sauce, puree of carrots, or pumpkin puree (Phase II only).

3) Missed doses must be taken within four hours of scheduled dose. If longer than four hours, or if dose vomited, skip dose.

Please call your doctor or research nurse before taking any new prescription or over-the-counter medications/supplements other than the study drugs.

For any problems, issues, or questions you may have, please contact: *Kari Nicolazzo, RRN 617-667-8350*

Study Participant Self-Administration Study Drug Diary

Please record how many capsules you take of study drug *Isoquercetin*, the time you take them and any comments here below and bring the completed Diary as well as your study drug supply, including empty bottles, to every study visit. This will help us keep track of your study drug and how well you are tolerating it.

Participant Identifier:
Protocol #: *14-114*
Doctor:
Nurse:

Cycle Number:
Assigned Dose:

You will take the following number of capsules each time (per dose) as listed in the table below:

Isoquercetin	**__ Capsules**	**Once a day**	**Within 4 hours of scheduled dose**

	Date	Number of Isoquercetin Capsules	Time of Dose	Explanation for missed dose
1			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
2			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
3			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
4			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
5			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
6			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
7			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
8			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
9			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
10			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
11			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
12			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
13			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
14			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
15			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	

16			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
17			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
18			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
19			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
20			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
21			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
22			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
23			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
24			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
25			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
26			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
27			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
28			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
29			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	
30			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken	

Participant/Caregiver Signature: _____Date:_____

FOR STUDY TEAM USE ONLY Staff Initials:	
Date Dispensed:	Date Returned:
# pills/caps/tabs dispensed:	# pills/caps/tabs returned:
# pills/caps/tabs that should have been taken:	
Discrepancy Notes:	

EORTC QLQ-C30 (versión 3)

Estamos interesados en algunas cosas sobre usted y su salud. Por favor, conteste todas las preguntas usted mismo/a marcando con un círculo el número que mejor se aplique a su caso. No hay respuestas "correctas" ni "incorrectas". La información que nos proporcione se mantendrá estrictamente confidencial.

Por favor, escriba sus iniciales: _____

Su fecha de nacimiento (día, mes, año): _____

La fecha de hoy (día, mes, año): 31 _____

	Para nada	Un poco	Bastante	Extremada-mente
1. ¿Tiene alguna dificultad para realizar actividades que requieran un gran esfuerzo como llevar una bolsa de compras pesada o una maleta?	1	2	3	4
2. ¿Tiene alguna dificultad para salir a caminar por largo tiempo?	1	2	3	4
3. ¿Tiene alguna dificultad para salir a caminar por corto tiempo fuera de la casa?	1	2	3	4
4. ¿Necesita quedarse en cama o en una silla durante el día?	1	2	3	4
5. ¿Necesita ayuda para comer, vestirse, bañarse o ir al baño?	1	2	3	4

Durante la última semana:

	Para nada	Un poco	Bastante	Extremada-mente
6. ¿Estuvo limitado/a al hacer su trabajo u otras actividades diarias?	1	2	3	4
7. ¿Estuvo limitado/a al hacer sus pasatiempos u otras actividades de tiempo libre?	1	2	3	4
8. ¿Le faltó el aire?	1	2	3	4
9. ¿Ha tenido dolor?	1	2	3	4
10. ¿Necesitó descansar?	1	2	3	4
11. ¿Ha tenido problemas para dormir?	1	2	3	4
12. ¿Se ha sentido débil?	1	2	3	4
13. ¿Le ha faltado el apetito?	1	2	3	4
14. ¿Ha sentido náuseas?	1	2	3	4
15. ¿Ha vomitado?	1	2	3	4
16. ¿Ha estado estreñido/a?	1	2	3	4

Por favor, continúe en la página siguiente

Durante la última semana:	**Para nada**	**Un poco**	**Bastante**	**Extremada-mente**
17. ¿Ha tenido diarrea?	1	2	3	4
18. ¿Estuvo cansado/a?	1	2	3	4
19. ¿Interfirió el dolor con sus actividades diarias?	1	2	3	4
20. ¿Ha tenido dificultad para concentrarse en cosas como leer el periódico o ver televisión?	1	2	3	4
21. ¿Se sintió tenso/a?	1	2	3	4
22. ¿Se preocupó?	1	2	3	4
23. ¿Se sintió irritable (enojado/a)?	1	2	3	4
24. ¿Se sintió deprimido/a?	1	2	3	4
25. ¿Ha tenido dificultad para recordar cosas?	1	2	3	4
26. ¿Ha interferido su condición física o su tratamiento médico con su vida familiar?	1	2	3	4
27. ¿Ha interferido su condición física o su tratamiento médico con sus actividades sociales?	1	2	3	4
28. ¿Le ha causado su condición física o su tratamiento médico dificultades económicas?	1	2	3	4

Para las siguientes preguntas, por favor, marque con un círculo el número del 1 al 7 que mejor se aplique a su caso

29. ¿Cómo calificaría su salud en general durante la última semana?

1	2	3	4	5	6	7
Muy mala						Excelente

30. ¿Cómo calificaría su calidad de vida en general durante la última semana?

1	2	3	4	5	6	7
Muy mala						Excelente

Firma del participante:_____

[redacted]