Phase 0 Pharmacodynamic Study of the Effects of Itraconazole on Tumor Angiogenesis and the Hedgehog Pathway in Early-stage Non-small Cell Lung Cancer

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The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

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LIST OF ABBREVIATIONS

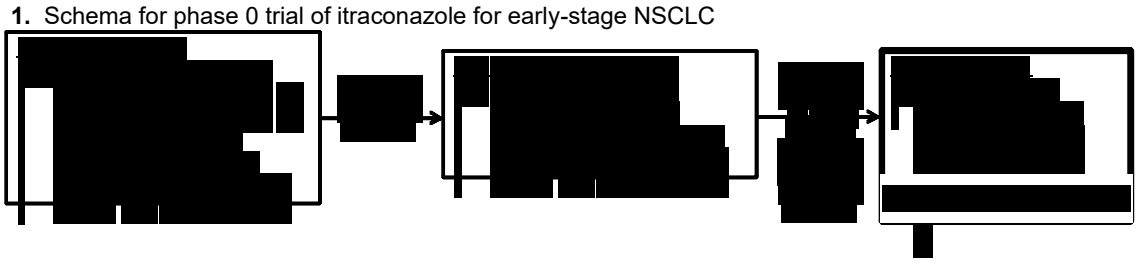
AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DOT	Disease Oriented Team
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IHC	Immunohistochemistry
IND	Investigational New Drug
IV (or iv)	Intravenously
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
pCR	Pathologic Complete Response
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
p.o.	per os/by mouth/orally
PR	Partial Response
RCB	Residual Cancer Burden
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SCCC	Simmons Comprehensive Cancer Center
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
WBC	White Blood Cells

STUDY SCHEMA

This will be a prospective phase 0 trial. Up to 15 eligible patients with previously diagnosed or suspected NSCLC planned for resection will undergo collection of archived biopsy tissue, imaging (dynamic contrast enhanced [DCE]-, diffusion weighted imaging [DWI]-, and arterial spin labeling [ASL] magnetic resonance imaging [MRI]), skin punch biopsy, and collection of peripheral blood. Subjects will then receive itraconazole 600 mg PO daily for 7-10 days, following which they will undergo repeat imaging, skin biopsy, and blood collection. Subsequently they will undergo surgical resection (see **Figure 1**). Due to the safety profile of itraconazole when used as an antifungal agent (1-3), all histologic subtypes of NSCLC will be eligible for the trial. The itraconazole dose of 600 mg, higher than an anti-angiogenic dose (4), has been shown to inhibit the Hedgehog (Hh) pathway (5).

Projected Outcomes

1. Schema for phase 0 trial of itraconazole for early-stage NSCLC



This is a phase 0 clinical trial. While clinical data including safety will be recorded, the principal outcomes are pharmacodynamic endpoints. Specifically, we seek to identify: (1) effects of itraconazole on tumor angiogenesis, (2) effects of itraconazole on the Hh pathway, (3) biomarker predictors of these effects, (4) the correlation between itraconazole pharmacokinetics and these effects, (5) the correlation between different biomarkers.

STUDY SUMMARY

Title	Phase 0 pharmacodynamic study of the effects of itraconazole on tumor angiogenesis and the Hedgehog pathway in early-stage non-small cell lung cancer
Short Title	Neoadjuvant Itraconazole in NSCLC
Protocol Number	STU 122011-038
Phase	Phase 0
Methodology	Prospective, open-label
Study Duration	3 years
Study Center(s)	Single-center
Objectives	Determine the pharmacodynamics effects of itraconazole in early-stage NSCLC
Number of Subjects	15
Diagnosis and Main Inclusion Criteria	Early-stage NSCLC
Study Product(s), Dose, Route, Regimen	Itraconazole 600 mg PO daily for 10-14 days
Duration of administration	10-14 days
Reference therapy	N/A
Statistical Methodology	Paired t-tests or Wilcoxon signed rank-tests will be used to investigate if there is a significant change in the values of tissue and peripheral samples, and imaging from pre-treatment to post-treatment in terms of itraconazole effects on tumor angiogenesis and hedgehog pathway signaling. The nonlinear mixed effects model will be used to determine the effect of itraconazole pharmacokinetics on the pharmacodynamic profile of itraconazole.

1.0 BACKGROUND AND RATIONALE

1.1 Background

Itraconazole, an orally available azole antifungal in clinical use for decades, has emerged as a promising, safe, convenient, and inexpensive treatment for lung cancer. In preclinical models, itraconazole has demonstrated inhibition of (1) tumor angiogenesis and (2) the Hedgehog (Hh) pathway, a critical axis in cancer stem cell development (see **Figure 2**). In multiple primary xenograft models, itraconazole demonstrates potent inhibition of endothelial cell proliferation and angiogenesis, resulting in over 50% reduction in micro vessel density and tumor vascular area. (6-9)

Itraconazole effects on angiogenesis

Itraconazole may have both mechanistic and clinical advantages over bevacizumab (Avastin), the anti-vascular endothelial growth factor (VEGF) monoclonal antibody approved by the U.S. Food and Drug Administration (FDA) for advanced non-squamous non-small cell lung cancer (NSCLC) and the only antiangiogenic agent approved to date for any lung cancer indication. In contrast to bevacizumab, itraconazole appears to affect multiple antiangiogenic pathways, including sterol biosynthesis, disruption of cholesterol trafficking, intratumoral induction of hypoxia inducible factor 1 (HIF1α), and inhibition of VEGF receptor 2 (VEGFR2) and mammalian target of rapamycin (mTOR) pathways(6, 8, 10). Conceivably, an agent with multi-targeted effects may be less susceptible to acquired resistance than are drugs with highly selective mechanisms such as VEGF inhibition. Indeed, in clinical settings, bevacizumab has demonstrated modest and inconsistent clinical benefit (11, 12), conveyed substantial toxicities (including fatal hemoptysis), has had restricted clinical use due to safety concerns (13), and results in a dramatic increase in the cost of care (incremental cost-utility ratio of more than $500,000 per quality adjusted life year [QALY]) (14).

Itraconazole effects on Hedgehog (Hh) pathway

The Hedgehog (Hh) pathway has been implicated in oncogenesis, maintenance of tumor progenitor cells, and tumor-stromal interactions in multiple tumor types (15). These include sporadic cancers such as lung cancer, pancreatic cancer, and prostate cancer(16-23), as well as malignancies with Hh pathway mutations such as basal cell carcinoma (BCC) and medulloblastoma (24, 25). Given the longstanding need for therapies targeting cancer stem cells, preclinical studies demonstrating efficacy in several cancer models, and cases of dramatic clinical benefit in BCC and medulloblastoma, the pharmaceutical industry has recently seen several large-scale efforts to develop Hh pathway antagonists for clinical use, including vismodegib (Erivedge), the first FDA-approved Hh pathway inhibitor, indicated for metastatic BCC.

Although inhibitors of Sonic Hedgehog (SHH) (26), GLI proteins (9, 27, 28), Smoothened (SMO) localization to primary cilia (29), and trafficking of ciliary motors (30) have been reported, these antagonists have been used only for research purposes thus far. SMO, as a central regulator of the pathway and an accessible cell membrane component, has been the primary focus for development of small molecule Hh pathway inhibitors (31). Cyclopamine, the archetypical SMO antagonist, was first described as a steroidal alkaloid teratogen associated with cyclopic lambs (32) and subsequently determined to be a SMO antagonist (33-36). All subsequent small-molecule SMO antagonists have been derived from the structural scaffold of cyclopamine or designed to mimic its mode of binding. In addition to vismodegib (GDC-0449; Genentech), a number of SMO inhibitors are in development and clinical trials, including NVP-LDE225 (Novartis), BMS-833923/XL-139 (BMS/Exelixis), and TAK-441 (Millenium). All of these inhibitors have been designed to be cyclopamine-competitive (36-38).

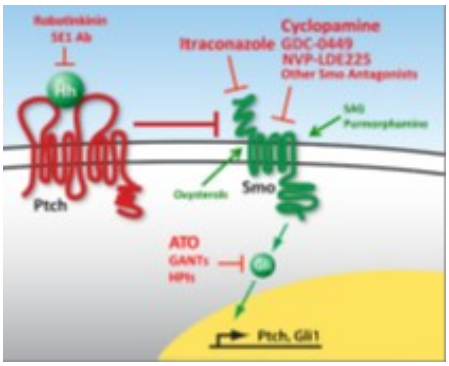


Figure 2. Hh signaling pathway. A simplified schematic view of the Hh signaling pathway. Small compound agonists (*green font*) and antagonists (*red font*) are shown.

Itraconazole may have mechanistic advantages over vismodegib and other Hh pathway antagonists.

Due to their limited mechanistic diversity resistance to these cyclopamine-competitive antagonists has already emerged (39-42). While efforts are underway to develop new small compounds to inhibit drug-resistant SMO mutants (40, 41), there is no clear time-line for when these drugs will enter into clinical trials. In contrast, UT Southwestern researchers have demonstrated that

itraconazole acts on SMO to inhibit the Hh pathway at a site distinct from cyclopamine (see **Figure 2**). Itraconazole inhibits *in vivo* tumor growth of Hh-dependent BCC and medulloblastoma in mice and inhibits the Hh pathway in human BCC (9, 43). We have also shown that itraconazole inhibits the activity of vismodegib-resistant SMO *in vitro* and *in vivo* and has inhibitory activity against all other drug-resistant SMO mutants that have been described (see **Figure 3**). The cyclopamine-independent mechanism by which itraconzole inhibits SMO appears distinct from its anti-fungal target of 14-α-lanosterol demethylase (14LDM) and from its activity as an anti-angiogenic agent (43, 44).

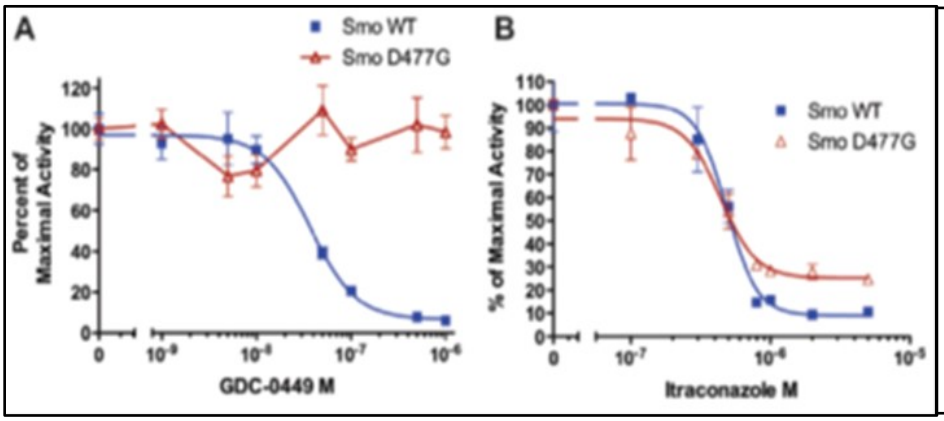


Figure 3. Itraconazole has inhibitory activity against mutant drug-resistant SMO (SMOD477G). Smo-/- cells expressing mouse SMOWT or SMOD477G and stimulated with SHHN-conditioned medium were treated with (A) GDC-0449 (vismodegib) or (B) itraconazole. Itraconazole has inhibitory activity against SMOWT and SMOD477G mediated Hh pathway activation whereas GDC-0449 cannot inhibit mutant SMOD477G activity (A).

The Hh pathway appears to be a relevant target in lung cancer.

Although most studies investigating the Hh pathway in lung cancer have focused on small cell histology (approximately 15% of lung cancers), we and others have demonstrated that transgenic mouse models of non-small cell lung cancer (NSCLC, approximately 85% of cases) and human NSCLC express high levels of Hh compared to normal bronchial epithelial tissue (see **Figure 4**) (22, 23, 45, 46). In pancreatic cancer cell lines, Hh pathway activation has been noted in tumor stroma. Treatment of these mice with SMO antagonists prolonged survival and decreased tumor-associated stroma, suggesting that the Hh pathway may act through a paracrine process (18, 45, 47). The recognition that Hh targeting may be relevant to NSCLC is beneficial not only because this histologic subtype is far more common, but also because early-stage NSCLC cases undergo surgical resection, permitting tumor-level pharmacodynamic assessment of target inhibition, as described in this proposal.

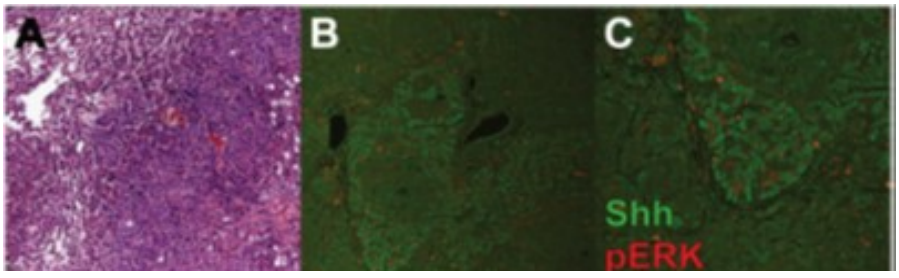


Figure 4. NSCLC expresses sonic hedgehog (Shh). *(A)* H&E section of lung tumors from an *LSL-K-rasG12D/+; Trp53fl/fl* mouse infected with adenovirus-Cre via nasal inhalation. 10x objective. *(B,C)* Shh expression (*green*) and pERK expression (*red*) at 20x *(B)* and 40x *(C)* magnification.

Clinical experience with itraconazole

Itraconazole has a number of favorable characteristics compared to existing antiangiogenic agents and Hh pathway antagonists. Decades of use have shown it to be well tolerated. Well before the agent was recognized for potential effects on angiogenesis, stem cells, and cancer growth, it was administered to lung cancer patients with invasive aspergillus infections without complications (1). Notably, most of these patients had cavitary squamous tumors and underwent invasive procedures while receiving itraconazole, suggesting that—in contrast to bevacizumab—this agent may be safely given to all histologic subtypes of lung cancer, even in the perioperative period.(2, 3) Principal toxicities of itraconazole include gastrointestinal events (nausea, vomiting, and diarrhea in ≤5%) and concern for drug-drug interactions via effects on CYP3A4 (48), PGP1 (48, 49), and protein glycosylation (10, 50). Importantly, adverse effects of vismodegib—which include taste loss, muscle cramps, hair loss, and weight loss—were noted to result in discontinuation of vismodegib treatment in

approximately half of subjects in a pivotal BCC trial (51). Finally, in an era of increasing attention to health care costs, itraconazole ($975/month when dosed 600 mg PO daily) presents a far less costly option than bevacizumab ($8,500/month) or vismodegib ($7,500/month).(52-54)

Clinical studies of itraconazole as an antineoplastic agent have been performed in prostate cancer, NSCLC, and BCC. A recent study using itraconazole 600 mg/day for castrate resistant prostate cancer resulted in decreased Hh pathway activity that correlated with decreased prostate-specific antigen (PSA) levels (5). In a preliminary phase 2 clinical trial, the addition of itraconazole to pemetrexed chemotherapy for second-line treatment of advanced nonsquamous NSCLC resulted in a doubling of progression-free survival (PFS) and a four-fold increase in overall survival (OS) (4). The survival difference reached statistical significance even though the trial was closed to enrollment before reaching target accrual. Based on our results that itraconazole inhibits the growth of endogenous *Ptch+/-; p53-/-* murine BCC and increases tumor necrosis (43), in collaboration with colleagues at Stanford University, members of this study team (Dr. James Kim) conducted an exploratory open label biomarker proof-of-concept study of itraconazole in BCC (NCT01108094), in which itraconazole demonstrated anti-tumor effects and antagonism of Hh pathway (see **Figure 5**). In that study, treatment with itraconazole 400 mg daily for one month produced decreases in Hh pathway activation, tumor size, and proliferation. Specifically, fourteen untreated patients and 15 patients treated with itraconazole 200 mg PO bid for 1 month, all with multiple BCCs, were monitored. Patients who received itraconazole showed decreases in tumor growth on average of 23%. The tumors showed a decrease in Hh pathway activity by 63% as measured by *GLI1* mRNA levels. Itraconazole decreased cell proliferation, measured by Ki-67 staining, by 45% in vismodegib-naïve subjects (*P*=0.04). In aggregate, this pilot trial showed that itraconazole inhibits the Hh pathway of BCCs in humans.

1.2 Rationale

Critical to the further development of itraconazole as a therapeutic agent in lung cancer is a clear understanding of its pharmacodynamic profile in patients. While a small phase 2 trial has demonstrated possible clinical benefit in this patient population (4), neither the mechanism nor predictors of this effect has been demonstrated in humans. The proposed phase 0 study will provide much needed and unprecedented insight via intensive and integrated real-time pharmacodynamic and pharmacokinetic analyses of tumor samples and surrogate tissues. These results will inform subsequent trials, both in terms of treatment selection and also biomarker implementation. Such studies are particularly essential in lung cancer, where perceived challenges to tissue acquisition may hinder implementation of essential bio correlatives. The molecular proof-of-concept investigations we propose may guide large-scale development of itraconazole for lung cancer therapy. Furthermore, because the Hh pathway and angiogenesis are relevant to multiple cancer types, our findings may be more broadly applicable to the field of medical oncology.

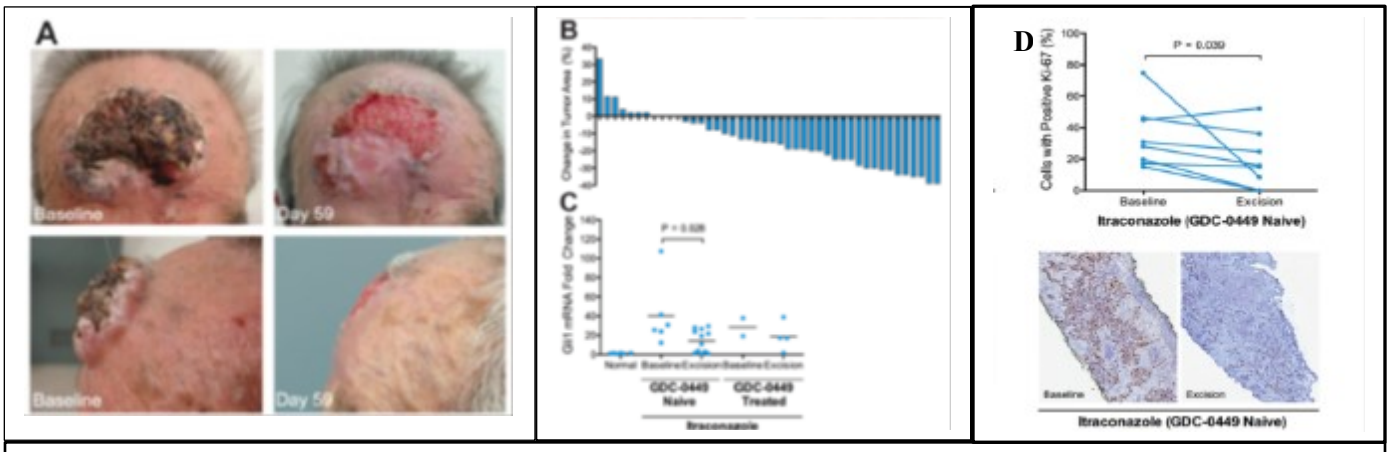


Figure 5. Itraconazole has activity against human BCC. (A) Nodular BCC at baseline and after treatment with itraconazole. (B) During itraconazole treatment, tumor area decreased by an average of 23% (95% CI: 17.2%, 28.1%). (C) Unpaired analysis of individual tumors of treated subjects showed inhibition of Hh pathway as measured by *GLI1* mRNA expression. "GDC-0449 Treated" patients had been treated with and were resistant to GDC-0449 (vismodegib) prior to itraconazole treatment. (D) Paired analysis of patient Ki67 (proliferation marker) averages and Ki67 tumor staining (brown) among itraconazole-treated GDC-0449 (vismodegib)-naïve subjects.

The design of this study incorporates the clinical and preclinical experience to date of itraconazole as a cancer therapeutic. The itraconazole dose was selected based on pharmacodynamic analyses in earlier human studies, suggesting that 600 mg daily provided optimal Hh pathway antagonism (5). The duration of itraconazole therapy is designed to provide sufficient drug exposure to reach steady-stage pharmacokinetics in plasma and tissues, and is also expected to be sufficient for target pharmacodynamic effects, as tumor *Gli1* mRNA has been shown to decrease after as little as 3 days of itraconazole treatment in a medulloblastoma xenograft model (43).

1.3 Correlative Studies

We hypothesize that itraconazole will inhibit tumor angiogenesis and will inhibit Hh pathway activation. The proposed trial will determine the impact of itraconazole on tumor angiogenesis and on the Hh pathway in lung cancer. For the first time in patients, we will determine itraconazole effects on (1) tumor vessel density, (2) tumor perfusion and cellular density, and (3) Hh pathway components including GLI1, SHH, and PTCH1. We will also investigate potential mechanisms of treatment resistance (SMO gene mutations, GLI2 and CCND1 copy number, PI3K-mTOR pathway activation), which have been demonstrated in other tumor types but not previously evaluated in lung cancer.

Separately, this will demonstrate the applicability of new molecular and imaging technologies to the clinical study of lung cancer. Specifically, we will evaluate use of RNAScope (Advanced Cell Diagnostics), a technology for detecting Hh pathway components that could be applied to patient selection in future clinical trials. We will also evaluate use of dynamic contrast enhanced (DCE)-, diffusion weighted imaging (DWI)-, and arterial spin labeling (ASL)-MRI. There has been limited experience in lung cancer with these start-of-the-art imaging techniques due to concerns for motion artifact. However, we have developed motion correction algorithms to address these limitations. Such imaging studies might be applied to patient selection and/or early determination of treatment efficacy in future trials.

2.0 STUDY OBJECTIVES

Primary Objective

- Determine the pharmacodynamic effects of itraconazole on tumor angiogenesis (assessed by changes in tumor tissue microvessel density [MVD])

Secondary Objectives

- Determine the pharmacodynamic effects of itraconazole on tumor angiogenesis (assessed by changes in HIF1α, VEGFR2, phospho-VEGFR2, and other plasma cytokine and angiogenic factor levels)
- Determine the pharmacodynamic effects of itraconazole on tumor angiogenesis (assessed by dynamic contrast enhanced [DCE], diffusion weighted imaging [DWI], and arterial spin labeling [ASL] MRI)
- Determine the pharmacodynamic effects of itraconazole on tumor Hedgehog (Hh) pathway (assessed by changes in tumor tissue GLI1, SHH, and PTCH1 levels)
- Assess predictors of effects of itraconazole on tumor Hedgehog (Hh) pathway (assessed by tumor SMO gene mutations, GLI2 and CCND1 copy number, PI3K-mTOR pathway activation)
- Determine the pharmacodynamic effects of itraconazole on systemic Hedgehog (Hh) pathway (assessed by changes in skin biopsy GLI1, SHH, and PTCH1 levels)
- Determine the effects of itraconazole on tumor cell proliferation and apoptosis (assessed by tumor Ki67 and cleaved caspase 3 levels) and correlate with pharmacodynamic effects on Hh pathway components
- Determine the effects of itraconazole pharmacokinetics on the pharmacodynamic profile of itraconazole (assessed by post-treatment serum, tumor tissue, and skin biopsy itraconazole levels)

3.0 STUDY AGENT

3.1 Drug class and Mechanism

Itraconazole is an anti-fungal drug in the same class of drugs as fluconazole (Diflucan), ketoconazole (Nizoral), and miconazole (Micatin, Monistat). Itraconazole inhibits the cytochrome P450-dependent synthesis of ergosterol and prevents growth of several types of fungi by

preventing the fungi from producing the membranes that surround the fungal cells The FDA approved Itraconazole in September 1992. Itraconazole is commercially available as Sporanox® capsules 100 mg.

3.2 Storage

Capsules and itraconazole patch should be stored at room temperature, 15-25 C (59-77 F) and protected from light and moisture.

3.3 Indication

Itraconazole is used for the treatment of fungal infections. It is active against fungal infections such as aspergillosis, blastomycosis, histoplasmosis, and candidiasis, as well as fungal infection localized to the toenails and fingernails (onychomycosis). It also is used for treating patients with fever and low white blood cell counts who are likely to develop a fungal infection. Do not take itraconazole if pregnant or nursing.

3.4 Dosing

The usual recommended dose is 400-600 mg daily as a single dose or two divided doses. Capsules should be taken with a full meal because food improves absorption. For systemic fungal infections, patients have been treated for one year without serious toxicities. For this study, patients will be treated with itraconazole 300 mg bid (600 mg/day total) for a total of10-14 days. Drug will be provided by the investigators.

3.5 Drug Interactions

Itraconazole reduces the liver metabolism (breakdown) of some drugs, resulting in increased blood levels and side effects from the affected drugs. Life threatening adverse effects occurred when Itraconazole was combined with cisapride (no longer available in the U.S.), pimozide (Orap), quinidine (Quinaglute, Quinidex), dofetilide (Tikosyn), or levomethadyl (Orlaam). Therefore, Itraconazole should not be combined with these drugs. Other drugs whose blood levels are increased by Itraconazole include warfarin (Coumadin), tolbutamide, glyburide (Micronase, Diabeta, Glynase), glipizide (Glucotrol), protease inhibitors [for example, indinavir (Crixivan), ritonavir (Norvir), saquinavir (Invirase, Fortovase)], midazolam (Versed), triazolam (Halcion), "statins" (for example, simvastatin or Zocor) and several others. Itraconazole increases blood levels of certain calcium channel blockers, for example, nisoldipine (Sular) and verapamil (Calan). Such combinations increase the occurrence of heart failure.

3.6 Side Effects

Stomach upset, diarrhea, headache, or dizziness may occur the first few days in adjustment to the medication. Other side effects reported include ringing in the ears, fever, sexual performance problems, depression, drowsiness, or trouble sleeping, severe nausea, yellowing eyes/skin, unusual weakness, dark urine, pale stools, numbness or tingling of the hands/feet. Symptoms of a serious allergic reaction include: rash, itching, swelling, dizziness, trouble breathing. Symptoms such as trouble breathing or ankle/foot swelling could also be due to a rare side effect—congestive heart failure.

3.7 Dose Reduction or Interruption for Toxicity

Subjects experiencing one or more AEs due to study treatment may require reductions in their doses or dosing interruptions in order to continue with study treatment. See section 5.2 for further details.

4.0 SUBJECT ELIGIBILITY

Eligibility waivers will be permitted on a case-by-case basis upon discussion with the Principal Investigator. Study treatment may not begin until a subject is registered.

4.1 Inclusion Criteria

4.1.1 Histologically or cytologically proven NSCLC planned for surgical resection. All NSCLC histologic subtypes are eligible. Alternatively, patients in whom a diagnosis of NSCLC is highly suspected based on history and imaging studies and who are, therefore, scheduled for diagnostic biopsy and/or surgical resection will also be eligible for screening, enrollment, and study treatment if they meet all additional eligibility criteria. In the event that biopsies do not confirm NSCLC, such patients will be removed from study but monitored for any adverse events resulting from study participation.

4.1.2 No prior therapy, but planned for surgical resection. Prior therapy for NSCLC is allowed, as long as no systemic or local therapy has been administered for the disease site planned for resection since the most recent biopsy was performed.

4.1.3 Age ≥ 18 years.

4.1.4 ECOG 0-2 performance status

4.1.5 Adequate organ function as defined below:
- total bilirubin within normal institutional limits
- AST(SGOT)/ALT(SPGT) ≤ 2.5 X institutional upper limit of normal
- creatinine ≤ 2 X institutional upper limit of normal

4.1.6 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, for the duration of study participation, and for 90 days following completion of therapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

4.1.6.1 A female of child-bearing potential is any woman (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- Has not undergone a hysterectomy or bilateral oophorectomy; or
- Has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

4.2 Exclusion Criteria

4.2.1 Subjects may not be receiving any investigational agents that would confound interpretation of study pharmacodynamic endpoints.

4.2.2 History of allergic reactions attributed to itraconazole or to compounds of similar chemical or biologic composition to itraconazole.

4.2.3 Uncontrolled, concurrent medical illness.

4.2.4 Active hepatitis or symptomatic liver disease.

4.2.5. History of or current evidence of uncontrolled cardiac ventricular dysfunction (congestive heart failure) or NYHA Class III or IV heart failure.

4.2.6 Patients currently taking any contraindicated medication listed in Appendix A. If a patient is already taking such a medication, a 4-5 half-life washout period is needed for enrollment.

4.2.7 Current evidence of hyperthyroidism (which would increase metabolism of itraconazole).

4.2.8 Pregnant or lactating female or any female trying to get pregnant.

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Itraconazole is commercially available as Sporanox® capsules 100 mg. Patients will be treated with itraconazole 300 mg bid (600 mg/day total) for a total of10-14 days. Drug will be provided by the investigators. Itraconazole should be taken during or right after a full meal.

Agent	Premedications; Precautions	Dose	Route	Schedule
Itraconazole	take during or right after a full meal	300 mg bid	PO	10-14 days total (see schema, **Figure 1**, above)

A study medication diary will be provided. For missed or vomited doses, if the missed dosed is within 6 hours of the next scheduled dose, the dose should be skipped and the patient should return to his/her regular schedule. Otherwise, the dose should be taken and the next scheduled dose should be pushed back slightly in an attempt to get the doses back on track.

5.2 Toxicities and Dosing Delays/Dose Modifications

Any subject who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events table (Section 5.11). Toxicity will be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

If a subject misses more than 3 doses, the subject may be replaced.

Non-hematological Toxicity Dose Reductions	
Event	**Action**
Nausea/Vomiting	
Grade 1-2	Allow attempt at control, e.g., with anti-emetics
Grade 3	Hold dose and use antiemetics until resolution or improvement to Grade 1-2.
Grade 4	Discontinue itraconazole and monitor.

Diarrhea	
Grade 1-2	Allow attempt at control, e.g., with anti-diarrheal agents
Grade 3	Hold dose and use anti-diarrheal agents until resolution or improvement to Grade 1-2.
Grade 4	Discontinue itraconazole and monitor.
Elevations in ALT or AST	
Grade 1-2	Decrease dose to 300mg PO qday.
Grade 3	Hold dose until resolution.
Grade 4	Discontinue itraconazole and monitor.
Rash	
Grade 1-2	Allow attempt at control, e.g. with topical agents or anti-histamines
Grade 3	Hold dose until resolution or improvement to Grade 1-2.
Grade 4	Discontinue itraconazole and monitor.
Headache	
Grade 1-2	Allow attempt at control with medication
Grade 3	Hold dose until resolution or improvement to Grade 1-2.
Edema	
Grade 1-2	Allow attempt at control with medication.
Grade 3	Hold dose until resolution or improvement to Grade 1-2.

5.3 Concomitant Medications/Treatments

Itraconazole reduces the liver metabolism (breakdown) of some drugs, resulting in increased blood levels and side effects from the affected drugs. Life threatening adverse effects occurred when Itraconazole was combined with cisapride (no longer available in the U.S.), pimozide (Orap), quinidine (Quinaglute, Quinidex), dofetilide (Tikosyn), or levomethadyl (Orlaam). Therefore, Itraconazole should **not** be combined with these drugs.
Other drugs whose blood levels are increased by Itraconazole include warfarin (Coumadin), tolbutamide, glyburide (Micronase, Diabeta, Glynase), glipizide (Glucotrol), protease inhibitors [for example, indinavir (Crixivan), ritonavir (Norvir), saquinavir (Invirase, Fortovase)], midazolam (Versed), triazolam (Halcion), "statins" (for example, simvastatin or Zocor) and several others. Itraconazole increases blood levels of certain calcium channel blockers, for example, nisoldipine (Sular) and verapamil (Calan). Such combinations increase the occurrence of congestive heart failure due to Itraconazole.

Additionally, itraconazole should be used with caution in patients also taking immunosuppressive drugs such as cyclosporin (due to increased plasma levels of these drugs when taken in conjunction with itraconazole). These patients require extra monitoring by the treatment team prescribing the immunosuppressive agent(s).

Drugs that may decrease itraconazole plasma concentrations

It is recommended that itraconazole capsules be administered with an acidic beverage (such as non-diet cola) upon co-treatment with drugs reducing gastric acidity.
It is recommended that acid neutralizing medicines (e.g. aluminum hydroxide) be administered at least 1 hour before or 2 hours after the intake of Itraconazole Capsules.

Coadministration of itraconazole with potent enzyme inducers of CYP3A4 may decrease the bioavailability of itraconazole and hydroxy-itraconazole to such an extent that efficacy may be reduced. Examples include:
Antibacterials: isoniazid, rifabutin, rifampicin
Anticonvulsants: carbamazepine, phenobarbital, phenytoin
Antivirals: efavirenz, nevirapine
Therefore, administration of potent enzyme inducers of CYP3A4 with itraconazole is not recommended. It is recommended that the use of these drugs be avoided from 2 weeks before and during treatment with itraconazole, unless the benefits outweigh the risk of potentially reduced itraconazole efficacy.

Drugs that may increase itraconazole plasma concentrations

Potent inhibitors of CYP3A4 may increase the bioavailability of itraconazole. Examples include:
Antibacterials: ciprofloxacin, clarithromycin, erythromycin
Antivirals: ritonavir-boosted darunavir, ritonavir-boosted fosamprenavir, indinavir, ritonavir.
It is recommended that these drugs be used with caution when coadministered with Itraconazole Capsules. It is recommended that patients who must take itraconazole concomitantly with potent inhibitors of CYP3A4 be monitored closely for signs or symptoms of increased or prolonged pharmacologic effects of itraconazole.

See Appendix A (Drugs that may have their plasma concentrations increased by itraconazole).

5.4 Duration of Therapy

Treatment will be for 10-14 days total with the last dose taken ≤ 12 hours prior to surgical resection OR until the event of

- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Subject decides to withdraw from the study, **OR**
- General or specific changes in the patient's condition render the subject unacceptable for further treatment in the judgment of the investigator

In the event that surgical resection is delayed, patients may continue taking itraconazole if tolerated up until ≤ 12 hours prior to surgical resection

5.5 Duration of Follow Up

All subjects will be followed for 3 weeks following resection. Subjects who experience study-related adverse events will be followed until resolution or stabilization of the adverse event.

Additionally, every year medical records will be evaluated and/or subjects will be contacted regarding disease recurrence and survival information.

5.6 Removal of Subjects from Protocol Therapy

Subjects will be removed from therapy when any of the criteria listed in Section 5.4 apply. Notify the Principal Investigator, and document the reason for study removal and the date the subject was removed in the Case Report Form. The subject should be followed-up according to protocol.

5.7 Subject Replacement

Subjects may be replaced if they do not complete study-directed therapy and surgical resection, or if their biospecimens are not evaluable for study endpoints

STUDY PROCEDURES

5.8 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed within 21 days prior to registration unless otherwise stated. The screening procedures include:

5.8.1 *Informed Consent*

5.8.2 *Medical history*

Complete medical and surgical history, history of infections

5.8.3 *Demographics*

Age, gender, race, ethnicity

5.8.4 *Review subject eligibility criteria*

5.8.5 *Review previous and concomitant medications*

5.8.6 *Physical exam including vital signs, height and weight*

Vital signs (temperature, pulse, respirations, blood pressure), height, weight

5.8.7 *Performance status*

Performance status evaluated prior to study entry according to Appendix D.

5.8.8 *Adverse event assessment*

Baseline adverse events will be assessed. See section 6 for Adverse Event monitoring and reporting.

5.8.9 *Hematology*

CBC with differential, PT/INR, aPTT

5.8.10 *Blood draw for correlative studies*

See Section 5.9 for details.

5.8.11 *Serum chemistries*

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

5.8.12 *Pregnancy test (for females of child bearing potential)*

See section 4.1.6.1 for definition.

5.9 Procedures During Treatment

- All screening procedures must take place within 21 days of trial registration unless otherwise stated.

- Following screening, pre-study ("Baseline") assessments will include history and physical exam, performance status evaluation, laboratory studies including a blood draw for the cytokine panelarchived tumor collection from diagnostic biopsy, skin biopsy, and the MRI evaluations (anticipated to last 45-50 minutes). In certain cases when there is a high suspicion of cancer prior to the diagnostic biopsy, the collection of study-required tissue can occur at the time of the diagnostic biopsy (see Inclusion Criteria 4.1.1).

- Once on study, itraconazole will be taken twice daily for a total of 10-14 days, After being on itraconazole for 7-10 days, "Post-treatment" assessments will include a skin biopsy, blood draw for the PK analyses and the cytokine panel, and also MRI evaluations (anticipated to last 45-50 minutes). Toxicity assessments and laboratory checks will also be conducted during that time. Following these assessments, itraconazole will be continued until the day of surgery.

- At the time of surgical resection, a tissue sample (with adjacent normal tissue as per standard resection technique) will be obtained to complete the analysis.

- Following resection, subjects will be followed per standard post-operative procedure.

Samples will be collected at the following time points per patient:

Baseline prior to study treatment:

- Collection of archive tissue from the diagnostic biopsy. A core-needle biopsy must have been completed. A fine needle aspiration (FNA) will not be sufficient. 12 unstained slides are needed for this study. Fresh tumor may be collected in cases where the diagnosis of NSCLC is highly suspected and the patient will be sent for a diagnostic biopsy.
- Skin biopsy- one 4mm punch biopsy
- Blood sample
  - One 3mL EDTA-containing tube for multiplex cytokine panel

7-10 days post itraconazole initiation:

- Skin biopsy- one 4mm punch biopsy
- Blood samples
  - One 3mL EDTA-containing tube for multiplex cytokine panel
  - One 3mL EDTA-containing tubes at each of the following PK time points: 4, 6, 8 (or as late as possible prior to discharge) hours.

Surgical Resection:

- 1 tissue sample will be obtained for analysis of itraconazole content (PK)
- 1 3mL EDTA containing tube for Itraconazole PK

5.10 Follow-up Procedures

Following resection, subjects will be followed per standard post-operative procedure. Subjects who experience study-related adverse events will be followed until resolution or stabilization of the adverse event.

Additionally, every year medical records will be evaluated and/or subjects will be contacted regarding disease recurrence and survival information.

5.11 Time and Events Table

Event	Pre-study		Days 1-10*	post-treatment assessments (after 7-10 days of itraconazole bid)	Day 8-14**	Resection (Non-study)
	Screen-ing	Base-line				
Archived tumor tissue^	X					
Phone assessment (to evaluate for adherence and any symptoms)			on day 3			
Informed Consent	X					
History and PE	X			X		
Performance Status	X					
Toxicity Evaluations				X		
Labs#	X		X (on day 5, 6, 7, or 8)			
Skin biopsy	X			X		
Blood draw for multiplex correlative studies	X			X		
Blood draw for PK studies				X		X
Imaging Studies §	X			X		
Itraconazole bid			X	X	X	
Tissue sample from non-study surgical biopsy						X

^ 12 unstained slides will be collected from the diagnostic core-needle biopsy. In cases where malignancy is highly suspected, tissue may be collected from the diagnostic biopsy to be performed.

***** To allow for scheduling, this phase will last between 7 and 10 days.

** To allow for scheduling, this phase will last between 4 and 7 days.

#Hematology and chemistry labs as detailed in Section 6.1; on day 5, 6, 7 or 8 only chemistry is required

§ MRI studies may be not be performed if the tumor is too small for imaging assessments. Patients may also be excluded from the imaging portion of the study for safety reasons. See section 7.0 for additional information.

5.12 Removal of Subjects from Study

Subjects can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The specific reason(s) for discontinuation will be documented and may include:

5.12.1 Subject voluntarily withdraws from treatment (follow-up permitted);

5.12.2 Subject withdraws consent (termination of treatment and follow-up);

5.12.3 Subject is unable to comply with protocol requirements;

5.12.4 Subject experiences toxicity that makes continuation in the protocol unsafe;

5.12.5 Treating physician deems continuation on the study would not be in the subject's best interest;

5.12.6 Subject becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

5.12.7 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

5.13. Safety/tolerability

Analyses will be performed for all subjects having received at least one dose of study drug. The study will use the CTCAE version 4.0 for reporting of adverse events (http://ctep.cancer.gov/reporting/ctc.html)

6.0 ADVERSE EVENTS

6.1 Experimental Therapy

Please see Section 3.0 and 5.3 for information on Itraconazole. For the most recent safety update, please refer to the Itraconazole drug packet insert.

6.1.1 Contraindications

Itraconazole reduces the liver metabolism (breakdown) of some drugs, resulting in increased blood levels and side effects from the affected drugs. Life threatening adverse effects occurred when Itraconazole was combined with cisapride (no longer available in the U.S.), pimozide (Orap), quinidine (Quinaglute, Quinidex), dofetilide (Tikosyn), or levomethadyl (Orlaam). Therefore, Itraconazole should **not** be combined with these drugs.

Itraconazole should not be administered to pregnant patients or to women contemplating pregnancy.

Itraconazole Capsules should not be administered in patients with evidence of ventricular dysfunction such as congestive heart failure (CHF) or a history of CHF.

Itraconazole is contraindicated for patients who have shown hypersensitivity to itraconazole. There is limited information regarding cross-hypersensitivity between itraconazole and other azole antifungal agents. Caution should be used when prescribing itraconazole to patients with hypersensitivity to other azoles.

6.1.2 Special Warnings and Precautions for Use

Itraconazole Capsules should not be used for other indications in patients with evidence of ventricular dysfunction unless the benefit clearly outweighs the risk.

Rare cases of serious hepatotoxicity have been observed with itraconazole treatment, including some cases within the first week. It is recommended that liver function monitoring be considered in all patients receiving itraconazole. Treatment should be stopped immediately and liver function testing should be conducted in patients who develop signs and symptoms suggestive of liver dysfunction.

If neuropathy occurs that may be attributable to Itraconazole Capsules, the treatment should be discontinued.

Transient or permanent hearing loss has been reported in patients receiving treatment with itraconazole. Several of these reports included concurrent administration of quinidine which is contraindicated. The hearing loss usually resolves when treatment is stopped, but can persist in some patients

6.1.3 Interaction with other medications

It is recommended that itraconazole capsules be administered with an acidic beverage (such as non-diet cola) upon co-treatment with drugs reducing gastric acidity.
It is recommended that acid neutralizing medicines (e.g. aluminum hydroxide) be administered at least 1 hour before or 2 hours after the intake of Itraconazole Capsules.
Upon coadministration, it is recommended that the antifungal activity be monitored and the itraconazole dose increased as deemed necessary.

Coadministration of itraconazole with potent enzyme inducers of CYP3A4 may decrease the bioavailability of itraconazole and hydroxy-itraconazole to such an extent that efficacy may be reduced. Examples include:
Antibacterials: isoniazid, rifabutin, rifampicin
Anticonvulsants: carbamazepine, phenobarbital, phenytoin
Antivirals: efavirenz, nevirapine
Therefore, administration of potent enzyme inducers of CYP3A4 with itraconazole is not recommended. It is recommended that the use of these drugs be avoided from 2 weeks before and during treatment with itraconazole, unless the benefits outweigh the risk of potentially reduced itraconazole efficacy. Upon coadministration, it is recommended that the antifungal activity be monitored and the itraconazole dose increased as deemed necessary.

Potent inhibitors of CYP3A4 may increase the bioavailability of itraconazole. Examples include:
Antibacterials: ciprofloxacin, clarithromycin, erythromycin
Antivirals: ritonavir-boosted darunavir, ritonavir-boosted fosamprenavir, indinavir, ritonavir.
It is recommended that these drugs be used with caution when coadministered with Itraconazole Capsules. It is recommended that patients who must take itraconazole concomitantly with potent inhibitors of CYP3A4 be monitored closely for signs or symptoms of increased or prolonged pharmacologic effects of itraconazole, and the itraconazole dose be decreased as deemed necessary.

Drugs whose blood levels are increased by Itraconazole include warfarin (Coumadin), tolbutamide, glyburide (Micronase, Diabeta, Glynase), glipizide (Glucotrol), protease inhibitors [for example, indinavir (Crixivan), ritonavir (Norvir), saquinavir (Invirase, Fortovase)], midazolam (Versed), triazolam (Halcion), "statins" (for example, simvastatin or Zocor) and several others. Itraconazole increases blood levels of certain calcium channel blockers, for example, nisoldipine (Sular) and verapamil (Calan). Such combinations increase the occurrence of congestive heart failure due to Itraconazole. **Please also see Appendix A: Drugs that may have their plasma concentrations increased by itraconazole**

6.1.4 Adverse Reactions

Please see Section 3.6.

6.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of subject safety and care.

All subjects experiencing an adverse event, regardless of its relationship to study drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

6.2.1 Definition

An adverse event is defined as any untoward or unfavorable medical occurrence in a human research study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, clinical event, or disease, temporarily associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

Adverse events encompass clinical, physical and psychological harms. Adverse events occur most commonly in the context of biomedical research, although on occasion, they can occur in the context of social and behavioral research. Adverse events may be expected or unexpected.

Severity

Adverse events will be graded by a numerical score according to the defined NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) and version number specified in the protocol. Adverse events not specifically defined in the NCI CTCAE will be scored on the Adverse Event log according to the general guidelines provided by the NCI CTCAE and as outlined below.

- Grade 1: Mild
- Grade 2: Moderate
- Grade 3: Severe or medically significant but not immediately life threatening
- Grade 4: Life threatening consequences
- Grade 5: Death related to the adverse event

Serious Adverse Events

ICH Guideline E2A and the UTSW IRB define serious adverse events as those events, occurring at any dose, which meets any of the following criteria:

- Results in death
- Immediately life-threatening

- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Note: A "Serious adverse event" is by definition an event that meets ***any*** of the above criteria. Serious adverse events may or may not be related to the research project. A serious adverse event determination does not require the event to be related to the research. That is, both events completely unrelated to the condition under study and events that are expected in the context of the condition under study may be serious adverse events, independent of relatedness to the study itself. As examples, a car accident requiring overnight hospitalization would be a serious adverse event for any research participant; likewise, in a study investigating end-stage cancer care, any hospitalization or death would be a serious adverse event, even if the event observed is a primary clinical endpoint of the study. Refer to the UTSW IRB website at http://www.utsouthwestern.net/intranet/research/research-administration/irb/study-management/adverse-events.html to determine when a serious adverse event requires reporting to the IRB.

Unanticipated Problems

The term "unanticipated problem" is found, but not defined in the regulations for the Protection of Human Subjects at 45 CFR 46, and the FDA regulations at 21 CFR 56. Guidance from the regulatory agencies considers unanticipated problems to include any incident, experience, or outcome that meets ***each*** of the following criteria:

- Unexpected (in terms of nature, severity or frequency) ***AND***
- Definitely, probably, or possibly related to participation in the research ***AND***
- Serious or a possible unexpected problem in that the research places subjects or others at greater risk of harm than was previously known or recognized. Note: Any serious adverse event would always suggest a greater risk of harm.

Follow-up

All adverse events will be followed up according to good medical practices.

6.2.2 Reporting

Local unanticipated problems require expedited reporting, and are submitted to the UTSW IRB through the UTSW eIRB and to the SCC DSMC Coordinator. Hardcopies or electronic versions of the eIRB report; FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator. The DSMC Coordinator forwards the information onto the DSMC Chairman who determines if immediate action is required. Follow-up eIRB reports, and all subsequent SAE documentation that is available are also submitted to the DSMC Chair who determines if further action is required.

All local serious adverse events which occur on research subjects on protocols for which the SCC is the DSMC of record require reporting to the DSMC regardless of whether IRB reporting is required. Hardcopies or electronic versions of the FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator.

If the event occurs on a multi-institutional clinical trial coordinated by the Cancer Center, the DOT Manager or lead coordinator ensures that all participating sites are notified of the event and resulting action, according to FDA guidance for expedited reporting. DSMC Chairperson reviews all serious adverse events within upon receipt from the DSMC Coordinator. The DSMC Chairperson determines whether action is required and either takes action immediately, convenes a special DSMC session (physical or electronic), or defers the action until a regularly scheduled DSMC meeting.

Telephone reports to: (David Gerber) UTSW SCC Data Safety Monitoring Committee Coordinator (if fax report is not available) within 2 working days to 214-648-7097.
Written reports to: (David Gerber) UTSW SCC Data Safety Monitoring Committee Coordinator Email: SCCDSMC@utsouthwestern.edu Fax: 214-648-7018 or deliver to NB 2.418 UTSW Institutional Review Board (IRB) Submit via eIRB with a copy of the final sponsor report as attached supporting documentation

1. SAEs

Serious adverse events (SAEs) will be reported to the DSMC coordinator within 2 working days of PI awareness.

2. Unanticipated Problems

Unanticipated problems will be reported to the UTSW IRB within 2 working days of PI awareness of the event.

For further guidance for Investigators regarding safety reporting requirements for INDs and BA/BE studies, refer to FDA Draft Guidance document: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf

6.3 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v4).

Step 2: Grade the adverse event using the NCI CTCAE v4.

Step 3: Determine whether the adverse event is related to the protocol therapy Attribution categories are 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.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

6.4 Role of the Research Monitor

The role and responsibilities of the research monitor will include:

1. May discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research.
2. Shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's report.
3. Shall have the responsibility to promptly report their observations and findings to the IRB or other designated official.
4. Is required to review all unanticipated problems involving risks to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor must comment on the outcomes of the event or problem and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The research monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and report of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO.

7.0 CORRELATIVES/SPECIAL STUDIES

Angiogenesis endpoints

Tissue (tumor): The proposed tissue correlates include multiple angiogenic biomarkers which will be assessed by standard immunohistochemical methods. The Brekken Lab has extensive experience in immunohistochemical analysis of human clinical tissue specimens) and in immunohistochemical analysis of angiogenic biomarkers in several preclinical tumor models (55-58).

Blood: As described previously (59), we will employ a commercially available kit to measure plasma cytokine and angiogenic factor levels. Plasma will be collected as detailed in "Biospecimen collection and processing."

Imaging: Imaging studies will include DCE- and DWI-MRI (both of which are established technologies to assess microvessel density and cellularity (60)) as well as ASL-MRI (an exploratory platform to assess perfusion and vascular density without contrast). Motion correction techniques will be employed (61). Together, these studies will take approximately 45-50 minutes per time-point. Patients may be excluded from MRI examinations for any of the following reasons:

1. Tumor size may be too small for imaging studies.
2. Claustrophobia that would interfere with MRI studies anticipated to last 45-50 minutes.

3. Metal implants deemed at risk for migration during MRI studies.
4. CrCl < 45 mL/min (increased risk of nephrogenic systemic fibrosis [NSF] from MRI Gadolinium contrast).
5. Known allergy to MRI contrast.

DCE-MRI. Each consented participant will undergo point of care testing for creatinine on the day of the examination. We will employ Gadavist, an FDA approved macrocyclic agent in order to minimize risk of nephrogenic systemic fibrosis (NSF). DCE-MRI will be carried out on a 3.0 Tesla Philips whole-body scanner using a turbo fast low-angle gradient echo sequence (TFE) combined with parallel acquisition SENSE technique. The images will be obtained in each patient so that the tumor moves along the imaging plane based on a method developed in our previous work (62). Images will be acquired after intravenous administration of a standard dose of 0.1 mmol per kg of body weight of Gadavist (Schering AG, Germany) over a 4-s period with subjects in the supine position, breathing normally during the scan.

DWI-MRI. MRI examinations will be performed on the 3 Tesla Philips Ingenia MRI scanner using a phased-array body coil. Data for apparent diffusion coefficient assessment on diffusion-weighted imaging will be acquired using an Echoplanar Imaging Inversion Recovery sequence acquired during either breath hold or using respiratory triggering in the desired plane to image the tumor.

ASL-MRI. Arterial spin labeling (ASL) has been used extensively to measure tissue perfusion in the brain (63, 64). ASL uses endogenous spins to measure tissue perfusion without having any complications due to permeability contributions. ASL in the lung has been developed and evaluated in normal volunteers (65, 66). However, its evaluation in lung pathology has been limited to date,. We will measure tumor perfusion using either pulsed ASL (e.g. FAIRER) or a high labeling efficient pseudo-continuous ASL (pCASL) (67). These approaches offer increased labeling efficiency and improved SNR. These characteristics should make ASL an attractive non-invasive method for measuring both absolute perfusion in lung cancer and changes in perfusion upon treatment.

Image data from all MRI examinations will be stored on a dedicated computer workstation for analysis. Image data will be correlated with other pharmacodynamic endpoints (eg, K_{trans} with MVD; ADC with cellularity).

Itraconazole pharmacokinetics (plasma, skin, tumor)

These analyses will be performed at the Clinical Pharmacology & Experimental Therapeutics Center at Texas Tech University School of Pharmacy. The Center laboratory includes a state-of-the-art mass spectrometry core including AB Sciex 5500 QTRAP®, AB Sciex 5600 TripleTOF™, and Agilent 7700x ICP-MS, and specializes in development of novel methods of analyses for analytes contained in complex biologic matrices, such as tumor tissue and skin biopsies. The Center is strategically located adjacent to the Simmons Cancer Center to support collaborative research initiatives such as the current proposal.

8.0 STATISTICAL CONSIDERATIONS

8.1 Sample size estimate

Paired t-tests or Wilcoxon signed rank-tests will be used to investigate if there is a significant change in microvessel density (MVD) and angiogenic cytokine levels from pre-treatment to post-treatment. We will enroll up to 15 patients. A sample size of 10 patients will have 90% power to detect a 30% reduction in MVD between the pre-and post-itraconazole tissue samples, assuming a standard deviation of 25% using a paired t-test with a two-sided significance level of 0.05. This is a relatively conservative estimate of effect size, as itraconazole reduces MVD by 50-75% in preclinical lung cancer models (6). Up to 5 additional patients will be accrued as needed to ensure that we have 10 matched pairs of imaging, blood, and tissue data for analysis. In exploratory analyses, we will evaluate the association between baseline biomarkers and pharmacodynamic effects of itraconazole.

The planned sample size of 15 patients is consistent with FDA recommendations for human PK studies. Specifically, the number of subjects is projected to provide (1) 20% precision (SEM)

within PK parameters, and (2) a reasonable understanding of intra-individual variability in pharmacokinetic parameters. (68)

Additionally, subjects who miss more than 3 doses may be replaced with additional patients to be included in the analyses.

8.2 Statistical analyses

To determine effects of itraconazole on tumor angiogenesis, paired t-tests or Wilcoxon signed rank-tests will be used to investigate if there is a significant change in the values of tissue and peripheral samples, and imaging from pre-treatment to post-treatment.

In order to determine effects of itraconazole on the Hedgehog pathway, pre- and post-treatment tissue samples and skin biopsies will be analyzed for GLI1 mRNA levels and compared using paired t-tests or Wilcoxon signed rank-tests. The nonlinear mixed effects model has been widely used to investigate the effect of pharmacokinetics (PK) on the pharmacodynamic profile of a drug compound.

The nonlinear mixed effects model will be used to determine the effect of itraconazole pharmacokinetics (PK) on the pharmacodynamic profile of itraconazole. Post-treatment serum and tissue will be analyzed for itraconazole concentrations. Mean serum and tissue concentration-time profiles of itraconazole will be plotted on linear and semi-logarithmic scales based on scheduled sample times. For values recorded above the upper limit of quantification (ULoQ) or below the lower limit of quantification (LLoQ), the ULoQ or LLoQ respectively will be used for any summaries and plots. The individual serum and tissue concentration over actual time data will be used to derive the pharmacokinetic (PK) parameters by non-compartmental PK analyses using Phoenix WinNonlin 6.1 or higher.

The following PK parameters will be computed: area under the concentration-time curve over the dosing interval (AUC), observed maximum serum and tissue concentration at steady state ($C_{max,ss}$), average concentration at steady state (C_{av}), calculated as AUC_{τ}/τ, minimum concentration immediately before the next application (C_{min}), and terminal elimination half-life calculated from the pharmacokinetic profile ($T_{1/2}$). Effects of these PK parameters will be compared to pharmacodynamic assay results using nonlinear mixed effects model.

9.0 STUDY MANAGEMENT

9.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the UTSW COI Committee and IRB according to UTSW Policy on Conflicts of Interest. All investigators will follow the University conflict of interest policy.

9.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB must approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form.

Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

9.3 Registration Procedures

All subjects must be registered with the Harold C. Simmons Cancer Center Clinical Research Office (CRO) before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the CRO Study Coordinator.

9.4 Data Management and Monitoring/Auditing

Trial monitoring will be conducted no less than annually and refers to a regular interval review of trial related activity and documentation performed by the DOT, which includes but is not limited to accuracy of case report forms, protocol compliance, timeliness and accuracy of Velos entries and AE/SAE management and reporting. Documentation of trial monitoring will be maintained along with other protocol related documents and will be reviewed during internal audit.

The UTSW Simmons Cancer Center (SCC) Data and Safety Monitoring Committee (DSMC) is responsible for overall monitoring of data quality and patient safety. As part of that responsibility, the DSMC reviews all local serious adverse events and unanticipated problems in real time as they are reported and reviews adverse events on a quarterly basis. The Quality Assurance activity of the Clinical Research Office provides for periodic auditing of clinical research documents to ensure data integrity and regulatory compliance based on risk.

The SCC-DSMC meets quarterly and conducts annual comprehensive reviews of ongoing clinical trials, for which it is the DSMC of record.

Further detail may be found in the SCC-DSMC Plan.

9.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

9.5.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

9.5.2 Other Protocol Deviations/Violations

All other planned deviations from the protocol must have prior approval by the Principal Investigator and the IRB. According to the IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs without prior approval from the Principal Investigator, please follow the guidelines below:

Protocol Deviations: Personnel will report to the SCC DSMC in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations: Study personnel should report violations within two (2) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

9.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. A summary of changes document outlining proposed changes as well as rationale for changes, when appropriate, is highly recommended. When an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

9.7 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study

with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

9.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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11.0 APPENDICES

Appendix A: Drugs that may have their plasma concentrations increased by itraconazole

Appendix B: Biospecimen collection and processing:

Appendix C: Pill Diary

Appendix D: ECOG performance status scale

Appendix A: Drugs that may have their plasma concentrations increased by itraconazole
[https://druginserts.com/lib/rx/meds/itraconazole/page/2/]

Itraconazole and its major metabolite, hydroxy-itraconazole, can inhibit the metabolism of drugs metabolized by CYP3A4 and can inhibit the drug transport by P-glycoprotein, which may result in increased plasma concentrations of these drugs and/or their active metabolite(s) when they are administered with itraconazole. These elevated plasma concentrations may increase or prolong both therapeutic and adverse effects of these drugs. CYP3A4-metabolized drugs known to prolong the QT interval may be contraindicated with itraconazole, since the combination may lead to ventricular tachyarrhythmias including occurrences of torsade de pointes, a potentially fatal arrhythmia. Once treatment is stopped, itraconazole plasma concentrations decrease to an almost undetectable concentration within 7 to 14 days, depending on the dose and duration of treatment. In patients with hepatic cirrhosis or in subjects receiving CYP3A4 inhibitors, the decline in plasma concentrations may be even more gradual. This is particularly important when initiating therapy with drugs whose metabolism is affected by itraconazole.

Examples of drugs that may have their plasma concentrations increased by itraconazole presented by drug class with advice regarding coadministration with itraconazole:

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
	Under no circumstances is the drug to be coadministered with itraconazole, and up to two weeks after discontinuation of treatment with itraconazole.	*It is recommended that the use of the drug be avoided during and up to two weeks after discontinuation of treatment with itraconazole, unless the benefits outweigh the potentially increased risks of side effects. If coadministration cannot be avoided, clinical monitoring for signs or symptoms of increased or prolonged effects or side effects of the interacting drug is recommended, and its dosage be reduced or interrupted as deemed necessary. When appropriate, it is recommended that plasma concentrations be measured. The label*	*Careful monitoring is recommended when the drug is coadministered with itraconazole. Upon coadministration, it is recommended that patients be monitored closely for signs or symptoms of increased or prolonged effects or side effects of the interacting drug, and its dosage be reduced as deemed necessary. When appropriate, it is recommended that plasma concentrations be measured. The label of the coadministered drug should be consulted for information on dose adjustment and adverse effects.*	

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
		of the coadministered drug should be consulted for information on dose adjustment and adverse effects.		
Alpha Blockers		tamsulosin		
Analgesics	methadone		alfentanil, buprenorphine IV and sublingual, fentanyl, oxycodone, sufentanil	The potential increase in plasma concentrations of methadone when coadministered with itraconazole may increase the risk of serious cardiovascular events including QTc prolongation and . **Methadone:** *torsade de pointes*
				The potential increase in plasma concentrations of fentanyl when coadministered with itraconazole may increase the risk of potentially fatal respiratory depression. **Fentanyl:**
				No human pharmacokinetic data of an interaction with itraconazole are available. In vitro data suggest that sufentanil is metabolized by CYP3A4 and so potentially increased sufentanil plasma concentrations would be expected when coadministered with itraconazole. **Sufentanil:**
Antiarrhythmics	disopyramide, dofetilide, dronedarone, quinidine		digoxin	The potential increase in plasma concentrations of these drugs when coadministered with itraconazole may increase the risk of serious cardiovascular events including QTc prolongation. **Disopyramide,**

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
				dofetilide, dronedarone, quinidine:
Antibacterials		rifabutin		**Rifabutin:**
Anticoagulants and Antiplatelet Drugs		rivaroxaban	coumarins cilostazol, dabigatran	Itraconazole may enhance the anticoagulant effect of coumarin-like drugs, such as warfarin. **Coumarins:**
Anticonvulsants		carbamazepine		studies have demonstrated an increase in plasma carbamazepine concentrations in subjects concomitantly receiving ketoconazole. Although there are no data regarding the effect of itraconazole on carbamazepine metabolism, because of the similarities between ketoconazole and itraconazole, concomitant administration of itraconazole and carbamazepine may inhibit the metabolism of carbamazepine. **Carbamazepine:** *In vivo*
Antidiabetics			repaglinide, saxagliptin	
Antihelmintics and Antiprotozoals			praziquantel	
Antimigraine Drugs	ergot alkaloids, such as dihydroergotamine, ergometrine (ergonovine), ergotamine, methylergometrine (methylergonovine)		eletriptan	The potential increase in plasma concentrations of ergot alkaloids when coadministered with itraconazole may increase the risk of ergotism, ie. a risk for vasospasm potentially leading to cerebral ischemia and/or ischemia of the extremities. **Ergot Alkaloids:**
Antineoplastics	irinotecan	dasatinib, nilotinib	bortezomib, busulphan, docetaxel, erlotinib, ixabepilone, lapatinib, trimetrexate, vinca alkaloids	The potential increase in plasma concentrations of irinotecan when coadministered with

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
				itraconazole may increase the risk of potentially fatal adverse events. **Irinotecan:**
Antipsychotics, Anxiolytics and Hypnotics	lurasidone, oral midazolam, pimozide, triazolam		alprazolam, aripiprazole, buspirone, diazepam, haloperidol, midazolam IV, perospirone, quetiapine, ramelteon, risperidone	Coadministration of itraconazole and oral midazolam, or triazolam may cause several-fold increases in plasma concentrations of these drugs. This may potentiate and prolong hypnotic and sedative effects, especially with repeated dosing or chronic administration of these agents. **Midazolam, triazolam** The potential increase in plasma concentrations of pimozide when coadministered with itraconazole may increase the risk of serious cardiovascular events including QTc prolongation and . **Pimozide:** *torsade de pointes*
Antivirals			maraviroc, indinavir, ritonavir saquinavir	**Indinavir, ritonavir**
Beta Blockers			nadolol	
Calcium Channel Blockers	felodipine, nisoldipine		other dihydropyridines, verapamil	can have a negative inotropic effect which may be additive to those of itraconazole. The potential increase in plasma concentrations of calcium channel blockers when co-administered with itraconazole may increase the risk of congestive heart failure. **Calcium channel blockers** Concomitant administration of itraconazole may cause several-fold increases in plasma

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
				concentrations of dihydropyridines. Edema has been reported in patients concomitantly receiving itraconazole and dihydropyridine calcium channel blockers. **Dihydropyridines:**
Cardiovascular Drugs, Miscellaneous	ranolazine	aliskiren		The potential increase in plasma concentrations of ranolazine when coadministered with itraconazole may increase the risk of serious cardiovascular events including QTc prolongation. **Ranolazine:**
Diuretics	eplerenone			The potential increase in plasma concentrations of eplerenone when coadministered with itraconazole may increase the risk of hyperkalemia and hypotension. **Eplerenone:**
Gastrointestinal Drugs	cisapride		aprepitant	The potential increase in plasma concentrations of cisapride when coadministered with itraconazole may increase the risk of serious cardiovascular events including QTc prolongation. **Cisapride:**
Immunosuppressants		everolimus, temsirolimus	budesonide, ciclesonide, cyclosporine, dexamethasone, fluticasone, methylprednisolone, rapamycin (also known as sirolimus), tacrolimus	
Lipid Regulating Drugs	lovastatin, simvastatin		atorvastatin	The potential increase in plasma concentrations of atorvastatin, lovastatin, and simvastatin when coadministered with itraconazole may

Drug Class	Contraindicated	Not Recommended	Use with Caution	Comments
				increase the risk of skeletal muscle toxicity, including rhabdomyolysis.
Respiratory Drugs		salmeterol		
Urological Drugs		vardenafil	fesoterodine. sildenafil, solifenacin, tadalafil, tolterodine	
Other	colchicine, in subjects with renal or hepatic impairment	colchicine	cinacalcet, tolvaptan	The potential increase in plasma concentrations of colchicine when coadministered with itraconazole may increase the risk of potentially fatal adverse events. **Colchicine:**

Appendix B: Biospecimen collection and processing:

Tumor specimens will be obtained from archived samples of the pre-treatment core-needle biopsy and core(s) obtained from the resected specimen at the time of surgery. To account for intratumoral heterogeneity, we will employ image guidance for pre- and post-treatment tissue sampling, thereby obtaining tissue from similar sites within the tumor. Specimens will be divided, with half to be (1) flash frozen in liquid nitrogen (for qPCR for Hh pathway components), and half designated to be (2) fixed in 4% paraformaldehyde (for RNAScope for Hh pathway components and IHC for angiogenic markers).

Blood samples will be collected in the Simmons Cancer Center laboratory. Plasma samples with EDTA will be collected at specified time-points. Samples will be centrifuged at 1,100 × g (relative centrifugal force) for 15 min at 4°C for separation of plasma and mononuclear cell layers. Plasma will be stored at −70°C. Before analysis, samples will be thawed overnight at 4°C and centrifuged at 1,500 × g to remove debris. PK time points have been selected to provide sufficient PK data but also to fit within hours of operation of this facility, thereby avoiding the need to admit subjects to our inpatient unit.

Skin biopsies will be performed by trained study personnel according to previously-described methods. (69) A 4-mm punch biopsy will be performed with a single suture placed after the procedure. Lidocaine will be used to numb the patient skin and antibiotic ointment will be applied to the suture site. The specimen will be flash frozen for RNA procurement (PCR).

Appendix C: Pill Diary

Itraconazole pill diary

Day	Date MM/DD/YYYY	Time hh:mm		Number of pills taken	
		AM	PM	AM	PM
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

Dosing instructions: Take itraconazole with a meal.

Appendix D: ECOG performance status scale:

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

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