

CC# 125513: Hedgehog Inhibition as a Non-Castrating Approach to Hormone Sensitive Prostate Cancer: A Phase II Study of Itraconazole in Biochemical Relapse

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ABSTRACT

Title	Hedgehog Inhibition as a Non-Castrating Approach to Hormone Sensitive Prostate Cancer: A Phase II Study of Itraconazole in Biochemical Relapse
Patient Population	Patients with biochemically relapsed hormone sensitive non-metastatic prostate cancer after prior definitive local therapy
Rationale for Study	<p>Preliminary data suggests itraconazole has anti-prostate cancer activity independent of androgen synthesis inhibition, thereby potentially avoiding the attendant castration-driven side effects. Its antagonistic properties within the Hedgehog signaling pathway and prior extensive safety record and demonstrated tolerability as an anti-fungal agent provide additional justification to test this agent in a rising PSA, non-metastatic disease setting.</p> <p>Favorable results with respect to modulation of serum PSA, along with parallel, exploratory findings demonstrating minimal impact on adrenal hormone synthesis, down-regulation of the Hedgehog pathway, and acceptable toxicity would form the preliminary basis and justification for the further study of itraconazole in biochemically relapsed hormone sensitive prostate cancer.</p>
Primary Objective	To determine whether the proportion of patients who achieve a $\geq 50\%$ decline in serum PSA after 12 weeks of protocol therapy with itraconazole dosed at 300 mg BID is superior to a historical control based upon the observed PSA response proportion in prior studies of non-castrating systemic therapy in men with biochemically relapsed hormone sensitive prostate cancer.
Secondary Objectives	<p>Clinical Activity</p> <ul style="list-style-type: none"> • To determine the median time to PSA progression from the start of protocol therapy with itraconazole among men with biochemically relapsed prostate cancer. • PSA progression will be defined as follows: (1) If no PSA decline is observed on therapy, PSA progression will be defined as an increase in serum PSA $> 50\%$ above the baseline PSA, and an absolute increase of $> 2 \text{ ng/mL}$ above baseline, confirmed by repeat measurement at least 2 weeks later (2) If PSA declines on therapy, PSA progression will be defined as an increase in serum PSA $> 50\%$ above the nadir PSA on therapy, and an absolute increase $> 2 \text{ ng/mL}$ above the nadir, confirmed by repeat measurement at least 2 weeks later. • To determine the median time to clinical progression measured from the start of protocol therapy with itraconazole among men with biochemically relapsed prostate cancer. Clinical progression will be defined as the first occurrence of either the development of metastases or initiation of non-protocol therapy. • To determine the median metastasis-free survival measured from the start of protocol therapy in patients treated with itraconazole for biochemically relapsed prostate cancer. • To determine the mean percent change from baseline after 12 weeks of protocol therapy compared with pre-treatment in PSA doubling time. The pre-treatment PSA doubling time will be determined based upon all PSA measurements obtained within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA measurements spaced at least 14 days apart. <p>Safety/Pharmacokinetics</p> <ul style="list-style-type: none"> • To characterize the safety profile of itraconazole in the biochemically relapsed hormone sensitive prostate cancer population, as graded by Common Toxicity Criteria (CTCAE) version 4.03 (14-JUN2010). • To determine the mean steady-state itraconazole and hydroxy-itraconazole serum levels after 4 weeks of therapy with itraconazole.
Correlative Objectives	<p>Endocrine Parameters</p> <ul style="list-style-type: none"> • To determine the mean percent change from baseline in serum androgen levels, including serum testosterone, DHEA-S, and androstenedione, after 4 and 12 weeks of protocol therapy with itraconazole. • To determine the mean percent change from baseline in additional serum hormone levels, including adrenocorticotropic hormone (ACTH), aldosterone, deoxycorticosterone (DOC), 11-deoxycortisol, and cortisol after 4 and 12 weeks of protocol therapy with itraconazole. • To determine if there is a relationship between baseline and/or percent change in serum hormone levels and achieving a decline of $> 50\%$ in serum PSA after 12 weeks of protocol therapy.

ABSTRACT

	<p>Hedgehog Pathway</p> <ul style="list-style-type: none"> To determine the proportion of patients treated with itraconazole who display a down-regulation of the Hedgehog pathway, as assessed by measurement of <i>GLII</i> mRNA expression by qRT-PCR on serial skin biopsies obtained at baseline and after 4 weeks of protocol therapy. Down-regulation will be defined as a decline of any magnitude in <i>GLII</i> mRNA expression after 4 weeks of protocol therapy compared to baseline expression level. To determine if there is a relationship between down-regulation of the Hedgehog pathway and PSA modulation, including proportion of patients achieving a decline of > 50% in serum PSA as well as time to PSA progression. To evaluate archived primary prostate cancer tissue (biopsy or prostatectomy specimen) for baseline Hedgehog pathway status, using both qRT-PCR-based mRNA expression analysis and protein-based immunohistochemical (IHC) analysis of key Hedgehog pathway components (<i>GLII</i>, <i>PCTH1</i>, <i>Smo</i>) and Sonic hedgehog ligand. To determine if there is a relationship between mRNA and IHC expression of components in the Hedgehog pathway in primary prostate cancer tissue and PSA modulation, including the proportion of patients achieving a decline of ≥ 50% in serum PSA as well as time to PSA progression.
Study Design	<p>This is a phase II, single arm study of itraconazole dosed at 300 mg PO BID in patients with noncastrate, non-metastatic, biochemically relapsed prostate cancer after prior definitive local therapy. Simon's two stage minimax design will be followed for accrual and include an interim test for lack of efficacy. There is a pre-specified stopping rule for safety after 10 patients have been treated for a minimum of 8 weeks of protocol therapy.</p> <p>Study Schema:</p> <pre> graph LR A["N = 40 evaluable patients"] --> B["Screening Period To Assess PSA Doubling Time"] B --> C["Itraconazole 600 mg/day"] C --> D["Disease Progression†"] C -.-> E["12 week cut-point for primary analysis†"] </pre> <p>The diagram illustrates the study schema. It starts with 40 evaluable patients. A screening period assesses PSA doubling time. Patients then receive Itraconazole 600 mg/day. The study continues until Disease Progression (marked with a double dagger †). A dashed line indicates a 12-week cut-point for primary analysis, which is also marked with a double dagger †.</p>
Number of patients	40 evaluable patients will be enrolled across three investigational sites
Duration of Therapy	Patients will continue protocol therapy until PSA progression, development of metastases, initiation of non-protocol therapy, unacceptable toxicity, or patient/physician decision, whichever comes soonest.
Duration of Follow up	Follow-Up lasts through patient survival
Duration of study	The duration from first patient enrolled to analysis of the primary objective is estimated to be approximately 18 months. The total study duration including follow up is estimated to be 3 years.
Study Drug	Itraconazole: 100 mg capsules administered orally, 300 mg PO BID

ABSTRACT

Safety Assessments	<p>Safety Assessments: History and physical exam, toxicity assessment, LFTs, sodium, and potassium level on day 8, week 4, then every 4 weeks thereafter. BUN and creatinine at baseline and every 4 weeks thereafter.</p> <p>Interim Safety Analysis: Will be performed after 10 patients have completed at least 8 weeks of protocol therapy. If 4 or more patients have a grade 3 or higher toxicity during the first 8 weeks of protocol therapy (excluding hypokalemia and hypertension) that the study investigator attributes as possibly, probably, or definitely related to study drug, further accrual will be halted. Study accrual will continue during the period of the interim safety analysis.</p>
Efficacy Endpoints	<ul style="list-style-type: none"> • Median time to PSA progression among men with biochemical relapse treated with itraconazole will be estimated. See Section 2 for the definition of PSA progression. • Median time to clinical progression will be estimated, with clinical progression defined as the first occurrence of either development of overt metastases or initiation of non-protocol therapy. The definition of clinical progression excludes PSA-only progression. • Median metastasis-free survival will be estimated among men with biochemical relapse treated with itraconazole. • Mean percent change after 12 weeks of protocol therapy compared with pre-treatment in PSA doubling time will be determined for those treated with itraconazole. The pre-treatment PSADT will be determined based upon all PSA measurements within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA values each spaced at least 14 days apart (see section 6 of the protocol). • Median time to PSA progression between patients with and without Hedgehog pathway over-activation in archived primary prostate cancer tissue.
Unique Aspects of the Study	This study is the first to evaluate itraconazole and Hedgehog pathway inhibition in hormone sensitive biochemically relapsed prostate cancer and will further investigate the impact of itraconazole on modulating PSA, dissect its impact on adrenal hormone synthesis, and measure its effect on Hedgehog pathway activity in patients with biochemically relapsed prostate cancer.

List of Abbreviations

ACTH	adrenocorticotrophic hormone
ADT	androgen deprivation therapy
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
ASTRO	American Society for Therapeutic Radiation Oncology
BCR	biochemical relapse, biochemically relapsed
BUN	blood urea nitrogen
CRPC	castrate resistant prostate cancer
CTEP	Cancer Therapy Evaluation Program
DES	diethylstilbestrol
DHEA-S	dihydroepiandrosterone-sulfate
DOC	deoxycorticosterone
EC	endothelial cell
ECOG	Eastern Cooperative Oncology Group
EMT	epithelial-to-mesenchymal transition
FDA	Food and Drug Administration
GM-CSF	granulocyte macrophage- colony stimulating factor
Hh	Hedgehog
HIF	hypoxia inducible factor
HIV	human immunodeficiency virus HUVEC
	human umbilical vein endothelial cell
IADT	intermittent androgen deprivation therapy
LHRH	luteinizing hormone releasing hormone
LLN	lower limit of normal
Na-F PET	Sodium-fluoride positron emission tomography
NYHA	New York Heart Association
PAB	peripheral androgen blockade
PCWG	Prostate Cancer Working Group
PDAR	pre-developed assay reagent
PSA	prostate specific antigen
PSADT	PSA doubling time
<i>PTCH1</i>	<i>patched</i> receptor
qRT-PCR	quantitative reverse transcriptase- polymerase chain reaction
SHH	Sonic hedgehog signaling protein
<i>SMO</i>	<i>Smoothened</i>
T	testosterone
ULN	upper limit of normal
VEGF	vascular endothelial growth factor

Table of Contents

1.0	Background.....	9
1.1	Rationale for Developing Non-Castrating Therapies for Biochemically Relapsed Prostate Cancer	9
1.2	Itraconazole	10
1.3	Demonstrating Clinical Activity in Initial Phase II Trials of Non-Hormonal Agents for Biochemically Relapsed Disease	17
1.4	Overall Study Rationale.....	19
2.0	2.0 Hypothesis and Study Objectives.....	20
2.1	Hypothesis	20
2.2	Study Objectives.....	20
3.0	Study Design	22
3.1	Overall Study Characteristics	22
3.2	Study Endpoints.....	23
3.3	Study Schema	25
3.4	Study Timeline	25
4.0	Study Population	25
4.1	Study Population.....	25
4.2	Inclusion Criteria.....	26
4.3	Exclusion Criteria	27
5.0	Study Rationale	28
5.1	Rationale for the Study Population.....	28
5.2	Rationale for the Study Design and Dose Selection of Itraconazole.....	29
5.3	Rationale for the Selection of Study Endpoints.....	30
6.0	Study Activities	34
6.1	Study Schedule	Error! Bookmark not defined.
6.2	Screening Period/Pre-Study Evaluation	37
6.3	Study Treatment	38
6.4	Reasons for Treatment Discontinuation	40
6.5	Follow Up	40
6.6	Duration of Study	41
7.0	Study Drug Information	41
7.1	Itraconazole	41
8.0	Dosage Modifications/Toxicity.....	43
8.1	Toxicity.....	43
8.2	Dose Modifications for Specific Toxicities	43
8.3	Dose Modification for Other Adverse Events	45
9.0	Laboratory Assessment and Correlative Studies	Error! Bookmark not defined.
10.0	Planned Statistical Methods	48
10.1	Study Design.....	48
10.2	Determination of Sample Size and Study Power.....	49
10.3	Accrual.....	49

10.4	Interim Analysis	50
10.5	Analysis Population.....	50
10.6	Demographics and Baseline Characteristics.....	50
10.7	Study Endpoints.....	51
10.8	Methods for Analysis.....	52
11.0	Reporting and Documentation of Adverse Events	55
11.1	Definitions of Adverse Events (21 CFR 312.32(a)).....	55
11.2	Recording of an Adverse Event.....	57
11.3	Follow-up of Adverse Events	58
11.4	Serious Adverse Events Monitoring.....	58
11.5	Serious Adverse Events Expedited Reporting.....	59
12.0	Study Management	60
13.0	References	65
Appendix 1.	List of Prohibited Medications and Supplements While Patients Are Taking Itraconazole	68
Appendix 2.	Potential Drug-Drug Interactions	69
Appendix 3.	Skin Biopsy Procedure and Specimen Handling/Shipping Instructions	70
Appendix 4.	Data and Safety Monitoring Plan for a Phase 2 Institutional Study.....	75

1.0 Background

An estimated 50,000 men/year in the U.S develop a rising PSA after definitive radical prostatectomy and/or radiation therapy for localized disease, a disease state termed biochemical relapse (BCR) [1,2]. The optimal timing and choice of treatment of men in this disease state is unknown and clinical practice varies widely. Salvage prostatectomy and radiation therapy have been shown to lead to long-term disease free survival in a minority of patients, especially those with a slow PSA doubling time (PSADT), positive surgical margins, and/or lower Gleason grade and serum PSA level at the time of initial diagnosis [3]. For patients that are not candidates for local salvage therapy, hormonal treatment via androgen deprivation therapy (ADT) is often employed to control serum PSA and potentially delay the development of symptomatic metastatic disease.

1.1 Rationale for Developing Non-Castrating Therapies for Biochemically Relapsed Prostate Cancer

Continuous androgen deprivation therapy (ADT), in which serum testosterone is decreased to a castrate level (less than 50 ng/dL) using luteinizing hormone releasing hormone (LHRH) agonists or antagonists, with or without the addition of an androgen receptor (AR) antagonist (i.e. bicalutamide), is a standard and commonly used treatment in BCR. There are no randomized data demonstrating an overall survival advantage for early continuous ADT versus deferred therapy until the time of progression to metastatic disease. Furthermore, while continuous, long term treatment with ADT is effective in terms of decreasing PSA levels, disease progression in the setting of castrate levels of testosterone is a near universal event. Additionally, over the past decade, there has been a growing appreciation for the significant short term and longer term toxicities of continuous ADT, including declining quality of life on treatment and increased risk of osteoporosis, diabetes, and potentially cardiovascular mortality with long term treatment.

Given the toxicities of long term, continuous ADT, more contemporary hormonally-based treatment strategies utilizing intermittent ADT (IADT) and peripheral androgen blockade (PAB) have emerged for men with BCR [4-8]. IADT, in which treatment with an LHRH agonist or antagonist is given in cycles consisting of “on” and “off” treatment phases, has been shown to lead to non-inferior overall survival and potentially improved quality of life in several prior phase 3 trials [4-8]. Though seemingly an improvement over continuous ADT with regards to preservation of overall quality of life, patients treated with IADT may experience delayed testosterone recovery during “off” treatment phases, and the percentage of time spent off treatment becomes progressively shorter with successive cycles of treatment, such that many patients are still at increased risk of long term metabolic derangements including osteoporosis, diabetes, and cardiovascular disease related to ADT[9].

Given the significant short- and long-term toxicities of castration-based therapy, which can impair quality of life and cause significant morbidity in this relatively asymptomatic population of men with BCR, there is an unmet medical need for well tolerated non-castrating therapies in

this disease setting. Non-castrating therapies may ultimately be used to replace the need for ADT in biochemically relapsed disease, delay the initiation of ADT, or potentially lengthen the duration of “off” treatment phase as part of an intermittent ADT treatment strategy, thereby prolonging the time to development of castrate-resistant disease.

Various non-castrating agents have been tested in prior phase 2 clinical trials in the biochemically relapsed disease setting, including a prostatic acid phosphatase vaccine, GM-CSF, as well as other therapies such as imatinib, rosiglitazone, lenalidomide, celecoxib, and superoxide dismutase inhibitors including marimastat and ATN-224 [10-16]. In general, the clinical activity of these agents has been modest, with less than 5% of patients achieving a decline in serum PSA $\geq 50\%$ during protocol therapy with these non-castrating agents. In addition, several of the therapies were associated with appreciable toxicity, including imatinib, which is especially undesirable in this population of patients without metastases and low disease burden. There exists a need for novel non-hormonal agents with improved biologic activity and better tolerability for use in biochemically relapsed disease.

1.2 Itraconazole

Itraconazole, a member of the azole family of anti-fungal antibiotics, has shown intriguing anti-cancer activity in pre-clinical and clinical studies, including prostate cancer [see further discussion below]. Unlike the related compound ketoconazole, which exerts its effects in prostate cancer via androgen synthesis inhibition, preliminary data suggests that itraconazole’s mechanism of action in prostate cancer may be independent of androgen synthesis blockade. Given the demonstrated biologic activity in prostate cancer with a potentially non-castrating mechanism of action, as well as its long term safety data and demonstrated tolerability as an anti-fungal agent in patients with chronic fungal infections, itraconazole is well-suited for evaluation in biochemically relapsed prostate cancer.

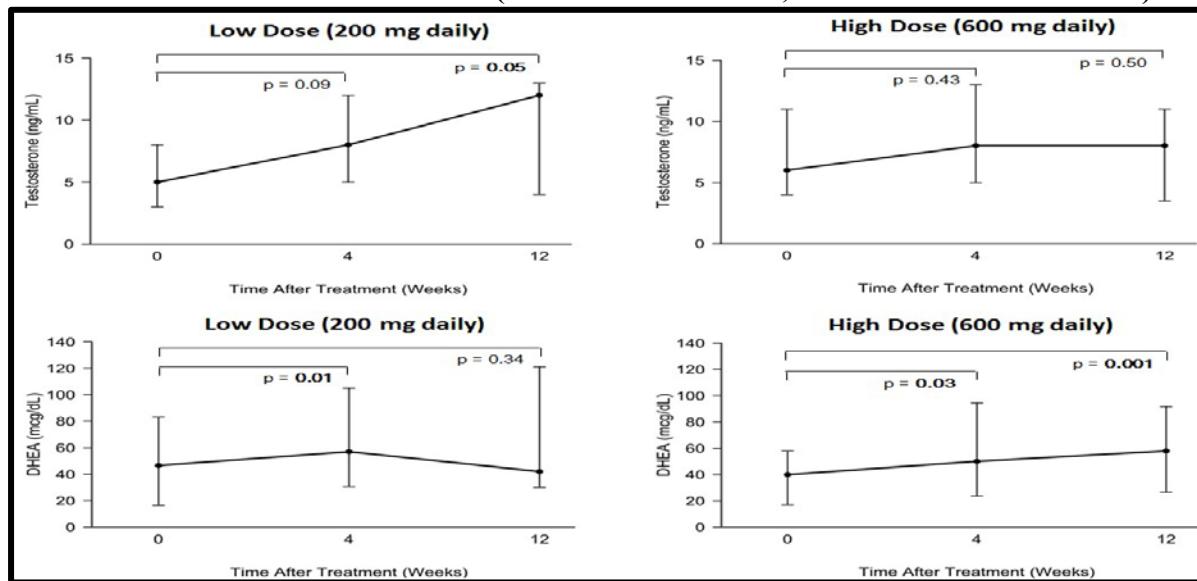
1.2.1 Mechanism of Action Distinct From Ketoconazole

The anti-fungal activity of the azole family of antibiotics stems from their ability to inhibit fungal wall ergosterol synthesis, via inhibition of the enzyme lanosterol 14 α -demethylase [17]. As compared with ketoconazole, itraconazole more selectively inhibits fungal wall ergosterol synthesis, and spares inhibition of human adrenal hormone synthesis. Consequently, itraconazole is not associated with the development of clinically significant adrenal insufficiency, nor is concomitant corticosteroid replacement required as it is for ketoconazole [18].

The results of a prior randomized, non-comparative study of low- (200 mg/day) and high-dose (300 mg PO BID) itraconazole in metastatic castrate-resistant prostate cancer (CRPC) provide preliminary evidence that itraconazole has a mechanism of action independent of androgen synthesis blockade, distinct from the mechanism of action of ketoconazole [19]. In this trial, there was no decrease in serum testosterone (T) or adrenal androgen dihydroepiandrosterone (DHEA) levels in patients treated with either low or high dose itraconazole [see figure 1 below]. It is important to note, however, that hormone levels were measured in the context of a medically

castrate patient population, with a different hormonal milieu than the proposed study population. In contrast to the effects of itraconazole on serum androgen levels in CRPC, a prior randomized clinical trial of antiandrogen withdrawal with or without ketoconazole in CRPC, serum levels of adrenal androgen hormones DHEA, DHEA-sulfate, and androstenedione decreased by 54%, 90%, and 58% respectively, and higher baseline levels of adrenal androgen levels were associated with PSA response to ketoconazole [20, 21].

Figure 1. Serum Testosterone and DHEA Levels On Itraconazole Therapy In Men With Castrate Resistant Prostate Cancer (from Antonarakis E, et al. J of ClinOncol 2011)



1.2.2 Non-Hormonal Mechanisms of Action of Itraconazole

There are several potential mechanisms of action of itraconazole as an anti-cancer agent that have been investigated in prior studies: (1) inhibition of angiogenesis and (2) Hedgehog (Hh) pathway suppression.

1.2.2.1 Itraconazole as a Potential Anti-Angiogenesis Agent

In a human umbilical vein endothelial cell (HUVEC) proliferation assay used to screen a library of FDA-approved drugs, itraconazole was unexpectedly identified as a potent and selective inhibitor of endothelial cell (EC) proliferation [22], only in part via inhibition of the enzyme lanosterol 14 alpha-demethylase. Itraconazole inhibited EC proliferation at a potency 40 times that of ketoconazole. In another study using a lung cancer xenograft model, treatment with itraconazole was shown to increase intra-tumoral levels of hypoxia inducible factor 1 alpha (HIF1 α) and decrease tumor microvessel density [23]. Whether the mechanism of action of itraconazole as a potential anti-neoplastic agent stems from its ability to inhibit angiogenesis remains an unresolved question. In the prior clinical trial of itraconazole in metastatic CRPC, serum VEGF levels did not consistently decline with treatment, nor were reductions in serum VEGF associated with declines in serum PSA on treatment. Furthermore, inhibiting angiogenesis

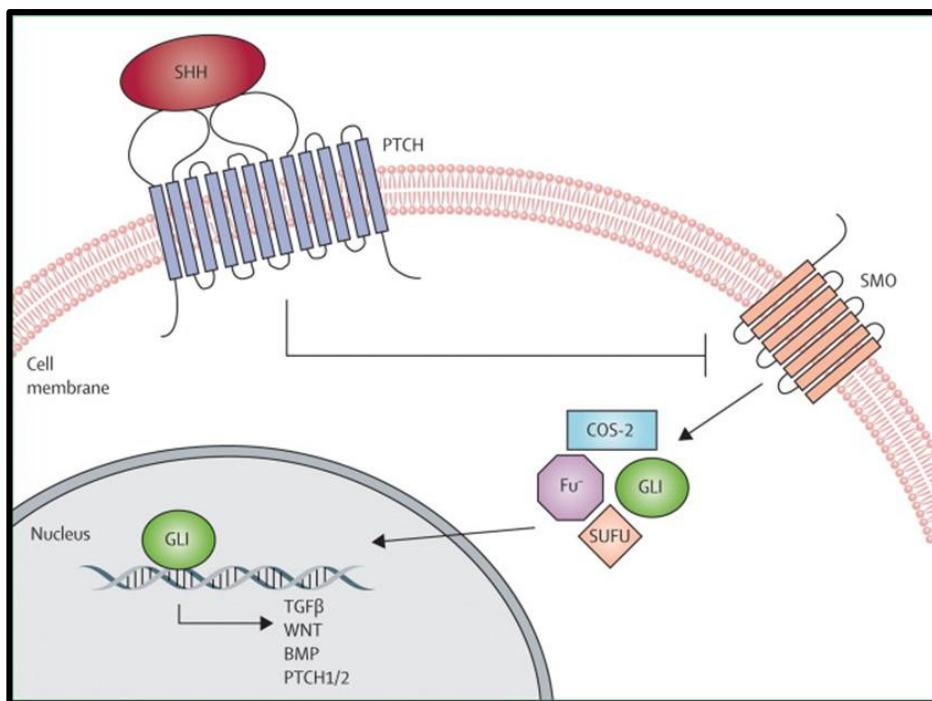
in non-metastatic biochemically relapsed disease, in which presumed micrometastases are present, may not be as biologically relevant as other anti-cancer mechanisms of action.

1.2.2.2 Hedgehog Pathway in Prostate Cancer

The hedgehog pathway (see figure 2 below) plays an important role in embryonic development and tissue polarity [24]. Secreted Hh molecules bind to the receptor *patched* (*PTCH*), thereby relieving *PTCH*-mediated suppression of *Smoothened* (*SMO*). *SMO* then translocates to the primary cilia, triggering a cascade of intracellular signal transduction events. This results in *GLI*-dependent transcription of various growth factors and signaling molecules, including *PTCH* and *GLI*, which act as negative and positive feedback loops respectively. Dysregulation of the Hh pathway via somatic mutations has been implicated in the tumorigenesis of various cancer subtypes, including basal cell carcinoma of the skin, in which loss-of-function mutations in *PCTH* and less commonly activating mutations in *SMO* lead to constitutive Hh pathway signaling and unrestrained proliferation of basal cells in the skin [25].

Figure 2. Hedgehog Pathway

(figure adapted from <http://www.thelancetstudent.com/wp-content/uploads/2010/02/fig1.jpg>)



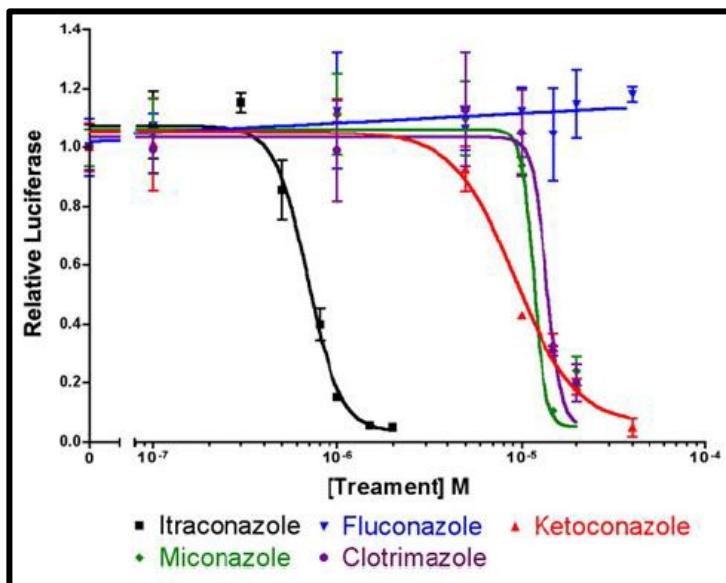
Preclinical studies in other solid tumors, including prostate and pancreatic adenocarcinoma, suggest that aberrant activation of the Hh pathway may promote tumor-stromal interaction and the epithelial-to-mesenchymal (EMT) transition, key steps in the progression to metastatic cancer [26]. Re-activation of the Hh pathway, which is quiescent in the normal adult male prostate gland, may be an important mechanism in the progression to metastases in prostate cancer.

In cell lines stably expressing *GLII*, Hh pathway activation was shown to promote proliferation of prostate cancer cells and malignant transformation of progenitor-like primary cells which formed diffuse visceral metastases in a mouse xenograft model [27]. Administration of cyclopamine, a small molecule inhibitor of *SMO*, inhibits metastasis in this mouse xenograft model. The level of E-cadherin, a molecule that maintains epithelial organization and cellular attachment, was downregulated in cell lines with forced *GLI* expression, suggesting that one mechanism by which Hh signaling promotes invasiveness and metastasis is by driving EMT. In human prostate cancer, metastatic tissue from 12/12 patients overexpressed *PTCH* and *GLII* by real time RT-PCR analysis; in contrast, only 3/12 localized prostate cancer samples and none of the benign prostate tissue samples overexpressed *PTCH* or *GLII*. A separate study reported that 70% of Gleason grade 8-10 human prostate cancer specimens displayed higher expression levels of two Hh pathway target genes, *PCTH1* and the hedgehog-interacting protein (*HIP*) compared with only 22% of Gleason grade 3-6 tumors [28], pointing to a role for the Hh pathway in aggressive tumor biology.

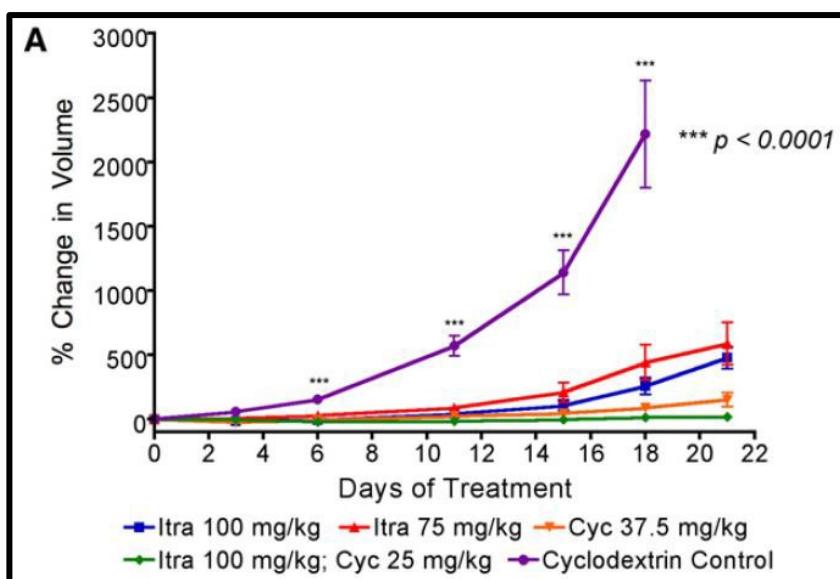
Thus, inhibition of the Hh pathway may target tumor-stromal interactions and inhibit the EMT, thereby delaying microscopic tumor growth and invasion. Biochemically relapsed disease, in which microscopic disease is present and potentially invasive in other tissues, therefore represents a logical disease setting in which to evaluate Hh pathway inhibition.

1.2.2.3 Itraconazole and Hedgehog Pathway Inhibition

Pre-clinical and emerging clinical data suggest that itraconazole is a potent inhibitor of the Hedgehog pathway. In a drug screen study of ~ 2400 FDA-approved or post-phase I drugs using a cell line containing a stably integrated *GLII*-luciferase reporter which responds to exogenous stimulation by Sonic hedgehog signaling protein (SHH), itraconazole was identified as a potent inhibitor of the Hh pathway with an IC₅₀ of approximately 800 nM, greater than 10-fold more potent than that of ketoconazole (see figure 3 below) [29].

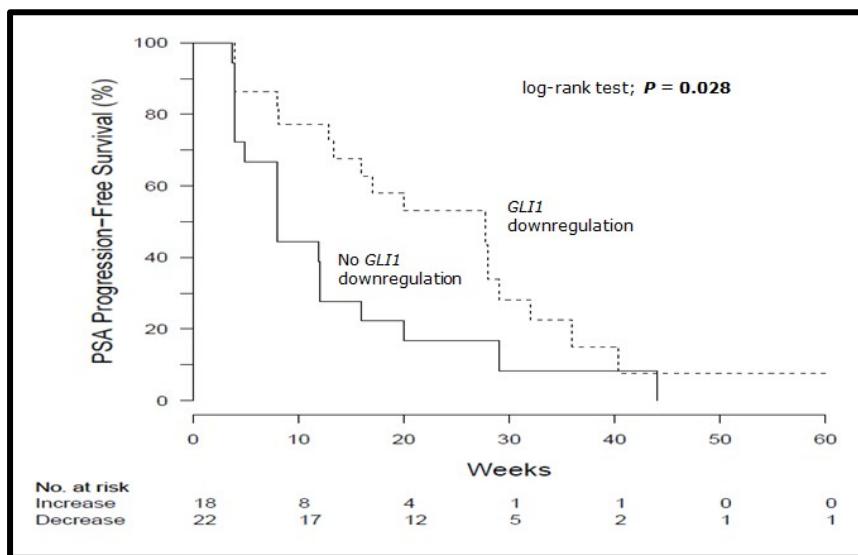
Figure 3.Itraconazole Potently Inhibits Hedgehog Pathway Relative to Other Azoles

Further experiments in *PTCH*-null and mutant *SMO* cell lines indicate that itraconazole inhibits the Hh pathway via a mechanism distinct from its effects on sterol biosynthesis by acting as an inverse agonist of *SMO*, thereby preventing its accumulation within the primary cilium. In a follow up study using a mouse xenograftmedullablastoma model with constitutively active Hhsignaling, itraconazole inhibited the growth of tumor allografts at steady-state serum concentrations comparable to those of patients treated with 200-300 mg PO BID on a chronic basis for fungal infections (see figure 4 below) [29].

Figure 4.Itraconazole Inhibits In Vivo Growth of a MedulloblastomaXenograft

In a recently presented phase II open label study of 19 patients with basal cell carcinoma, treatment with itraconazole (dosed at either 200 or 400 mg/day) led to a 23% reduction in clinical tumor area and a 65% reduction in Hedgehog activity as assessed by *GLI1* mRNA expression [30]. In an intriguing correlative study from the previously described phase II trial of low- and high-dose itraconazole in patients with CRPC, modulation of Hh pathway activity was assessed by serial skin biopsy. Tissue samples were assayed for *GLI1* mRNA expression by qRT-PCR, using previously validated methods [25]. In this analysis, 33% of patients treated with itraconazole 200 mg/day and 68% of patients treated with 300 mg PO BID experienced a decrease in *GLI1* expression [31]. Interestingly, *GLI1* downregulation appeared to be associated with an improvement in PSA progression-free survival, the primary endpoint of the study (see figure 5 below).

Figure 5. Correlation of *GLI1* Downregulation with Outcomes in a Prior Study of Itraconazole in Castrate Resistant Prostate Cancer



In summary, itraconazole appears to have a distinct mechanism of action from ketoconazole and an emerging role as a non-castrating prostate cancer therapeutic agent. The anti-neoplastic activity of itraconazole may act via inhibition of the Hedgehog pathway, which is implicated in the progression to metastatic prostate cancer. Inhibiting Hh signaling may be especially relevant in patients with biochemically relapsed prostate cancer, who have micrometastases that ultimately progress to macrometastatic disease in the axial skeleton and other organs.

1.2.3 Clinical Efficacy and Safety of Itraconazole

1.2.3.1 Clinical Efficacy in CRPC

Itraconazole dosed at 300 mg PO BID exhibited promising clinical activity in the phase II, randomized, non-comparative trial of low- and high-dose itraconazole (200 mg/day and 300 mg BID respectively) in patients with metastatic castrate resistant prostate cancer [19]. In this study, the mean age was approximately 72 years old, approximately 30% of patients had prior treatment

with ketoconazole, and patients had a relatively high burden of disease (mean number of metastases was ~ 5; > 50% with both bone + soft tissue metastases). 17 and 29 patients were enrolled onto the low- and high-dose itraconazole arms respectively. In the 200 mg/day treatment arm, there was minimal activity demonstrated with 1/17 patients (6%) achieving a > 30% decline in serum PSA on therapy. In the high-dose arm (300 mg BID), however, significant clinical activity was demonstrated. 15/28 (54%) patients on this treatment arm had any decline in serum PSA, and 8/28 (29%) achieved a > 30% PSA decline. 48.4% of men were PSA-progression free at 24 weeks, and the median progression-free survival was 35.9 weeks.

1.2.3.2 Safety

In the study of itraconazole in metastatic CRPC, itraconazole dosed at either 200 or 300 mg BID was well tolerated, and most adverse events were of grade 2 or less in severity (see table 1 below) [19]. Common adverse events included fatigue and various gastrointestinal symptoms, including nausea, anorexia, constipation, or diarrhea. These gastrointestinal adverse events were all of grade 2 or lower in severity in the 300 mg BID study arm. The triad of hypokalemia, hypertension, and peripheral edema observed raises the possibility of induced mineralocorticoid excess. Serum aldosterone levels actually declined on treatment; however levels of 11-deoxycortisol and corticosterone were not measured in this study. There were no grade 4 or higher toxicities.

Table 1. Adverse Events Associated with Low- and High-Dose Itraconazole

Adverse Event	Low Dose: 200 mg/d (N=17)				High Dose: 600 mg/d (N=29)			
	All grades	%	Grade 3	%	All grades	%	Grade 3	%
Fatigue	9	52.9	1	5.9	15	51.7		
Pain (all types)	7	41.2			13	44.8		
Nausea	4	23.5			11	37.9		
Constipation	2	11.8			10	34.5		
Hypertension	0	0.0			9	31.0	2	6.9
Edema (periph)	6	35.3			8	27.6		
Diarrhea	1	5.9			8	27.6		
Anorexia	2	11.8	1	5.9	7	24.1		
Headache	2	11.8			6	20.7		
Rash	3	17.6	1	5.9	5	17.2	1	3.4
Vomiting	2	11.8			5	17.2		
Dyspnea	1	5.9			5	17.2		
Hypokalemia	0	0.0			5	17.2	3	10.3

Itraconazole is FDA-approved as an anti-fungal agent at doses ranging from 100-600 mg/day, and has been widely used to treat chronic invasive fungal infections since the early 1990s. Treatment duration often exceeds 6 months, and in certain clinical scenarios, patients are placed on lifelong suppressive therapy (i.e. HIV cryptococcal meningitis secondary prophylaxis). This affords the opportunity to have gathered a large volume of safety data with this agent, which has demonstrated that itraconazole is well tolerated at chronic doses up to and including 600 mg/day. In a prior study of 189 patients treated withitraconazole for chronic fungal infections (44% for

coccidioidomycosis; 28% with cryptococcosis, 12% with aspergillosis), with the majority of patients treated with a dose of 400 mg/day, the medication was well tolerated and patients received treatment for a median duration of 5 months (range < 1 to 42 months) [32]. As in the Antonorakis et al. trial outlined above, gastrointestinal symptoms were among the most common adverse events, with 10% of patients experiencing nausea/vomiting, 2% with diarrhea. Elevated transaminases occurred in 5% of patients, none with elevations > 3 times the upper limits of normal. Toxicity led to discontinuation of itraconazole in 4% of patients.

In another study of high dose itraconazole (600 mg/day) delivered for a mean duration of 5.5 months to 8 patients, none experienced gastrointestinal symptoms attributable to itraconazole [33]. No elevations in transaminases were noted. Mild hypokalemia and peripheral edema were observed in 3/8 patients, though not in association in hypertension and again without an increase in serum aldosterone concentration. Another study of ten patients receiving doses of itraconazole ranging from 200-400 mg/day for chronic mycotic infections, serial cosyntropin assays failed to detect subclinical adrenal insufficiency in any of the ten patients [22]. In contrast, patients treated with ketoconazole at high doses (i.e. 800-1200 mg/day in divided doses) developed clinically significant adrenal insufficiency in a prior prospective observational study [34].

Taken together, the clinical efficacy and safety data of itraconazole at a dose of 600 mg/day indicates promising clinical benefit in advanced prostate cancer. In a lower tumor burden setting such as biochemical relapse, itraconazole may have an even greater impact on disease control and/or may be a less toxic alternative to castration therapy. Its long track record of demonstrated tolerability and safety as an anti-fungal agent, even with chronic daily dosing, serves as additional justification to explore its potential for clinical benefit in this disease setting.

1.3 Demonstrating Clinical Activity in Initial Phase II Trials of Non-Hormonal Agents for Biochemically Relapsed Disease

Developing novel non-castrating therapies for men with biochemical relapse presents several unique study design challenges in initial phase I/II trials of new agents tested in this setting. Using clinically relevant study endpoints such as time to metastatic disease, time to castrate-resistant disease, or overall survival require long term study follow up and large patient numbers for adequate statistical power. Such endpoints are best utilized in larger phase II and confirmatory phase III clinical trials. Changes in serum PSA represent the primary means to assess the preliminary biologic activity of novel agents tested in phase I/II trials in the biochemically relapsed disease setting, yet changes in serum PSA have not been validated as a surrogate marker of clinical outcome. Furthermore, the specific choice of PSA-based study endpoint has varied in prior trials of non-castrating agents in biochemical relapse.

1.3.1 Decline in Serum PSA

The proportion of patients achieving PSA declines of > 50% is a widely utilized endpoint as a means for screening the biologic activity of novel agents in the castrate-resistant disease setting.

PCWG2 recommended that phase II trials in the CRPC setting adopt time-to-event variables such as progression-free survival based on radiographic/clinical variables rather than declines in serum PSA as the primary means of assessing biologic activity in this disease setting. For biochemically relapsed disease, however, declines in serum PSA may still have utility as a means to determine which agents show anti-cancer activity, as was demonstrated in prior trials of imatinib and GM-CSF in biochemically relapsed prostate cancer [12, 15]. Though PSA declines are not a validated surrogate marker, a retrospective analysis of hormonal therapy for biochemically relapsed hormone sensitive prostate cancer demonstrated that greater declines of PSA while on therapy were significantly associated with longer prostate cancer specific survival [35]. Preliminary data indicating that a novel therapy leads to substantial PSA declines in a significant proportion of patients, along with acceptable safety data, would form the basis for larger, placebo-controlled, randomized phase II/III trials with primary efficacy endpoints including time to first metastasis or overall survival. Several caveats to the use of PSA decline as a study endpoint are the fact that it is not a validated surrogate endpoint for overall survival in the CRPC disease setting, certain agents may affect PSA expression without demonstrable anti-tumor activity, and conversely, cytostatic agents may have a beneficial effect with respect to control of micrometastases without affecting serum PSA.

1.3.2 PSA Progression-Free Survival

Time-to-event variables such as radiographic or PSA progression-free survival have been increasingly used as primary endpoints in initial phase II trials of novel agents in the castrate-resistant prostate cancer setting, in accordance with updated guidelines from the Prostate Cancer Working Group. In the hormone sensitive, biochemically relapsed disease setting there is no standardized definition of PSA progression, with PSA Working Group guidelines suggesting the definition of PSA progression must be individualized for each study depending upon the mechanism of the agent being tested (i.e. hormonal therapy, chemotherapy, immunotherapy, etc.) [36]. In a prior phase II study of ATN-224, a novel targeted superoxide dismutase inhibitor, progression was defined as an increase in serum PSA over baseline of $\geq 50\%$ (and by $> 5 \text{ ng/mL}$) [16]. This definition is acknowledged to be arbitrary but does reflect clinical practice with intermittent androgen deprivation therapy, in which patient's baseline PSA prior to initiation of ADT is taken into account when deciding at what PSA level to re-initiate therapy. In contrast, in an ongoing phase III trial of ADT +/- docetaxel in the biochemically relapsed disease setting, the primary endpoint is progression-free survival, with PSA progression defined as a detectable PSA of any level after completion of treatment (NCT00514917). This definition is more appropriate for chemohormonal therapy than for non-castrating targeted agents.

Extrapolation from the PCWG2 recommendations for castrate-resistance prostate cancer can be applied to the biochemically relapsed disease setting, in which PSA progression is defined by the nadir PSA achieved on therapy. According to PCWG2 recommendation, a rise in serum PSA $> 25\%$ and an absolute increase of at least 2 ng/mL above the nadir PSA defines PSA progression in the castrate-resistant disease setting. Whether the same threshold is appropriate in the

hormone-sensitive biochemically relapsed disease setting is debatable, given the lower burden of disease and sensitivity to androgen deprivation in the latter disease state.

At the current time, given the absence of a standard definition of PSA progression in the BCR disease setting, choosing a threshold that is consistent with the mechanism of the agent being tested, and then uniformly applying the same definition of progression to all patients included in the clinical trial or observational study is recommended to avoid potential measurement bias.

1.3.3 Change in PSA Kinetics on Therapy

A prior retrospective, combined analysis of 4 clinical trials of non-castrating agents (lenalidomide, marimastat, imatinib, and ATN-224) in biochemically relapsed prostate cancer provides preliminary evidence for the potential clinical significance of within-subject changes in serum PSA slope during treatment [37]. In this analysis, four factors were predictive of metastasis-free survival on multivariate analysis: baseline PSA doubling time, baseline PSA slope, on-study change in PSA doubling time, and on-study change in PSA slope. In a landmark Kaplan-Meier analysis, median metastasis-free survival was 63.5 months (95% CI 34.6-not reached) and 28.9 months (95% CI 13.5-68.0) for men with or without any decrease in PSA slope by 6 months of treatment, respectively. These results, however, are retrospective and need to be validated in prospective study prior to widespread acceptance of change in PSA slope as an alternative endpoint for screening new non-hormonal therapies in patients with biochemical relapse.

A prior randomized, placebo-controlled, phase II study of rosiglitazone highlights several of the methodological issues with the use of changes in PSADT as the primary study endpoint in biochemical relapse [14]. The primary study endpoint was a positive PSA doubling time (PSADT) outcome, defined as either a post-treatment PSADT > 150% compared to baseline PSADT or a declining PSA on treatment in the absence of development of new metastases. Patients were excluded if baseline PSADT was > 24 months. Yet, there was no standardization of serum PSA measurement prior to and after study enrollment, both with respect to frequency of PSA measurement and potential inter-laboratory variability, thus making within-subject pre-treatment and on-treatment calculations of PSADT more difficult to interpret. A second issue highlighted by this study is that increases in PSADT can occur even in the absence of treatment, due to the inherent variability of PSA measurement over time. In this study, 40% of the men in the placebo group experienced an increase in post-treatment PSADT to > 150%, a result that was much higher than expected and may have contributed to an overall negative study (38% of patients in the rosiglitazone arm had an increase in PSADT > 150%; p = 1).

1.4 Overall Study Rationale

The properties of itraconazole - single agent activity in prostate cancer, a potentially non-castrating mechanism of action distinct from ketoconazole, Hedgehog pathway inhibition which may block the formation of metastases, and an established safety record at an FDA-approved dose level for chronic fungal infections - make it a compelling agent to test in biochemically

relapsed prostate cancer. The proposed clinical trial is a single arm phase 2 study of itraconazole dosed at 300 mg PO BID in men with biochemically relapsed non-metastatic hormone sensitive prostate cancer. The primary endpoint of the study is the proportion of patients achieving $\geq 50\%$ PSA decline after 12 weeks of therapy. Secondary endpoints include change in PSA doubling time, time to PSA or clinical progression, metastasis-free survival, and toxicity. Correlative endpoints will include serum hormone levels and Hedgehog pathway activity. Favorable results with respect to modulation of serum PSA, along with parallel, exploratory findings demonstrating minimal impact on adrenal hormone synthesis, down-regulation of the Hedgehog pathway, and acceptable toxicity would form the preliminary basis and justification for the further study of itraconazole in biochemically relapsed hormone sensitive prostate cancer. Its use in this setting may ultimately either be to delay the need for initiation of ADT or to lengthen the duration of “off” treatment cycles as part of an intermittent ADT treatment strategy. Furthermore, demonstrating that the anti-prostate cancer activity of itraconazole is not primarily related to androgen synthesis inhibition would form the impetus for further study into the mechanism of action of itraconazole in prostate cancer.

2.0 Hypothesis and Study Objectives

2.1 Hypothesis

A significantly greater proportion of patients treated withitraconazole will achieve at least a 50% decline from baseline in serum PSA after 12 weeks of therapy, as compared to a historical control based upon the PSA response proportion observed in prior phase II clinical trials of other non-castrating therapiesin men with biochemically relapsed hormone sensitive prostate cancer. Favorable results with respect to PSA modulation, along with parallel findings demonstrating acceptable toxicity, potent Hedgehog pathway inhibition, and absence of significant impact on serum hormone levels,would form the basis for the further study of itraconazoleas a potentially viable non-castrating treatment option for men with biochemically relapsed prostate cancer.

2.2 Study Objectives

2.2.1 Primary Objective

To determine whether the proportion of patients who achieve a $\geq 50\%$ decline in serum PSA after 12 weeks of protocol therapy with itraconazole dosed at 300 mg PO BID is superior to a historical control based upon the observed PSA response proportion in prior studies of non-castrating systemic therapy in men with biochemically relapsed hormone sensitive prostate cancer.

2.2.2 Secondary Objectives

Clinical Activity

- To determine the median time to PSA progression from the start of protocol therapy with itraconazole among men with biochemically relapsed prostate cancer.
PSA progression will be defined as follows: (1) If no PSA decline is observed on therapy, PSA progression will be defined as an increase in serum PSA > 50% above the baseline PSA, and an absolute increase of > 2 ng/mL above baseline, confirmed by repeat measurement at least 2 weeks later (2) If PSA declines on therapy, PSA progression will be defined as an increase in serum PSA > 50% above the nadir PSA on therapy, and an absolute increase > 2 ng/mL above the nadir, confirmed by repeat measurement at least 2 weeks later.
- To determine the median time to clinical progression measured from the start of protocol therapy with itraconazole among men with biochemically relapsed prostate cancer. Clinical progression will be defined as the first occurrence of either the development of metastases or initiation of non-protocol therapy, and will exclude PSA-only progression.
- To determine the median metastasis-free survival measured from the start of protocol therapy in patients treated with itraconazole for biochemically relapsed prostate cancer.
- To determine the mean percent change from baseline after 12 weeks of protocol therapy compared with pre-treatment in PSA doubling time. The pre-treatment PSA doubling time will be determined based upon all PSA measurements obtained within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA measurements spaced at least 14 days apart.

Safety/Pharmacokinetics

- To characterize the safety profile of itraconazole in the biochemically relapsed hormone sensitive prostate cancer population, as graded by Common Toxicity Criteria (CTCAE) version 4.03 (14-JUN-2010).
- To determine the mean steady-state itraconazole and hydroxy-itraconazole serum levels after 4 weeks of therapy with itraconazole.

2.2.3 Correlative Objectives

Endocrine Parameters

- To determine the mean percent change from baseline in serum androgen levels, including serum testosterone, DHEA-S, and androstenedione, after 4 and 12 weeks of protocol therapy with itraconazole.
- To determine the mean percent change from baseline in additional serum hormone levels, including adrenocorticotropic hormone (ACTH), aldosterone, deoxycorticosterone (DOC), 11-deoxycortisol, and cortisol after 4 and 12 weeks of protocol therapy with itraconazole.

- To determine if there is a relationship between baseline and/or percent change in serum hormone levels and achieving a decline of $\geq 50\%$ in serum PSA after 12 weeks of protocol therapy.

Hedgehog Pathway:

- To determine the proportion of patients treated with itraconazole who display a down-regulation of the Hedgehog pathway, as assessed by measurement of *GLII* mRNA expression by qRT-PCR on serial skin biopsies obtained at baseline and after 4 weeks of protocol therapy. Down-regulation will be defined as a decline of any magnitude in *GLII* mRNA expression after 4 weeks of protocol therapy compared to baseline expression level.
- To determine if there is a relationship between down-regulation of the Hedgehog pathway and PSA modulation, including proportion of patients achieving a decline of $\geq 50\%$ in serum PSA as well as time to PSA progression.
- To evaluate archived primary prostate cancer tissue (biopsy or prostatectomy specimen) for baseline Hedgehog pathway status, using both qRT-PCR-based mRNA expression analysis and protein-based immunohistochemical (IHC) analysis of key Hh pathway components (*GLII*, *PCTH1*, *SMO*) and Sonic hedgehog ligand.
- To determine if there is a relationship between mRNA and ICH expression of components in the Hedgehog pathway in primary prostate cancer tissue and PSA modulation on itraconazole therapy, including the proportion of patients achieving a decline of $\geq 50\%$ in serum PSA as well as time to PSA progression.

3.0 Study Design

3.1 Overall Study Characteristics

This is a phase II, single arm study of itraconazole dosed at 300 mg PO BID in patients with non-castrate, non-metastatic, biochemically relapsed prostate cancer after prior definitive local therapy. Simon's two stage minimax design will be followed for accrual and include an interim test for lack of efficacy (see Sections 10.2 and 10.4). There is a pre-specified stopping rule for safety after 10 patients have been treated for a minimum of 8 weeks of protocol therapy (see Section 10.4). Patients will remain on protocol therapy until the first occurrence of any of the following: PSA progression, as defined in Section 2, development of metastasis, unacceptable toxicity, initiation of non-protocol therapy, or patient/physician withdrawal from study. Patients will remain on study until development of metastasis, initiation of non-protocol therapy, or patient/physician withdrawal from study, whichever occurs soonest. The total study duration including follow up is expected to be 3 years from the date of first patient enrolled onto study.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The proportion of patients with biochemically relapsed disease after prior definitive local therapy who achieve a $\geq 50\%$ decline from baseline in serum PSA after 12 weeks of therapy with itraconazole, confirmed by repeat measurement at least 2 weeks later, will be calculated.

3.2.2 Secondary Endpoints

Clinical Activity

- Median time to PSA progression among men with biochemical relapse treated with itraconazole will be estimated. See Section 2 for the definition of PSA progression.
- Median time to clinical progression will be estimated, with clinical progression defined as the first occurrence of either development of overt metastases or initiation of non-protocol therapy. The definition of clinical progression excludes PSA-only progression.
- Median metastasis-free survival will be estimated among men with biochemical relapse treated with itraconazole.
- Mean percent change after 12 weeks of protocol therapy compared with pre-treatment in PSA doubling time will be determined for those treated with itraconazole. The pre-treatment PSADT will be determined based upon all PSA measurements within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA values each spaced at least 14 days apart (see Section 6.2.1).

Safety/Pharmacokinetics

- Maximum grade toxicities observed during treatment with itraconazole, as graded by CTCAE version 4.03.
- Mean steady-state trough level of serum itraconazole and its active metabolite, hydroxyitraconazole, after 4 weeks of therapy with itraconazole.

3.2.3 Correlative Endpoints

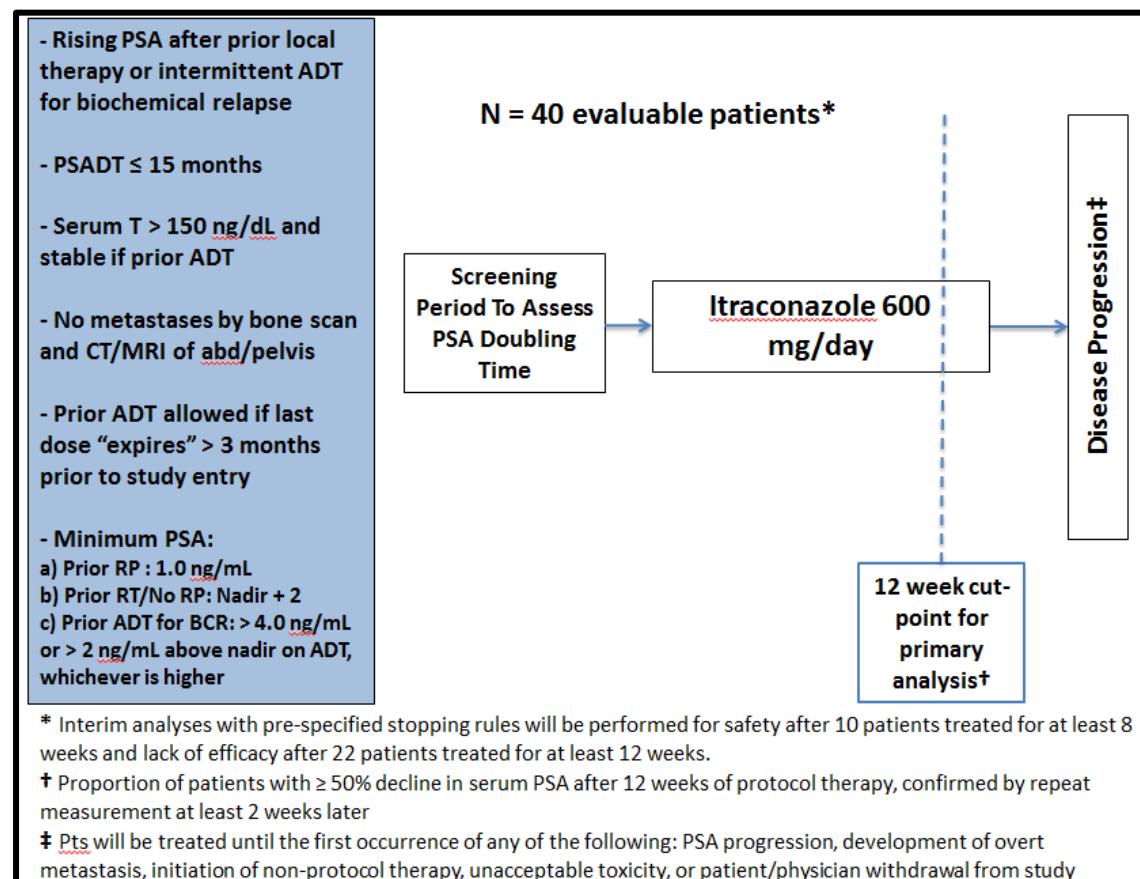
Endocrine Parameters

- Mean percent change from baseline in serum androgen levels, including serum testosterone, DHEA-S, and androstenedione, after 4 and 12 weeks of protocol therapy.
- Mean percent change from baseline in additional serum hormone levels, including ACTH, aldosterone, deoxycorticosterone (DOC), 11-deoxycortisol, and cortisol after 4 and 12 weeks of protocol therapy.
- Mean baseline and mean percent change in serum hormone levels among patients achieving $\geq 50\%$ decline from baseline in serum PSA after 12 weeks of itraconazole therapy and among those not achieving this decline.

Hedgehog Pathway (assessed by serial skin biopsies on protocol therapy and analysis of archived primary prostate cancer tissue including prior biopsy or prostatectomy specimen)

- Proportion of patients who display a down-regulation of the Hedgehog pathway, as assessed by measurement of *GLI1* mRNA expression by RT-PCR on serial skin biopsies obtained at baseline and after 4 weeks of protocol therapy with itraconazole. Down-regulation will be defined as a decline of any magnitude in *GLI1* mRNA expression after 4 weeks of protocol therapy compared to baseline expression level.
- Proportion of patients with and without at least a 50% decline from baseline after 12 weeks of protocol therapy in PSA exhibiting a down-regulation of the Hedgehog pathway.
- Median time to PSA progression between patients with and without down-regulation of the Hedgehog pathway on itraconazole therapy.
- Proportion of patients with and without at least 50% decline from baseline after 12 weeks of protocol therapy in PSA exhibiting over-activation of Hedgehog pathway components as assessed by immunohistochemical (IHC) and mRNA expression analysis of primary prostate cancer tissue (prior biopsy or prostatectomy specimen). Patients will be dichotomized (immunohistochemical staining 0 to 1+ vs. 2 to 3+ staining intensity; mRNA expression above and below median) for the purposes of analysis of the Hedgehog pathway in primary prostate cancer tissue.
- Median time to PSA progression between patients with and without Hedgehog pathway over-activation in archived primary prostate cancer tissue.

3.3 Study Schema



3.4 Study Timeline

3.4.1 Primary Completion:

The study will reach primary completion approximately 18 months from the date the first patient is enrolled.

3.4.2 Study Completion:

The total study duration including follow up is estimated to be 3 years.

4.0 Study Population

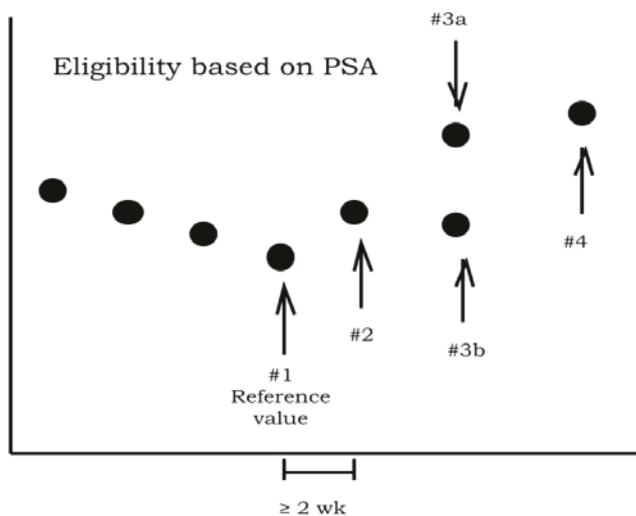
4.1 Study Population

40 evaluable patients with biochemically relapsed hormone sensitive non-metastatic prostate cancer after prior definitive local therapy will be enrolled onto this study across three investigational sites, including the University of California San Francisco, Dana-Farber Cancer Institute, and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Hospital.

Patients with prior salvage local therapy and/or those with prior androgen deprivation therapy for biochemical relapsed disease will be allowed onto study, provided that the eligibility requirements pertaining to pre-treatment PSA doubling time and serum testosterone level are met (see below).

4.2 Inclusion Criteria

1. Histologic confirmation of adenocarcinoma of the prostate
2. Biochemically relapsed disease with a rising PSA on at least two successive measurements at least two weeks apart after prior definitive local therapy (radical prostatectomy, external beam radiation, or brachytherapy) or combination of radical prostatectomy and radiotherapy (RT) with curative intent. If the confirmatory PSA value is less than the screening PSA value, then an additional test for rising PSA will be required to documents progression (see Figure below):



3. Prior primary or salvage radiation or not a candidate for salvage radiation due to patient preference or clinical assessment based upon disease characteristics and/or patient comorbidities.
4. Minimum PSA:
 - *If no prior androgen deprivation therapy (ADT) for biochemical relapse:*
 - 1.0 ng/mL if prior radical prostatectomy with or without adjuvant/salvage radiation therapy, confirmed by repeat measurement at least 2 weeks later, or
 - Nadir + 2 ng/mL if prior RT alone without prior radical prostatectomy, confirmed by repeat measurement at least 2 weeks later
 - *If prior ADT for biochemical relapse:*
 - 4.0 ng/mL or > 2 ng/mL above nadir on prior cycle of ADT, whichever is higher, confirmed by repeat measurement at least 2 weeks later

5. No evidence of metastatic disease on imaging by whole body bone scan (technetium-99 or Na-F PET bone scan) and cross-sectional imaging of the abdomen/pelvis (CT or MRI) within 6 weeks of Day 1 of protocol therapy
6. Prior androgen deprivation therapy (ADT) with LHRH agonist and/or antagonist allowed for either (neo)adjuvant treatment with local therapy or for biochemical relapse
7. Last effective dose of LHRH agonist/antagonist "expired" > 3 months prior to study entry.
8. For example, a patient receiving LHRH agonist injection every 3 months would be eligible provided their last injection was > 6 months prior to Day 1 of protocol therapy. A patient receiving LHRH agonist injections every 4 months will be eligible provided last injection was >7 months prior to Day 1 of protocol therapy.
9. Serum testosterone level:
 - *If no prior androgen deprivation therapy:*
 - A single measurement greater than 150 ng/dL within 3 months of day 1 of protocol therapy
 - *If prior androgen deprivation therapy (either in adjuvant or biochemical relapse setting):*

The two most recent measurements of serum testosterone prior to Day 1 of protocol therapy must fulfill the following criteria:

 - Both measurements are greater than 150 ng/dL.
 - The two measurements are spaced at least 14 days apart.
 - Both must be measured within 3 months of Day 1 of protocol therapy.
 - There must not be an increase of > 50 ng/dL between these two successive measurements.
10. PSA doubling time (PSADT) ≤ 15 months, calculated based upon all serum PSA measurements obtained within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA measurements spaced at least 14 days apart (see section 6). PSA values obtained when serum testosterone was known to be less than 150 ng/dL, prior to local therapy, or within three months of last dose of LHRH agonist/antagonist or antiandrogen will be excluded from the calculation of the PSADT. PSADT calculation to be carried out using the following website:
<http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx>
11. Total bilirubin less than 1.5 times upper limit of normal (ULN), or less than 3 times ULN at study entry in a patient with documented Gilbert's disease.
12. ALT and AST levels less than 1.5 times ULN at study entry
13. Serum potassium greater than 3.5 mmol/L without oral supplementation
14. No history of uncontrolled hypertension (blood pressure > 160/100 mm Hg despite anti-hypertensive medication)
15. ECOG performance status of 0 or 1
16. Estimated life expectancy greater than 5 years
17. Age greater than or equal to 18 years at time of study entry
18. Ability to sign written informed consent

19. Ability to swallow study drug whole as a capsule
20. Primary prostate cancer tissue available for analysis is not required for inclusion onto this study but is strongly encouraged.
21. Patients who have partners of childbearing potential must be willing to use a method of birth control with adequate barrier protection as determined to be acceptable by the principal investigator and sponsor during the study and for 1 week after last study drug administration.

4.3 Exclusion Criteria

1. Castrate-resistant disease, as evidenced by either:
 - Rising PSA on 2 consecutive measurements at least 2 weeks apart with concurrent documented serum testosterone < 50 ng/dL at the time of PSA measurement, or
 - Rising PSA on 2 consecutive measurements at least 2 weeks apart measured within 3 months after last LHRH agonist/antagonist injection
1. Prior bilateral orchiectomy
2. Congestive heart failure of NYHA class III or higher severity at study entry
3. History of chronic active hepatitis
4. Grade 2 or higher peripheral neuropathy at the time of study entry
5. Use of 5-alpha reductase antagonist (i.e. finasteride, dutasteride) or antiandrogen (i.e. flutamide, bicalutamide) within 6 weeks of Day 1 of protocol therapy
6. Use of systemic steroids at an equivalent dose of prednisone 5 mg/day or higher within 6 weeks of Day 1 of protocol therapy
7. Use of medications or herbal supplements which are known to potentially lower serum PSA within 6 weeks of Day 1 of protocol therapy (see appendix 1 for list of medications)
8. Use of other medications that may potentially interact with itraconazole within 1 week of study entry (see appendix 2 for list of medications)
9. Use of other investigational agents within 6 weeks of Day 1 of protocol therapy
10. Prior pathology consistent with small cell carcinoma or prostate cancer with predominantly neuroendocrine differentiation

5.0 Study Rationale

5.1 Rationale for the Study Population

Patients with biochemically relapsed, non-castrate, non-metastatic prostate adenocarcinoma after prior definitive local therapy will be enrolled onto this study. Both patients without prior ADT for biochemical relapse, or those on the “off” phase of a cycle of intermittent ADT will be allowed to enroll on the current study. Though allowing prior ADT for BCR may add heterogeneity with respect to expected disease outcomes within the study population, the primary objective of the current study is to investigate whether or not itraconazole has significant biologic activity in this disease setting, as reflected by decline in serum PSA after 12 weeks of therapy. Prior ADT for BCR is not hypothesized to significantly decrease the chance of detecting

PSA decline on itraconazole therapy, though it is acknowledged that prior studies of intermittent ADT have shown that nadir PSA tends to be higher and “off” phase length shorter with successive cycles of intermittent ADT [9]. Furthermore, the role for non-hormonal agents in BCR may eventually fit within the framework of an intermittent ADT strategy, to lengthen the period “off” therapy. As such, including patients with prior ADT for BCR in the current study reflects this potential future application of these agents.

Patients with up to 15 months baseline PSADT can be enrolled onto the current study. Prior studies have shown that pre-treatment PSADT is an independent prognostic factor in the BCR disease setting, and patients with a progressively shorter PSADT have an increasing risk of distant metastases and prostate-cancer specific mortality [38, 39]. Patients with a slow PSA doubling time > 15 months may be more suited to observation alone or local salvage therapies, and as such will be excluded from the current clinical trial.

The minimum PSA required for study entry varies depending on the modality of prior treatment, in keeping with guidelines set forth by the PSA Working Group [36]. In patients with prior radiation therapy (external beam or brachytherapy), the minimum PSA required for study entry is nadir PSA after RT + 2.0 ng/mL, consistent with ASTRO guidelines to define biochemical recurrence [40]. For patients with prior radical prostatectomy, definition of BCR varies by institution, but in general is usually defined as a PSA > 0.4 using a sensitive assay. A minimum PSA of 1.0 ng/mL is chosen for this study to allow for a more accurate determination of baseline

PSADT. For patients with prior ADT for biochemically relapsed disease, as outlined in section 1 of the protocol, there is no standard and commonly accepted definition of PSA progression. In prior clinical trials of intermittent ADT, widely varying PSA thresholds have been used as a “trigger” to re-initiate therapy, ranging from 2-20 ng/mL in absolute value or 25-100% of baseline or nadir PSA. The minimum PSA after prior ADT for BCR utilized in the current study (4.0 ng/mL or > 2 ng/mL above nadir PSA on prior ADT, whichever is higher) is acknowledged to be arbitrary but reflects current clinical practice and prior clinical trials of intermittent ADT, in which the second and subsequent cycles of ADT are often initiated upon reaching a pre-specified absolute PSA level that ranges from 2-20 ng/mL.

Patients with a history of prior androgen deprivation therapy will be required to have a stable serum testosterone level, as defined by the absence of an increase of more than 50 ng/dL between consecutive measurements using the two most recent measurements prior to the initiation of protocol therapy. A rising serum testosterone level may drive the rise in PSA during the period of testosterone recovery, which would then confound efforts to interpret pre-treatment vs. on-treatment changes in PSA doubling time and PSA kinetics, one of the secondary endpoints of the current study. Without attempting to exclude patients in the testosterone recovery phase, a lengthening of the PSA doubling time on therapy may be reflective of a plateau in testosterone recovery rather than a biologic effect of the therapeutic agent being tested.

5.2 Rationale for the Study Design and Dose Selection of Itraconazole

5.2.1 Rationale for the Study Design

The current study is a single arm, phase II trial of itraconazole in patients with biochemically relapsed disease. The primary aim of the study is to assess biological activity of itraconazole at dose of 300 mg PO BID as evidenced by decline in serum PSA $\geq 50\%$ from baseline after 12 weeks of protocol therapy. Estimates of historical control PSA response proportions can be fairly accurately estimated based upon the numerous prior studies of non-castrating agents in biochemically relapsed prostate cancer, including the recently published trial of the superoxide dismutase inhibitor ATN-224 [10-16]. In these studies, the biologic activity was modest, with the proportion of patients achieving $> 50\%$ decline in serum PSA on therapy usually 5% or less. Preliminary evidence demonstrating substantial modulation of serum PSA with itraconazole, along with acceptable toxicity and minimal impact on the adrenal hormone synthetic pathway, would potentially demonstrate that itraconazole is a viable non-castrating treatment alternative for patients with biochemical relapse and may additionally form the basis for further study of itraconazole in a larger placebo-controlled, randomized phase II/III trial, with adequate statistical power to detect a difference in clinically relevant efficacy endpoints such as time to first metastasis.

Interim analyses with pre-specified stopping rules for safety and lack of efficacy will be carried out after 10 patients have been treated for a minimum of 8 weeks and 30 patients for a minimum of 12 weeks, respectively (see Section 10). Study accrual will continue during the two

planned interim analyses, which we feel is acceptable due to the previously well-documented safety profile of itraconazole, continuous safety data monitoring, the relatively asymptomatic nature of the biochemically relapsed non-castrate patient population, and the previously utilized single stage study designs of other non-castrating therapies in the biochemically relapse disease setting.

5.2.2 Rationale for Dose Selection of Itraconazole

Itraconazole is FDA-approved at doses ranging from 100 mg/day to 300 mg PO BID for chronic treatment of invasive fungal infections. High-dose itraconazole (300 mg PO BID) has demonstrated acceptable safety and tolerability in immunosuppressed patients with chronic fungal infections, as well as in a prior phase II trial in patients with CRPC [19], and thus has acceptable prior safety data to justify dosing at 300 mg PO BID in the current study. The protocol contains pre-specified dose reductions for anticipated toxicities (see Section 8) and a stopping rule for safety after the first 10 patients have been treated for a minimum of 8 weeks (see Section 10).

From an efficacy and pharmacodynamic standpoint, the optimal dose of itraconazole in prostate cancer is not yet well-characterized. However, based on the prior randomized phase II study of low- and high-dose itraconazole (200 mg/day and 300 mg PO BID respectively) in metastatic castrate resistant prostate cancer, low-dose itraconazole appeared to have only minimal biologic activity and down-regulated *GLII* expression in only one third of patients (see Section 1). In contrast, the high-dose study arm demonstrated significant biologic activity and down-regulated *GLII* expression in approximately two-thirds of patients. Thus, the dose of 300 mg PO BID will be chosen for further study in the current trial.

5.3 Rationale for the Selection of Study Endpoints

5.3.1 Primary Endpoint

The primary endpoint of this trial will be the proportion of patients who achieve a $\geq 50\%$ decline in serum PSA after 12 weeks of protocol therapy, which can serve as a marker of biological activity of itraconazole in biochemically relapsed hormone sensitive prostate cancer. Although decline in serum PSA has not been prospectively validated as a surrogate marker for clinical outcomes in this disease setting, a prior retrospective analysis identified PSA decline on therapy as an independent predictor of prostate-cancer mortality in patients with biochemically relapsed prostate cancer treated with androgen deprivation therapy [35]. In addition, PSA decline was chosen as the primary endpoint in several prior trials of non-hormonal agents with varying mechanisms of action in this disease setting, including studies investigating the small molecule tyrosine kinase inhibitor imatinib and immune-modulating therapy with GM-CSF [12, 15]. Furthermore, PSA decline on therapy as a primary endpoint may be less prone to inter-patient variability than other endpoints involving PSA modulation such as time to PSA progression or change in PSA doubling time, as has been observed in prior trials of non-hormonal therapies in biochemical relapse [14].

Favorable results with respect to decline in PSA, along with acceptable safety and endocrinologic data, would form the basis for further study of itraconazole in biochemically relapsed disease, with the larger subsequent studies adequately designed (with sufficient power) for primary efficacy endpoints of clinical relevance, including time to first metastasis and overall survival.

5.3.2 Secondary Endpoints

A number of additional endpoints will be evaluated to assess the overall impact of itraconazole on PSA kinetics, clinical outcome, safety, and pharmacokinetics.

5.3.2.1 PSA Kinetics

Mean Change From Baseline in PSADT

The mean change from baseline in PSADT after 12 weeks of protocol therapy will be measured for patients treated with itraconazole. A prior retrospective analysis of four non-hormonal agents tested in the biochemically relapsed setting identified change in the pre-treatment vs. on-treatment natural log PSA slope as an independent prognostic factor with respect to development of metastatic disease [37]. Novel agents tested in BCR may have clinical benefit without causing PSA decline but by slowing the rate of PSA rise instead, especially for agents with a cytostatic effect on tumor cells. It is not known if itraconazole has either cytoidal or cytostatic activity (or neither) in biochemically relapsed prostate cancer, and thus measuring both declines in PSA and changes in PSA kinetics is warranted.

Time to PSA Progression

The PCWG2 has suggested that duration of clinical activity is a key intermediate endpoint in initial phase II trials of agents conducted in the BCR disease population [36]. Indeed, in an exploratory analysis of a trial of IADT in BCR patients, Yu and colleagues demonstrated that in patients who completed the first cycle of IADT, a shorter duration of the first “off treatment” interval was associated with a shorter time to developing castrate resistant prostate cancer and shorter overall survival [41]. In the proposed trial, the median time to PSA progression on itraconazole therapy will be evaluated. The definition of PSA progression, as outlined in Section 2 ($> 50\%$ and > 2 ng/mL above nadir (or above baseline if no PSA decline), is acknowledged to be arbitrary but does reflect PCWG2 guidelines for PSA progression in the castrate-resistant disease setting.

Given the heterogeneity of the study populations in prior studies of non-hormonal agents evaluated in BCR, as well as the variable definition of PSA progression used in prior clinical trials, using historical data to generate accurate estimates of median time to PSA progression is problematic. The analysis in the current study is exploratory in nature, aimed at generating preliminary estimates of the impact of itraconazole on the time to PSA progression.

5.3.2.2 Clinical Outcome

The median time to clinical progression, as defined by the first occurrence of either initiation of non-protocol therapy (including initiation or resumption of ADT) or development of overt

metastatic disease, as well as the median metastasis-free survival, will be determined for patients treated with itraconazole. As was the case for time to PSA progression, this analysis is exploratory in nature, and the aim is to generate preliminary estimates of time to disease progression to potentially aid in the planning of future study of itraconazole in biochemically relapsed prostate cancer. Serial radiographic scans will not be mandated by the study, as this does not reflect standard clinical practice (see Section 6). Scans will be required at baseline, at the time of PSA progression, and as clinically indicated for signs/symptoms on history and physical exam. If itraconazole is further evaluated in a definitive, placebo-controlled phase II/III trial in BCR, periodic scans would need to be mandated to ensure balanced evaluations per treatment arm.

5.3.2.3 Pharmacokinetics

The mean steady-state serum concentration of itraconazole and hydroxyl-itraconazole, its major active metabolite, will be measured after 4 weeks of protocol therapy. Prior studies have demonstrated variability in the oral bioavailability of the oral capsule formulation, dependent mostly upon the degree of gastric acidity (lower pH enhances oral absorption) at the time of ingestion. In an exploratory analysis of low- and high-dose itraconazole in CRPC, higher steady-state serum concentrations of itraconazole and hydroxyl-itraconazole were associated with a higher likelihood of being free of PSA progression at 24 weeks and displaying greater percentage PSA decline on therapy [31]. A similar analysis will be carried out in the current study.

5.3.3 Correlative Endpoints

5.3.3.1 Endocrine Parameters

The mean change from baseline in serum androgen levels, including testosterone, DHEA-S, and androstenedione after 12 weeks of therapy with itraconazole will be determined to investigate further the mechanism of action of itraconazole in prostate cancer. Prior study in CRPC provided preliminary evidence that itraconazole, unlike the related azole compound ketoconazole, has a mechanism of action distinct from androgen synthesis inhibition [19], albeit in a castrate patient population. Demonstrating in the current study, with a non-castrate patient population, that itraconazoletherapy concurrently does not significantly impact serum androgen levels yet modulates serum PSA will lend further support to the hypothesis that itraconazole has a mechanism of action distinct from ketoconazoleand androgen synthesis inhibition. The main limitations of this particular analysis is that serum hormone levels may not reflect intra-tumoral androgen concentrations, and that this patient population is non-castrate with a different systemic hormonal milieu than the previous study in CRPC.

In the prior clinical trial of itraconazole in CRPC, there were several patients with a constellation of adverse events including hypokalemia, hypertension, and peripheral edema [19]. This constellation of symptoms has also occasionally been observed in prior studies of high dose itraconazole in patients with chronic fungal infections, and raises the possibility of induced mineralocorticoid excess. Serum aldosterone levels were measured in the prior study of itraconazole in CRPC (and were not elevated on therapy); however upstream compounds within

the adrenal synthetic pathway with mineralocorticoid activity, including deoxycorticosterone (DOC) and 11-deoxycortisol were not measured. A more comprehensive panel of adrenal and pituitary hormones, including ACTH, DOC, aldosterone, and 11-deoxycortisol, will be measured on a serial basis in the current study and the mean change from baseline will be determined.

Evaluation for a relationship between baseline and percent change in serum hormone levels with PSA modulation will be evaluated in the current study. Prior studies of ketoconazole and abiraterone in metastatic CRPC demonstrated a relationship between higher baseline levels of adrenal androgen levels with clinical response [21, 42], and a prior trial of estrogenic compounds (DES and the herbal supplement PC-SPES) in CRPC demonstrated that a decline in adrenal androgen DHEA-S was related to a decline in serum PSA on therapy [43]. Whether the same relationships apply to itraconazole, which is hypothesized to have a mainly non-hormonal mechanism of action, and whether the same relationships between serum hormone levels and clinical response apply to the biochemically relapsed disease setting as it does in CRPC, will be evaluated in the current study.

5.3.3.2 Hedgehog Pathway

The impact of itraconazole on Hedgehog pathway activity will be assessed by serial skin biopsies obtained at baseline and after 4 weeks of therapy. Hedgehog pathway activity will be assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of *GLII*mRNA transcripts in the skin biopsy specimens. *GLII* is a downstream transcription factor upregulated by activation of the Hh pathway that functions in a positive feedback loop. This method for analysis of Hh pathway activity has been previously validated in a study of the small molecule *SMO* inhibitor GDC-0449 in basal cell carcinoma of the skin [25]. In patients with CRPC treated with itraconazole, down-regulation of *GLII* expression was shown to be associated with a longer PSA progression-free survival [31].

In an exploratory fashion, for patients with prior archived primary prostate cancer tissue available, the relationship between mRNA and immunohistochemical expression of components involved in the Hh pathway, including Sonic hedgehog ligand, *PCTH1*, *GLII*, and *SMO*, and PSA modulation on subsequent itraconazole therapy will be determined. Such an analysis may eventually lead to the development of a predictive biomarker for itraconazole, which would require prospective validation in larger placebo controlled trials.

In summary, the proposed trial will evaluate the effects of itraconazole in patients with non-metastatic biochemically relapsed prostate cancer with a variety of biologically relevant endpoints. Favorable results with respect to PSA modulation, along with acceptable toxicity and lack of adverse impact on endocrine parameters, along with demonstration of Hedgehog pathway down-regulation, would demonstrate itraconazole as a viable non-castrating treatment strategy in this disease setting, and may help to form the justification for a larger placebo-controlled clinical trial of itraconazole in BCR, with primary endpoints potentially including time to metastatic disease and overall survival.

6.0 Study Activities

6.1 Study Schedule

	Pre-Study	Cycle 1		Cycle 2	Cycle 3	Cycle 4	Cycle 5 and beyond	End of Study
Procedure	Screening	Day 1	Day 8 (+/- 3 days)	Day 1 (+/- 7 days)	PSA or Clinical Progression			
Informed Consent ^a	X							
Within 3 months of Cycle Day 1								
PSA ^b	X ^c	X		X	X	X	X	X
Serum Testosterone ^d	X ^e			X		X		X
Within 6 weeks of Cycle Day 1								
Whole body bone scan + CT or MRI of and/pelvis ^f	X							X ^g
Skin biopsy (optional) ^h	X			X				
Within 28 days (4 weeks) of Cycle Day 1								
Demographics	X							
History / Physical	X	X	X	X	X	X	X	
Height/Weight	X	X	X	X	X	X	X	
ECOG Performance Status	X	X	X	X	X	X	X	
Toxicity Assessment	X	X	X	X	X	X	X	X
CBC w/diff and platelets	X						X	
BUN / Creatinine	X						X	
ALT, AST, AlkP, total bilirubin	X		X	X	X	X	X	
Other serum hormones ^d	X			X		X		X

6.1 Study Schedule

Procedure	Pre-Study	Cycle 1		Cycle 2	Cycle 3	Cycle 4	Cycle 5 and beyond	End of Study
	Screening	Day 1	Day 8 (+/- 3 days)	Day 1 (+/- 7 days)	PSA or Clinical Progression			
Plasma/serum for banking (optional) ⁱ	X			X		X		X
PBMCs for banking (optional) ⁱ	X							
Concomitant Medications	X	X	X	X	X	X	X	
Serum sodium and potassium	X		X	X	X	X	X	
Archived primary prostate cancer tissue (optional) ^j	X							
Itraconazole dispensed ^k		X		X	X	X	X	
After Cycle 1								
Trough itraconazole& [OH]-itraconazole serum levels ^l				X				

^a Informed Consent prior to any Screening procedures

^b Measured in same laboratory at each time point during study (if possible).

^c All serum PSA measurements within 3 months prior to Day 1 of protocol therapy will be included for the calculation of baseline PSA doubling time, with a minimum of three values each spaced at least 14 days apart.

^d Serum hormone levels should be collected between 8:00 am and 12:00pm if possible; will be shipped to UCSF and banked for later analyses; includes testosterone, cortisol, aldosterone, deoxycorticosterone, 11-deoxycortisol, adrenocorticotropic hormone (ACTH), dihydroepiandrosterone-sulfate (DHEA-S), and androstenedione (see Appendix 3, section IV for processing, storage, and shipping instructions).

^e For serum testosterone measurement, if the patient does not have a history of ADT, a single measurement > 150 ng/dL within 3 months prior to Day 1 of protocol therapy will be required for study entry. For patients with any prior history of ADT, the two most recent serum testosterone measurements prior to Day 1 of protocol therapy must fulfill the following requirements:

- Both measurements > 150 ng/dL
- Both measurements obtained within 3 months prior to day 1 of protocol therapy
- Measurements must be spaced at least 14 days apart
- There must not be an increase of > 50 ng/dL between the two consecutive measurements

^f IV contrast with CT or MRI per individual investigator discretion. Whole body bone scan may consist of either technetium-99 labeled scan or sodium-fluoride PET bone scan.

^g May be omitted if completed within the past 30 days.

^h 3 mm punch biopsy non-scalp skin containing hair follicles

ⁱ Collection of PBMCs at baseline and serum/plasma at baseline, cycle 2 day 1, cycle 4 day 1, and at disease progression is optional. Blood will be shipped to UCSF and stored within HDFCCC Tissue Bank. See appendix 3, section IV for processing and shipping instructions).

6.1 Study Schedule

Procedure	Pre-Study	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5 and beyond	End of Study	
	Screening	Day 1	Day 8 (+/- 3 days)	Day 1 (+/- 7 days)	PSA or Clinical Progression			

^j Archived formalin-fixed paraffin embedded prostate biopsy or prostatectomy specimen (optional)

^k Itraconazole to be taken as three (100 mg) capsules twice daily with food (6 capsules total per day; 168 capsules dispensed every 4 weeks during study visits)

^l Hold am dose of itraconazole

ALT = alanine aminotransferase, AST = aspartate aminotransferase, AlkP = alkaline phosphatase, PBMC = peripheral blood mononuclear cells

6.2 Screening Period/Pre-Study Evaluation

All patients must sign a written informed consent form before study specific screening procedures are performed. Screening procedures to evaluate patient eligibility will be conducted as per the schedule outlined below. If the patient meets eligibility and screening requirements he will return to the site for the day 1 visit and drug dispensation.

6.2.1 Establish baseline PSA doubling time and serum testosterone level for eligibility determination (within 3 months prior to day 1 of protocol therapy)

- Estimation of baseline PSA doubling time
 - Calculated based upon all serum PSA measurements obtained within 3 months prior to day 1 of protocol therapy, with a minimum of three PSA measurements each spaced at least 14 days apart.
 - PSA values obtained when serum testosterone was known to be less than 150 ng/dL, prior to local therapy, or within three months of last dose of LHRH agonist/antagonist or antiandrogen will be excluded from the calculation of the PSA doubling time.
 - Measurement of all PSA values included in determination of PSA doubling time recommended to be performed in the same laboratory
 - Actual calculation of the PSA doubling time can be obtained from the following online calculator: <http://nomograms.mskcc.org/Prostate/PsaDoublingTime.aspx>
 - Patients eligible if PSA doubling time is less than or equal to 15 months.
- Serum testosterone level
 - *If no prior androgen deprivation therapy:*
 - A single measurement greater than 150 ng/dL within 3 months of day 1 of protocol therapy
 - *If prior androgen deprivation therapy (either in adjuvant or biochemical relapse setting):*

The two most recent measurements of serum testosterone prior to study entry must fulfill the following criteria:

 - Both measurements greater than 150 ng/dL,
 - The two measurements are spaced at least 14 days apart
 - Both must be measured within 3 months of day 1 of protocol therapy
 - There must not be an increase of > 50 ng/dL between these two successive measurements

6.2.2 Clinical (within 28 days of day 1 of protocol therapy)

- Complete history and physical examination
- Baseline demographics

Version 4.0: October 8, 2014

- Height and weight
- ECOG Performance Status
- Adverse Event Assessment

6.2.3 Laboratory (within 28 days of day 1 of protocol therapy)

- Complete blood count including differential and platelet count
- Total bilirubin, alkaline phosphatase, AST, ALT, BUN, creatinine, sodium, potassium
- Serum testosterone, androstenedione, DHEA-S, cortisol, aldosterone, deoxycorticosterone, 11-deoxycortisol, and ACTH levels
- Plasma/serum and PBMCs for banking (Optional)

6.2.4 Radiographic Studies and Procedures (within 6 weeks of day 1 of protocol therapy)

The following will only be conducted if the patient is otherwise eligible for the study as per the laboratory/clinical parameters outlined above and has given written, informed consent.

- Radionuclide bone scan(either technetium-99 or Na-F radiolabel)
- Cross-sectional imaging of the abdomen and pelvis (CT or MRI). Use of IV contrast per individual investigator discretion.
- Skin biopsy (if additional consent is obtained).

6.3 Study Treatment

Patients will commence protocol therapy on Day 1 of study treatment. Patients will continue protocol therapy and remain on study until PSA progression (see Section 2 for definition), development of metastases, initiation of non-protocol therapy, unacceptable toxicity, or patient/physician withdrawal from the study, whichever occurs soonest. The study timeline is defined as Day 1 = first day of study treatment initiation. Subsequent study time points will be defined based on study calendar, irrespective of subsequent dose delays/interruptions (see Section 8 for discussion of dose delays due to toxicity).

6.3.1 Day 1 of Study Treatment

Patients will return to study site for a history and physical exam including weight, adverse event and con meds assessment, ECOG, measurement of serum PSA, and dispensing of study drug. The starting dose of itraconazole will be 300 mg PO BID, taken as 3 (100 mg) capsules twice daily with meals.

6.3.2 Dose Modifications

Both dose interruptions and dose reductions will be permitted for toxicity, as outlined in Section 8.

Version 4.0: October 8, 2014

6.3.3 Concomitant Medications

Concomitant medications will be recorded at each monthly clinic visit. No concomitant use of any anti-neoplastic therapy will be permitted.

This will include:

- Surgery
- Radiation therapy
- Other secondary hormonal therapies including antiandrogens, 5-alpha reductase inhibitors (i.e. Finasteride, dutasteride), megestrol acetate
- Immunotherapy
- Chemotherapy
- Or other investigational agents

Nutritional supplements containing saw palmetto, pomegranate extract, or pomegranate juice are also specifically prohibited. See Appendix 1 for a full list of medications and substances that are prohibited while taking itraconazole.

Patients may not currently be taking drugs that significantly interact with CYP3A4. A list of medications and substances with the potential to interact with itraconazole are provided in Appendix 2. Patients taking digoxin should have serum digoxin levels monitored frequently; patients on warfarin should have INR levels measured regularly.

Because of the potential for drug-drug interaction, the electronic case report form (eCRF) must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

6.3.4 Safety Assessment

Patients will be assessed for adverse events at Day 1 (for baseline) and Day 8, week 4, and every 4 weeks thereafter while receiving protocol therapy. Adverse events will be graded according to CTCAE version 4.03 (available at <http://ctep.cancer.gov>). Adverse events will be assessed by the individual investigator as definitely, probably, possibly, unlikely, or not related to study drug. Laboratory measurement is outlined below in Section 6.3.5. Additional laboratory measurements are at the discretion of the treating physician.

6.3.5 Laboratory/Radiographic Assessment

The frequency and schedule of laboratory and radiographic assessment is outlined above in the study schedule. Serum PSA will be measured on a monthly basis while patients are receiving protocol therapy. A whole body bone scan (either Na-F PET or technetium-99 bone scan), along with cross-sectional imaging of the abdomen/pelvis (CT or MRI) will be obtained at baseline and at the time of PSA or clinical progression, whichever occurs first.

Version 4.0: October 8, 2014

Liver function tests and serum sodium and potassium levels will be measured pre-study, on day 8 +/- 3 days, day 28 +/- 7 days, then every 4 weeks +/- 7 days thereafter. BUN/serum creatinine will be measured pre-study and every 4 weeks +/- 7 days.

Serum itraconazole and hydroxy-itraconazole levels will be measured at the day 28 (+/- 7 days) study visit. Hormone levels (outlined above in the study schedule) will be measured at baseline, day 28 (+/- 7 days), and week 12 (+/- 7 days) of protocol therapy.

6.3.6 Skin Biopsy

For patients who sign additional consent form, a 3 mm punch biopsy of non-scalp skin containing hair follicles (will be obtained at baseline (within 6 weeks of Day 1 of protocol therapy) and day 28 +/- 7 days of protocol therapy. Serial skin biopsies are an optional part of this study.

6.4 Reasons for Treatment Discontinuation

Patients will discontinue protocol therapy and betreated per individual investigator discretion at the first occurrence of any of the following:

- PSA progression
- Development of overt metastases
- Patient or treating physician decision
- Unacceptable toxicity
- Non-protocol therapy

Patients who discontinue therapy due to development of metastases detected on radiographic tests, initiation of non-protocol therapy, or patient/physician decision will be removed from study at the time of protocol therapy discontinuation.

Patients who discontinue protocol therapy due to PSA progression or unacceptable toxicity will remain on study in follow up phase (see next section) until clinical progression.

6.5 Follow Up

Patients will have a safety follow up visit 30 days after the last dose of study drug.

If patients discontinue protocol therapy due to PSA progression or unacceptable toxicity, they will be followed until the time of initiation of non-protocol therapy, development of metastases, or patient/physician withdrawal from study, whichever occurs soonest, at which point they will be taken off study.

During the follow up phase, the frequency of clinical/laboratory/radiographic assessment will beper individual investigator discretion.

6.6 Duration of Study

The total duration of the study including follow up is expected to be approximately 3 years from the date the first patient is enrolled onto study.

7.0 Study Drug Information

7.1 Itraconazole

Drug Substance: Itraconazole is an almost white to slightly yellow powder.

Chemical Name: (\pm)-cis-4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3H-1,2,4-triazol-3-one

Molecular Formula: C₃₅H₃₈Cl₂N₈O₄

Molecular Weight: 705.64

Formulation: Itraconazole is available as pink and blue capsules containing 100 mg of itraconazole in a pellet formulation. Each capsule contains 100 mg of itraconazole as well as: sugar spheres (NF), hydroxypropylmethylcellulose, gelatin, polyethylene glycol, titanium dioxide, FD&C Blue No. 1, FD&C Blue No. 2, D&C Red No. 22 and D&C Red No. 28. The pKa of itraconazole is 3.7 with a melting range of 165-169 degrees Celsius. It is highly hydrophobic and lipophilic, with a log partition coefficient of 5.66 in the n-octanol/aqueous buffer solution of pH=8.1. Itraconazole is very poorly soluble in water (<1 µg/mL) and in diluted acidic solutions (<5 µg/mL).

Route of Administration: Itraconazole 100 mg capsules are administered orally. Absorption of itraconazole is impaired when the gastric pH is increased. Capsules should be taken after a meal. If a patient is taking a concurrent proton pump inhibitor or H₂-antagonist, it is advisable that itraconazole be taken in conjunction with an acidic beverage such as a cola product or orange juice to increase absorption. Grapefruit juice is not recommended due the potential drug interaction with itraconazole.

Pharmacokinetics:

Absorption: Better absorbed with food and lower gastric pH.

Distribution: V_d (average): 796 ± 185 L or 10 L/kg; highly lipophilic and tissue concentrations are higher than plasma concentrations. The highest concentrations: adipose, omentum, endometrium, cervical and vaginal mucus, and skin/nails. Aqueous fluids (eg, CSF and urine) contain negligible amounts.

Protein binding, plasma: 99.8%; metabolite hydroxy-itraconazole: 99.5%

Metabolism: Extensively hepatic via CYP3A4 into >30 metabolites including hydroxy-itraconazole (major metabolite); appears to have *in vitro* antifungal activity. Main metabolic pathway is oxidation; may undergo saturation metabolism with multiple dosing.

Version 4.0: October 8, 2014

Bioavailability: Variable, ~30-40% (capsule).

Half-life elimination: Oral: Single dose: ~21 hours, steady state: 64 hours; Cirrhosis (single dose): 37 hours (range 20-54 hours)

Time to peak, plasma: 3-5 hours

Excretion: Urine (<0.03% active drug, 40% as inactive metabolites); feces (~3% to 18%)

Storage and Handling: Itraconazole capsules are supplied in HDPE bottles of 30. They should be stored at room temperature (15-30 degrees Celsius) and protected from light and moisture.

Dose: 300 mg PO BID. Three 100 mg capsules taken twice daily with food (6 capsules per day in total).

Possible Anticipated Adverse Events: The most frequently reported adverse events in prior clinical trials of itraconazole were gastrointestinal in nature, including diarrhea, nausea and/or vomiting, anorexia, or abdominal pain. Other potential adverse events include peripheral edema, hypertension, hypokalemia, fatigue, and elevated liver enzymes. Rare but serious and potentially life threatening adverse events include adrenal insufficiency, allergic reactions including Stevens-Johnson syndrome, congestive heart failure, and liver failure. Itraconazole may also potentially interact with a number of other medications and herbal supplements, so a careful review of the concurrent medications is warranted during therapy (see Appendix 2 for list of potential interacting medications to avoid while on itraconazole therapy).

Dispensing of Drug: A 28 day supply of study drug will be provided to patients at each study visit every 4 weeks.

Inventory and Control: The itraconazole used for this study must be maintained under adequate security and stored in the pharmacy at the study site until dispensing or their return to the UCSF Helen Diller Family Comprehensive Cancer Center investigational pharmacy. Investigators may not supply study medication to any person not enrolled in this study or to any person not named as a sub-investigator.

In addition, the investigator must maintain an accurate, running inventory of all drug supplies received and dispensed during conduct of the study. At the completion of the study, a copy of the final inventory will be supplied to the UCSF Helen Diller Family Comprehensive Cancer Center investigational pharmacy. The original will be retained by the investigator in the study files.

Return of Clinical Supplies: Upon completion or termination of the study, all used and unused original study drug bottles, whether empty or containing study drug, will be returned to the UCSF Helen Diller Family Comprehensive Cancer Center investigational pharmacy. Return shipments must provide proof of delivery, and will be accompanied by the Drug Accountability Form and a memo noting the number of bottles and quantity of study drug being returned.

8.0 Dosage Modifications/Toxicity

8.1 Toxicity

Toxicities and Adverse Events will be assessed during each monthly clinic visit using the NCI CTCAEversion 4.03. Since CTEP has standardized the CTC, the NCI does not require the inclusion of the CTCAE within this protocol document. However, all appropriate treatment areas will have access to a copy of the CTCAEversion 4.03. A copy can be downloaded from the CTEP home page: <http://ctep.cancer.gov>

Dose re-escalation after prior dose reduction due to toxicity will not be permitted.

8.2 Dose Modifications for Specific Toxicities

8.2.1 Management of Hypokalemia

Every patient that experiences Grade 1 hypokalemia (serum potassium < 3.5 mM or below lower limit of normal range, but \geq 3.0 mM) will require oral potassium supplementation, and will be monitored bi-weekly until serum potassium is between 3.5 to 5.0 for \geq 4 weeks. The frequency of testing after supplementation is at Investigator discretion. The dose of potassium supplement must be titrated to maintain serum potassium at \geq 3.5mM but \leq 5.0 mM. If any patient experiences Grade 3 hypokalemia (serum potassium levels < 3.0 mM but \geq 2.5 mM) study treatment will be held, and the patient will be given intravenous and/or oral potassium replacement. If hypokalemia resolves and potassium level remains $>$ 3.5 mM on a stable oral dose for a period $>$ 1 week, itraconazole may be restarted at 200 mg/day per discretion of study investigator. If hypokalemia recurs to grade 3 or higher, itraconazole will be permanently discontinued. If any patient experiences Grade 4 hypokalemia (< 2.5 mM), itraconazole will be permanently discontinued and the patient will be hospitalized for IV potassium supplementation and cardiac monitoring. Any treatment delay greater than 4 weeks will result in permanent discontinuation of the study drug.

Serum Potassium	Grade of Hypokalemia	Action	Further Action/Maintenance
< 3.8 mM – 3.0 mM	1/2	Initiate oral K+ supplementation and increase frequency of K+ lab monitoring to bi-weekly	Titrate oral K+ dose to maintain concentration 3.5 – 5.0 mM
< 3.0 – 2.5 mM	3	Hold itraconazole; initiate standing oral supplementation +/- temporary IV supplementation	If K+ > 3.5 mM for > 1 week, re-initiate itraconazole at 200 mg/day per investigator discretion
< 2.5 mM	4	Permanently discontinue itraconazole; hospitalize for IV supplementation and cardiac monitoring	Permanently discontinue itraconazole

8.2.2 Management of Elevated Liver Function Tests

If Grade 1 increases in AST, ALT, or total bilirubin occur (AST or ALT > ULN – 3.0 x ULN or bilirubin > ULN – 1.5 x ULN), the frequency of LFT monitoring will be increased to bi-weekly but dose reduction and/or delay will not be mandated. If Grade 2 increases in AST, ALT or total bilirubin occur (AST or ALT > 3.0 – 5.0 x ULN or bilirubin > 1.5 – 3.0 x ULN, itraconazole will be held until the LFT abnormality improves to Grade 1 or lower, at which point the study drug will be restarted at 400 mg/day. If a grade 2 increase in AST, ALT, or bilirubin recurs, itraconazole will be held until improved to Grade 1 or lower in severity, and then restarted at 200 mg/day. If Grade 3 increases in AST, ALT, or bilirubin occur (AST or ALT > 5.0 – 20.0 x ULN or bilirubin > 3.0 – 10.0 x ULN, itraconazole will be held until the LFT abnormality improves to Grade 1 or lower, at which point study drug may be restarted at 200 mg/day per individual investigator discretion. If a grade 3 increase in AST, ALT, or bilirubin recurs, itraconazole will be permanently discontinued. If a grade 4 increase in AST, ALT, or bilirubin occurs, itraconazole will be permanently discontinued. Any treatment delay greater than 4 weeks will result in permanent discontinuation of itraconazole.

AST or ALT Bilirubin	Grade of LFT Abnormality	Action	Further Action/Maintenance
>ULN – 3X ULN > ULN – 1.5 X ULN	1	Increase frequency of LFT monitoring to bi-weekly	None
> 3.0 – 5.0 X ULN > 1.5 – 3.0 X ULN	2	Hold itraconazole; increase frequency of LFT monitoring to weekly	If improves to Grade 1 or lower, re-initiate at 400 mg/day. If recurrent grade 2 AE, hold until Grade 1 or lower, then re-start at 200 mg/day.
> 5.0 – 20.0 X ULN > 3.0 – 10.0 X ULN	3	Hold itraconazole; increase frequency of LFT monitoring to weekly	If improves to Grade 1 or lower, re-initiate at 200 mg/day per investigator discretion. If recurrent grade 3 AE, permanently discontinue itraconazole.
> 20.0 X ULN > 10.0 X ULN	4	Permanently discontinue itraconazole	N/A

8.2.3 Management of Hypertension

For grade 1-2 hypertension, management will be per study investigator discretion and institutional guidelines. Treatment with eplerenone should be avoided due to the potential drug interaction with itraconazole. Spironolactone should likewise be avoided due to the potential to contribute to prostate cancer progression. No dose reduction or interruption will be mandated for grade 1 or 2 hypertension. For grade 3 hypertension, itraconazole will be held until hypertension is grade \leq 1 in severity, at which point itraconazole will be restarted at 400 mg/day. BP will be

Version 4.0: October 8, 2014

monitored at investigator's discretion. If there is a recurrence of grade 3 hypertension, itraconazole will be held until grade ≤ 1 in severity, and then restarted at 200 mg/day. If there is a second recurrence of grade 3 hypertension, itraconazole will be permanently discontinued. If grade 4 hypertension occurs during study treatment, itraconazole will be permanently discontinued. If there is a greater than 4 week delay in treatment, itraconazole will be permanently discontinued.

8.3 Dose Modification for Other Adverse Events

For adverse events of grade 1 in severity, treatment will be per institutional guidelines, and no dose interruption will be mandated. For adverse events of grade 2 in severity that the study investigator deems as possibly, probably or definitely related to study drug, treatment will be interrupted until the adverse event resolves to grade ≤ 1 in severity, at which point itraconazole will be re-started at a reduced dose of 400 mg/day. If a patient experiences a recurrent adverse event of grade 2 in severity, itraconazole will be held until the adverse event resolves to grade ≤ 1 in severity, at which point itraconazole will be re-started at 200 mg/day.

Treatment will be interrupted for grade 3 adverse events that study investigator attributed as possibly, probably, or definitely related to study medication. Treatment will be held until the adverse event resolves to grade ≤ 1 in severity, at which point therapy will be re-instituted at a reduced dose of 200 mg/day. If the adverse event recurs to grade 2 in severity, treatment will be held until the adverse event resolves to grade ≤ 1 in severity, at which point therapy will be re-instituted at 200 mg/day per individual investigator discretion. If the adverse event recurs to grade 3 or higher in severity, treatment will be permanently discontinued. Treatment will be permanently discontinued and patients will not be re-challenged for any grade 4 adverse events occurring while receiving protocol therapy.

If there is a greater than 4 week delay in treatment, protocol therapy will be permanently discontinued.

Grade of Other AE	Action	Further Action/Maintenance
1	<ul style="list-style-type: none"> Treatment per institutional guidelines No dose interruption will be mandated 	None
2	<ul style="list-style-type: none"> Treatment interrupted until the adverse event resolves to grade ≤ 1 in severity @ ≤ 1 in severity, re-start itraconazole at reduced dose of 400 mg/day 	<ul style="list-style-type: none"> If recurrent grade 2 AE, hold itraconazole until resolves to grade ≤ 1 in severity @ ≤ 1 in severity, re-start itraconazole at reduced dose of 400 mg/day
3	<ul style="list-style-type: none"> Treatment interrupted and held until resolves to grade ≤ 1 in severity @ ≤ 1 in severity, re-start itraconazole at reduced dose of 200 mg/day 	<ul style="list-style-type: none"> If recurrent grade 2 AE, hold itraconazole until resolves to grade ≤ 1 in severity @ ≤ 1 in severity, re-start itraconazole at reduced dose of 200 mg/day If the AE recurs to grade 3 or higher in severity, permanently discontinue treatment

Version 4.0: October 8, 2014

Grade of Other AE	Action	Further Action/Maintenance
4	Treatment permanently discontinued and patients will not be re-challenged	N/A

Dose re-escalation after prior dose reduction due to toxicity will not be permitted.

9.0 Laboratory Assessment and Correlative Studies

9.1 Serum Hormone Levels

Serum hormone levels will be measured at baseline, week 4, week 12, and at the time of PSA or clinical progression for patients enrolled onto this study. Serum hormone levels should be drawn between 8:00 am and 12:00pm, please see table below for all collection parameters. Peripheral blood for serum hormone levels will be processed and stored using procedures outlined below and in appendix 3 section IV. The blood will be shipped to UCSF (see appendix 3 section IV) and subsequently analyzed at UCSF according to the methods outlined below.

	Time of Day	Method	Volume	Container	Processing
Testosterone	Any	LC/MS/MS	2 mL	Red Top	Separate serum and freeze at -20 degrees C
Androstenedione	AM*	LC/MS/MS	2 mL	Red Top	Separate serum and freeze at -20 degrees C
DHEA-S	Any	RIA after hydrolysis	1 mL	Gold Top	Separate serum and freeze at -20 degrees C
ACTH	AM	CLIA	3 mL	Lavender Top (pre-chilled); deliver on ice to laboratory	Separate serum and freeze plasma in plastic tube at -20 degrees C
Cortisol	AM	CLIA	1 mL	Gold Top	Separate serum and freeze at -20 degrees C
Aldosterone	AM	LC/MS/MS	2 mL	Red Top	Separate serum and freeze at -20 degrees C
11-Deoxycortisol	AM	RIA	2 mL	Gold Top	Separate serum and freeze at -20 degrees C

	Time of Day	Method	Volume	Container	Processing
Deoxycorticosterone	AM	RIA	2 mL	Gold Top	Separate serum and freeze at -20 degrees C

* Between 8:00 am and 12:00pm* Between 8:00 am and 12:00pm

DHEA-S = dihydroepiandrosterone-sulfate; LC = liquid chromatography; MS = mass spectroscopy; RIA = radioimmunoassay; CLIA = chemiluminescent immunoassay

9.2 Hedgehog Pathway Assessment By mRNA Expression Analysis

Because Hedgehog signaling is present in skin and hair follicles, *GLII* mRNA expression (a marker of Hedgehog pathway activation) will be analyzed using 3-millimeter skin punch-biopsies from hair-containing non-scalp skin (e.g. upper back or arms) obtained at baseline and after 4 weeks on study, using previously described and validated methodology [25]. The skin sample should be placed immediately into a 5 mL Tissue Protect Tube containing RNAlater solution, and care must be taken to ensure that the skin sample is fully submerged in RNAlater. Samples should then be frozen at -20 degrees Celsius or colder until the time of RNA extraction. Additionally, mRNA expression analysis of archived, formalin-fixed paraffin embedded primary prostate cancer tissue will be performed when tissue is available. Please see Appendix 3 for details regarding the skin biopsy procedure as well as specimen handling/shipping instructions.

*GLII*mRNA expression levels in tissue samples will be analyzed using the RT² Profiler Custom PCR Array protocol (SABiosciences, Qiagen). RNA will be extracted from tissue samples using RNeasy Fibrous Tissue kit (Qiagen). The RT² PCR Array First Strand kit (SuperArray Bioscience), will be used to synthesize cDNA. Quantitative PCR will be performed using RT² Real-Time SYBR Green PCR Master Mix (SABiosciences, Qiagen), in an ICycler PCR System (Bio-Rad Laboratories), according to the manufacturer's protocol. The housekeeper gene *GAPDH* will be used, as the expression level of this gene has been previously shown to not change with itraconazole therapy using a housekeeper array (Cat#: PHAS-000, SABiosciences, Qiagen). Cycling threshold (*C_t*) values for *GLII*and *GAPDH* from each experiment will be analyzed using the SABiosciences software and fold change of *GLII*expression, normalized to *GAPDH*, will be derived for each sample.

Patients will be dichotomized for the purposes of statistical analysis. The full statistical analytic plan for *GLII* expression data is described in Section 10.

9.3 Immunohistochemical Analysis of the Hedgehog Pathway in Primary Prostate Cancer Tissue

Availability of archived primary prostate cancer tissue (either prior biopsy or prostatectomy specimen) is recommended but not required for study entry. The goal is to obtain archival primary prostate cancer tissue from 50% of the patients enrolled onto study. Representative

Version 4.0: October 8, 2014

formalin fixed and paraffin embedded tissue sections will be used for this immunohistochemical analysis (see Appendix 3 for shipping instructions). Tissue sections will be deparaffinized, followed by rehydration with decreased concentrations of ethanol, and immersed in 3% H₂O₂ (in distilled H₂O) for 10 minutes. Following antigen retrieval in citrate buffer, the tissue sections will be incubated with normal goat serum to block non-specific antibody binding (20 min at room temperature). The sections will then be incubated with primary antibodies (at 1:200 dilution) at 37 degrees C in humid chambers for 2 hours. After washing with PBS three times, the sections will be incubated with biotinylated secondary antibody (goat anti-rabbit IgG or monkey anti-goat IgG) and streptavidin conjugated to horseradish peroxidase for 20 min at 37 degrees C, followed by PBS wash. Hematoxylin will be used for counter-staining. Negative controls will be performed in all cases by omitting the first antibodies. Specific antibodies to Sonic hedgehog ligand, *SMO*, *GLII*, and *PTCH1* (Cat. No. 9024 for Sonic hedgehog ligand, Cat. No. 6149 for *PTCH1*, Cat. No. 166685 for *SMO*, and Cat. No. 20291 for *GLII*, Santa Cruz Biotechnology Inc., Santa Cruz, CA) will be utilized. The intensity of immunohistochemical staining will be scored by pathology reviewers blinded to clinical outcomes on a scale from 0, 1+, 2+, and 3+. Patients will be dichotomized for the purposes of statistical analysis (0 and 1+ staining vs. 2-3+; see Section 10).

9.4 Serum Drug Levels

The plasma trough concentrations of itraconazole and hydroxyitraconazole will be assessed by a validated liquid chromatography-mass spectrometry assay developed by the Analytical Pharmacology Core Laboratory at Johns Hopkins University. Trough levels will be drawn after 4 weeks of protocol therapy, collected before the morning dose of itraconazole. Patients should hold their morning dose until the collection is completed.

Patients will have about 5 ml of blood collected into tubes containing K2-EDTA as an anticoagulant (lavender top). Following extraction, blood will be immediately placed on ice or refrigerated until processed. The actual day and time of collection will be recorded. Plasma will be separated from whole blood as soon as possible following extraction. This will be done by centrifuging whole blood at 1000 x g for 10 minutes at 4°C. Plasma will then be transferred to a screw-cap polypropylene cryotube for determination of itraconazole and hydroxyitraconazole concentrations. The polypropylene tube will be labeled with patient's initials, study number, day, time, and protocol day and then frozen at -70°C until the time of analysis (see appendix 3 for additional information regarding processing and shipping instructions).

10.0 Planned Statistical Methods

10.1 Study Design

This is a phase II, single arm study of itraconazole dosed at 300 mg BID in patients with non-castrate, non-metastatic, biochemically relapsed prostate cancer after prior definitive local therapy. Simon's two stage minimax design will be followed for accrual and include an interim test for lack of efficacy (see Sections 10.2 and 10.4). There is a pre-specified stopping rule for

Version 4.0: October 8, 2014

safety after 10 patients have been treated for a minimum of 8 weeks of protocol therapy (see Section 10.4). Patients will remain on protocol therapy until the first occurrence of any of the following: PSA progression, as defined in Section 2, development of metastasis, unacceptable toxicity, initiation of non-protocol therapy, or patient/physician withdrawal from study. Patients will remain on study until development of metastasis, initiation of non-protocol therapy, or patient/physician withdrawal from study, whichever occurs soonest. The total study duration including follow up is expected to be 3 years from the date of first patient enrolled onto study.

10.2 Determination of Sample Size and Study Power

This is a single arm phase II study to test the hypothesis for an improvement in the proportion of patients achieving a PSA decline of at least 50% from baseline with itraconazole therapy. The null hypothesis is that 10% of patients treated with itraconazole will achieve a $> 50\%$ decline in serum PSA after 12 weeks of protocol therapy. This response proportion is based upon the overall PSA response proportions observed in prior clinical trials of non-castrating agents in biochemically relapsed prostate cancer, which demonstrated modest activity with 0-10% of patients achieving a $\geq 50\%$ PSA decline on therapy [10-16]. A response proportion of 10% or less in the current study would indicate minimal disease activity and would not justify further testing of itraconazole in this disease setting. The alternative hypothesis is that 25% of patients treated with itraconazole will achieve a $\geq 50\%$ decline in serum PSA after 12 weeks of protocol therapy, a clinically meaningful result that would represent promising biologic activity of a novel potentially non-castrating agent tested in the biochemically relapsed disease setting. The sample size is based upon the primary study endpoint and will follow a two stage design for accrual following Simon's two-stage minimax approach. Twenty-two patients will be accrued at the first stage. If more than two patients achieve a PSA decline of at least 50% within 12 weeks of starting protocol therapy, accrual will continue to the second stage and an additional 18 patients will be entered. If at least 8 of the 40 patients entered on study achieve a PSA decline of at least 50%, then the null hypothesis will be rejected in favor of the alternative. This test with a total evaluable accrual of 40 patients has a power of 80% and a directional level of significance of 0.05. The probability of stopping accrual at the first stage is 0.62 if the null hypothesis is true. A drop-out rate for reasons not due to treatment or disease progression of 5% prior to the cut-off point for the primary analysis is assumed for a total study accrual of 42 patients.

10.3 Accrual

A total of 40 evaluable patients will be enrolled across three investigational sites. The estimated enrollment rate across the three sites is 3 patients per month, leading to anticipated enrollment duration of approximately 14 months. The duration from first patient enrolled to primary analysis is approximately 18 months, and the total study duration including follow ups is expected to be 3 years.

Version 4.0: October 8, 2014

10.4 Interim Analysis

There will be an interim analysis for safety after 10 patients have received a minimum of 8 weeks of protocol therapy. Study accrual will continue during the interim safety analysis.

If, after 8 weeks of protocol therapy, four or more out of ten patients have a grade 3 or higher adverse event that the study investigator deems as possibly, probably, or definitely related to study drug (excluding hypokalemia or hypertension), the study will close to further accrual.

An interim analysis for lack of efficacy will be performed after completion of the first stage of accrual of 22 patients. Study accrual will not stop for this analysis, consistent with the study design of prior non-hormonal therapies in biochemically relapsed prostate cancer, the majority of which were single stage phase II studies without pre-specified stopping rules for lack of efficacy despite observed PSA response proportions of less than 5%.

10.5 Analysis Population

The primary efficacy analysis will include all patients who have at least one PSA follow-up measurement. In addition, if a patient comes off study prior to obtaining one PSA follow-up measurement for disease progression or toxicity, they will be included in the analysis of PSA response as a treatment failure. All patients who receive at least one dose of study drug will be included in the analysis of safety.

10.6 Demographics and Baseline Characteristics

Demographic variables will include age, race, ethnicity, height, and weight. Baseline characteristics will include the following:

- Number of years since diagnosis of prostate cancer
- Gleason grade/clinical stage/pathologic stage (if prior prostatectomy)/PSA at the time of diagnosis
- Type and timing of local therapy (surgery and/or radiation) for prostate cancer
- Nadir PSA after definitive local therapy and time to nadir PSA
- Time from definitive local therapy to PSA progression (as defined by first PSA ≥ 0.2 ng/mL in patients with prior radical prostatectomy with or without adjuvant radiation therapy; first PSA rise of 2.0 ng/mL or more above the nadir in patients with prior primary definitive radiation therapy)
- Time interval from biochemical relapse to study entry
- PSA doubling time at the time of study entry
- Use of prior ADT, nadir PSA on ADT, duration of ADT, time interval from last dose of ADT and study enrollment, and indication (i.e. adjuvant or in biochemical relapse) (if applicable)
- Use of prior antiandrogen therapy, time interval since prior therapy to study enrollment, and indication (if applicable)

10.7 Study Endpoints

10.7.1 Primary Endpoint

The proportion of patients with biochemically relapsed disease after prior definitive local therapy who achieve a $\geq 50\%$ decline from baseline in serum PSA after 12 weeks of therapy with itraconazole, confirmed by repeat measurement at least 2 weeks later, will be calculated.

10.7.2 Secondary Endpoints

A) Clinical Activity:

- Median time to PSA progression among men with biochemical relapse treated with itraconazole will be estimated. See Section 2 for the definition of PSA progression.
- Median time to clinical progression will be estimated, with clinical progression defined as the first occurrence of either development of overt metastases or initiation of non-protocol therapy. The definition of clinical progression excludes PSA-only progression.
- Median metastasis-free survival will be estimated among men with biochemical relapse treated with itraconazole.
- Mean percent change after 12 weeks of protocol therapy compared with pre-treatment in PSA doubling time will be determined for those treated with itraconazole. The pre-treatment PSADT will be determined based upon all PSA measurements within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA values each spaced at least 14 days apart (see Section 6.2.1).

B) Safety/Pharmacokinetics:

- Maximum grade toxicities observed during treatment with itraconazole, as graded by CTCAE version 4.03.
- Mean steady-state trough level of serum itraconazole and its active metabolite, hydroxyitraconazole, after 4 weeks of therapy with itraconazole.

10.7.3 Correlative Endpoints

A) Endocrine Parameters:

- Mean percent change from baseline in serum androgen levels, including serum testosterone, DHEA-S, and androstenedione, after 4 and 12 weeks of protocol therapy.
- Mean percent change from baseline in additional serum hormone levels, including ACTH, aldosterone, deoxycorticosterone (DOC), 11-deoxycortisol, and cortisol after 4 and 12 weeks of protocol therapy.
- Mean baseline and mean percent change in serum hormone levels among patients achieving $\geq 50\%$ decline from baseline in serum PSA after 12 weeks of itraconazole therapy and among those not achieving this decline.

Version 4.0: October 8, 2014

B) Hedgehog Pathway (assessed by serial skin biopsies on protocol therapy and analysis of archived primary prostate cancer tissue including prior biopsy or prostatectomy specimen):

- Proportion of patients who display a down-regulation of the Hedgehog pathway, as assessed by measurement of *GLI1* mRNA expression by RT-PCR on serial skin biopsies obtained at baseline and after 4 weeks of protocol therapy with itraconazole. Down-regulation will be defined as a decline of any magnitude in *GLI1* mRNA expression after 4 weeks of protocol therapy compared to baseline expression level.
- Proportion of patients with and without at least a 50% decline from baseline after 12 weeks of protocol therapy in PSA exhibiting a down-regulation of the Hedgehog pathway.
- Median time to PSA progression between patients with and without down-regulation of the Hedgehog pathway on itraconazole therapy as assessed by serial skin biopsy.
- Proportion of patients with and without at least 50% decline from baseline after 12 weeks of protocol therapy in PSA exhibiting over-activation of Hedgehog pathway components as assessed by immunohistochemical (IHC) and mRNA expression analysis of primary prostate cancer tissue (prior biopsy or prostatectomy specimen). Patients will be dichotomized (immunohistochemical staining 0 to 1+ vs. 2 to 3+ staining intensity; mRNA expression above and below median) for the purposes of analysis of the Hedgehog pathway in primary prostate cancer tissue.
- Median time to PSA progression between patients with and without Hedgehog pathway over-activation in archived primary prostate cancer tissue.

10.8 Methods for Analysis

10.8.1 Analytic Plan for the Primary Objective

The primary objective will be evaluated following the decision rules for Simon's minimax 2 stage design as defined above (Section 10.2). The proportion of patients who achieve a $\geq 50\%$ decline in serum PSA compared to baseline after 12 weeks of protocol therapy, confirmed by repeat measurement at least two weeks later, will be calculated. Patients with at least one PSA follow-up measurement who discontinue protocol therapy prior to 12 weeks due to any reason will be counted as failures for analysis of the primary objective. In addition, patients who discontinue protocol therapy due to disease progression or toxicity prior to obtaining one PSA follow-up measurement will be counted as failures for analysis of the primary objective. Patients who withdraw from the study for other reasons prior to one PSA follow-up measurement will be excluded in the analysis of the primary objective. The null hypothesis is that 10% of patients will achieve a $\geq 50\%$ decline in serum PSA after 12 weeks of protocol therapy, with an alternative hypothesis that 25% of patients will achieve such a decline in serum PSA. If accrual is completed and at least 8 patients achieve a $\geq 50\%$ decline in serum PSA from their baseline measurement then the null hypothesis will be rejected based upon a test with 80% power and a

Version 4.0: October 8, 2014

directional level of significance of 5%. The results will be summarized by the proportion of patients who achieve a $\geq 50\%$ decline in serum PSA along with a 95% confidence interval.

10.8.2 Analytic Plan for the Secondary Objectives

Clinical Activity

The probability distribution of the time to PSA progression will be estimated using the Kaplan-Meier product limit method measured from the start of protocol therapy. The results will be summarized by the estimated median with 95% confidence intervals. PSA progression will be defined as follows: (1) If no PSA decline is observed on therapy, PSA progression will be defined as an increase in serum PSA $> 50\%$ above the baseline PSA, and an absolute increase of $> 2\text{ng/mL}$ above baseline, confirmed by repeat measurement at least 2 weeks later (2) If PSA declines on therapy, PSA progression will be defined as an increase in serum PSA $> 50\%$ above the nadir PSA on therapy, and an absolute increase $> 2\text{ng/mL}$ above the nadir, confirmed by repeat measurement at least 2 weeks later. Durations will be measured from Day 1 of study treatment to the first date of PSA progression. Patients who discontinue study therapy prior to PSA progression due to unacceptable toxicity, patient/physician withdrawal, initiation of non-protocol therapy, or development of metastases will be censored at the time of last PSA measurement prior to the event.

The probability distribution of the time to clinical progression and time to first metastasis will be estimated using the Kaplan-Meier product limit method measured from the time of start of protocol therapy. The results will be summarized by the estimated median with 95% confidence intervals. Clinical progression will be defined as the first occurrence of either development of metastasis detected by radiographic scans or initiation of non-protocol therapy, whichever occurs soonest. PSA-only progression will not be counted as clinical progression. If a patient withdraws from study, discontinues therapy due to unacceptable toxicity, or is lost to follow up prior to clinical progression, he will be censored at the date of the last recorded study visit. Additionally, for the analysis of time to first metastasis, if a patient initiates non-protocol therapy prior to development of metastatic disease, he will be censored at the start date of non-protocol therapy.

The mean percent change in PSA doubling time after 12 weeks of protocol therapy from pre-treatment PSA doubling time will be calculated. Calculation of baseline PSA doubling time will be based upon all PSA measurements obtained within 3 months prior to Day 1 of protocol therapy, with a minimum of three PSA values spaced at least 14 days apart (see Section 6). The PSA doubling time both pre- and on-treatment will be estimated using the relationship of natural log (Ln) 2 divided by the slope of lnPSA versus time. Descriptive statistics will be presented to summarize the PSA doubling time at baseline and after 12 weeks of protocol therapy as well as the change between time points. Pearson's correlation will be used to estimate the relationship between pre-treatment PSA doubling time and percent change in PSA doubling time after 12 weeks of protocol therapy. In an exploratory fashion, the mean percent change in PSA doubling time after 12 weeks of protocol therapy from pre-treatment PSA doubling time will be calculated.

Version 4.0: October 8, 2014

for the subsets of patients with and without a decline in serum PSA on therapy. Either a dependent t statistic or the Wilcoxon matched pairs test will be performed for the subset of patients without a PSA decline to describe any change in PSA doubling time from pretreatment and for the full study cohort.

Safety and Pharmacokinetics:

All patients who receive at least one dose of study drug will be analyzed for safety. Safety endpoints including vital signs, adverse events, and clinical laboratory parameters will be summarized using descriptive statistics. All adverse events will be tabulated by grade according to CTCAE version 4.03, according to the worst grade experienced.

Descriptive statistics including the mean, standard deviation, and range of steady-state trough serum levels of itraconazole and its active metabolite hydroxy-itraconazole will be determined.

10.8.3 Analytic Plan for the Correlative Objectives

Endocrine Parameters

Serum hormone levels, including testosterone, androstenedione, DHEA-S, cortisol, aldosterone, 11-deoxycortisol, ACTH, and deoxycorticosterone, will be measured at baseline, at week 4, week 12, and at the time of PSA or clinical progression. To summarize the outcome, descriptive statistics including the mean percent change from baseline in serum hormone levels at 4 and 12 weeks will be presented with a standard deviation and Pearson's correlation will be used to estimate the relationship between the baseline hormone level and percent change in hormone level after 4 and 12 weeks of treatment. Analysis of variance (ANOVA) methods for mixed models allowing for variable number of follow-up measurements with linear contrasts will be used to evaluate the change in serum hormone levels after 4 and 12 weeks of protocol therapy. It is hypothesized that approximately 15% of patients treated with protocol therapy will achieve a PSA decline $\geq 50\%$. Depending upon the observed frequency, the ANOVA model will include a factor to test for the effect of PSA decline on change in serum hormone levels. Otherwise, analyses will compare the baseline and percent change in serum hormone levels at 4 and 12 weeks between patients achieving a PSA decline $\geq 50\%$ or not using a two group t statistic. For these exploratory analyses, graphic presentations will also be used to explore the overall pattern of change in serum hormone levels between those with and without a PSA decline.

Hedgehog Pathway

The quantitative expression of *GLII*, a downstream product of the Hedgehog signal transduction pathway, will be measured by quantitative RT-PCR from serial skin biopsies obtained at baseline and after 4 weeks of therapy. The fold change in expression level after 4 weeks of protocol therapy will be calculated for each patient. Descriptive statistics will be used to summarize the proportion of patients with down-regulation of *GLII* expression after 4 weeks of treatment with itraconazole. Down-regulation will be defined as any magnitude of decrease in *GLII* mRNA expression after 4 weeks of protocol therapy compared to baseline expression level. The patient

Version 4.0: October 8, 2014

cohort will be dichotomized by whether there is up- or down-regulation of *GLII* expression. Fisher's exact test will be used to test for an association between down-regulation of *GLII* expression and PSA decline $\geq 50\%$ on protocol therapy. The probability distributions for each *GLII* expression subset (as defined above) of the time to PSA progression will be estimated using the Kaplan-Meier product limit method. The results will be summarized by the estimated median with 95% confidence intervals. An exploratory analysis using the log-rank test will be used to test for differences in the distributions between patients with down-regulation of *GLII* expression vs. those without *GLII* down-regulation on therapy.

The quantitative expression of *GLII* will also be measured by quantitative RT-PCR from archived primary prostate cancer tissue. The patient cohort will be dichotomized by expression level of *GLII* (above vs. below the median). Fisher's exact test will be used to test for an association between *GLII* expression in primary prostate cancer tissue and PSA decline $\geq 50\%$ on itraconazole therapy. The probability distributions for each *GLII* expression subset of the time to PSA progression will be estimated using the Kaplan-Meier product limit method. The results will be summarized by the estimated median with 95% confidence intervals. An exploratory analysis using the log-rank test will be performed to test for differences in the distributions between patients with *GLII* expression above vs. below the median from primary prostate cancer tissue specimens.

Immunohistochemical analysis of primary prostate cancer tissue for protein expression of *PCTH1*, *GLII*, *SMO*, as well as Sonic hedgehog ligand will be carried out for patients with evaluable tissue. It is estimated that approximately 50% of patients will have tissue available for analysis. Immunohistochemical expression will be graded as 0, 1+, 2+, and 3+ staining intensity by independent, blinded pathologic assessment. For each protein, the patient cohort will be dichotomized based on intensity of IHC staining (0 and 1+ vs. 2+-3+). Fisher's exact test will be used to test for an association between IHC expression and PSA decline $> 50\%$ on protocol therapy. The probability distributions for each expression subset of the time to PSA progression will be estimated using the Kaplan-Meier product limit method. The results will be summarized by the estimated median with 95% confidence intervals.

No adjustment for multiple comparisons will be made for the analysis of the secondary and correlative objectives.

11.0 Reporting and Documentation of Adverse Events

11.1 Definitions of Adverse Events (21 CFR 312.32(a))

Adverse Event

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Version 4.0: October 8, 2014

An *adverse event* (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

Adverse reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction

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

Unexpected

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or package insert(s) or, is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

“Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the investigator brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect,

Version 4.0: October 8, 2014

the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

Serious

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

- Death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. 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.

Life-threatening

An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

11.2 Recording of an Adverse Event

All adverse events will be entered into OnCore®, UCSF’s Clinical Trial Management System, whether or not the event is believed to be associated with use of the investigational drug. Data about these events and their severity will be recorded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) (V4.0), when applicable, on the appropriate case report forms (CRFs). The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into OnCore using the classification system listed below:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention ¹	Unrelated	The AE is <i>clearly NOT related</i> to the intervention
	Unlikely	The AE is <i>doubtfully related</i> to the intervention
Related to investigational agent/intervention ¹	Possible	The AE <i>may be related</i> to the intervention
	Probable	The AE is <i>likely related</i> to the intervention
	Definite	The AE is <i>clearly related</i> to the intervention

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they are to be graded by the Investigator as none, mild, moderate or severe according to the following grades and definitions:

- Grade 0: No AE (or within normal limits).
- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

11.3 Follow-up of Adverse Events

All adverse events must be followed with appropriate medical management until resolved. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

11.4 Serious Adverse Events Monitoring

All serious adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, as noted above.

The Principal Investigator will assess each Serious Adverse Event (SAE) and determine reportability requirements to the Data and Safety Monitoring Committee (DSMC), UCSF's Committee on Human Research (CHR), and, when the study is conducted under an Investigational Drug Application (IND), to the Food and Drug Administration.

Version 4.0: October 8, 2014

All serious adverse events entered into OnCore® will be reviewed by the Site Committee on a weekly basis. The Site Committee will review and discuss at each weekly meeting the selected toxicity, the toxicity grade, and the attribution of relationship of the SAE to the administration of the study drug(s).

In addition, all serious adverse events entered into OnCore® will be reviewed and monitored by the Data and Safety Monitoring Committee on a monthly basis and prior to dose escalation. At the time of dose escalation, a written report will be submitted to the DSMC Chair outlining the cohorts, dose, AEs and SAE reports, and any Dose Limiting Toxicities observed, in accordance with the protocol. The report will be reviewed and approved by the DSMC Chair (or designee) prior to dose escalation.

11.5 Serious Adverse Events Expedited Reporting

Reporting to the Data and Safety Monitoring Committee (DSMC)

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair within 24 hours of knowledge of the event. The contact may be by phone or e-mail.

Reporting to the Committee on Human Research (CHR)

The Investigator must report SAEs meeting the CHR definition of “Unanticipated Problem” (UP) within 10 working days of his/her awareness of the event.

Specific instructions on SAE reporting may be found at the UCSF CHR website at:
http://www.research.ucsf.edu/chr/Guide/Adverse_Events_Guidelines.asp

Expedited Reporting to the IND

If the study is being conducted under an Investigational Drug Application, the Sponsor-Investigator is responsible for determining whether or not the SAE meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The Investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Serious
- Unexpected

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

Version 4.0: October 8, 2014

The timeframe for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction must be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report must be submitted to FDA as a Follow Up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

For additional information and guidance on IND Safety Reports, please refer to the FDA Guidance for Industry: [Safety Reporting Requirements for INDs](#).

12.0 Study Management

Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Prior to implementing this protocol at UCSF HDFCCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the UCSF Committee on Human Research (CHR). Prior to implementing this protocol at the participating sites, approval for the UCSF CHR approved protocol must be obtained from the participating site's IRB.

The following documents must be provided to UCSF HDFCCC before the participating site can be initiated and begin enrolling participants:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normals

Version 4.0: October 8, 2014

Upon receipt of the required documents, UCSF HDFCCC will formally contact the site and grant permission to proceed with enrollment (a detailed listing of the documents required from participating sites is provided in Appendix 4).

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR) Parts §50, §54, and §312, GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR 56 and applicable regulatory requirements.

Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Committee on Human Research (UCSF's IRB). Prior to obtaining CHR approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

Informed Consent

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB-approved informed consent form prior to participation in any study specific procedure. The participant must receive 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.

Changes in the Protocol

Once the protocol has been approved by the UCSF Committee on Human Research (CHR), any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Investigator and approved by PRC and the CHR prior to implementation. If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to CHR approval. In this circumstance, however, the Investigator must then notify the CHR in writing within five (5) working days after implementation.

Handling and Documentation of Clinical Supplies

The Investigator shall maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the

Version 4.0: October 8, 2014

drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included.

The Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. All study data will be entered into OnCore®, UCSF's Clinical Trial Management System (CTMS) via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. Each CRF must be reviewed for accuracy by the Investigator, corrected as necessary, and then approved. Alternatively, the Investigator may sign individual, printed CRFs. These signatures attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the Data and Safety Monitoring Committee (DSMC) and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement among the Principal Investigator, the Trial Statistician, and the Protocol Project Manager.

Oversight and Monitoring Plan

The Helen Diller Family Comprehensive Cancer Center Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The Data and

Version 4.0: October 8, 2014

Safety Monitoring Plan for Multicenter Institutional Study (Phase 2 or 3) is described in Appendix 4. The DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable.

Retention of Records

The Investigator will retain a copy of all study documents in accordance with FDA regulations:

- for a minimum of two years following the date a marketing application (New Drug Application or NDA) is approved for the drug for the proposed clinical indication; or
- if no marketing application is filed, or if the marketing application has been filed but is not approved by the FDA, then for a minimum of two years following the release date of the final report; or

The Investigator agrees to maintain a complete and current record of all documentation associated with the study. All of the documents shall be kept together. Each should be available for ready review. These study documents will include the:

- protocol
- protocol amendments, when applicable
- signed FDA Form 1572
- Investigator's current curriculum vitae
- documentation of CHR approvals for the protocol, amendments, informed consents and advertisements used to recruit patients
- Investigator's Brochure(s)
- site visit log
- correspondence (all "to" and "from" correspondence)
- drug accountability records, drug shipment forms, and drug disposal records
- case report forms and informed consent documents for individual patients
- final report for the study if available.

13.0 Protection of Human Subjects

Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the CHR mechanism and the process of informed consent. The CHR reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The CHR also reviews the

Version 4.0: October 8, 2014

informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

14.0 References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer Journal Clin. 2011; 61(2):69.
2. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer Journal Clin. 2010; 60(5):277.
3. Stephenson AJ, Scardino PT, Kattan MW, et al. Predicting the outcome of salvage radiation therapy for recurrent prostate cancer after radical prostatectomy. Journal of Clinical Oncology 2007;25(15):2035-2041.
4. Albrecht W, Collette L, Fava C, et al. Intermittent maximal androgen blockade in patients with metastatic prostate cancer: an EORTC feasibility study. European Urology 2003; 44(5):505-511.
5. Peyromaure M, Delongchamps NB, Debre B, Zerbib M. Intermittent androgen deprivation for biologic recurrence after radical prostatectomy: long-term experience. J of Urology 2005; 65(4):724-729.
6. Bruchovsky N, Klotz L, Crook J, et al. Final results of the Canadian prospective phase II trial of intermittent androgen suppression for men in biochemical recurrence after radiotherapy for locally advanced prostate cancer: clinical parameters. Cancer 2006; 107(2):389-395.
7. Da Silva FC, Bono A, Whelan P, et al. Phase III study of intermittent MAB vs. continuous MAB- an international cooperative study – quality of life. European Urology 2006; 5:289, Abstract no. 1066.
8. Crook JM, O'Callaghan CJ, Ding K, et al. A phase III randomized trial of intermittent versus continuous androgen suppression for PSA progression after radical therapy (NCIC CTG PR.7/SWOG JPR.7/CTSU JPR.7/ UK Intercontinental Trial CRUKE/01/013). Abstract # 4514, ASCO Annual Meeting. June 2011; Chicago, IL USA
9. Grossfeld GD, Chaudhary UB, Reese DM, Carroll PR, and Small EJ. Intermittent androgen deprivation: update of cycling characteristics in patients without clinically apparent metastatic prostate cancer. J of Urology 2001; 58(2):240-245.
10. McNeel DG, Dunphy EJ, Davies JG, et al. Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer. J ClinOncol 2009; 27(25):4047-4054.
11. Smith MR, Manola J, Kaufman DS, et al. Celecoxib versus placebo for men with prostate cancer and a rising serum prostate-specific antigen after radical prostatectomy and/or radiation therapy. J ClinOncol 2006; 24(18):2723-2728.
12. Rini BI, Weinberg V, Bok R, and Small EJ. Prostate-specific antigen kinetics as a measure of the biologic effect of granulocyte-macrophage colony-stimulating factor in patients with serologic progression of prostate cancer. J ClinOncol 2003; 21:99-105.
13. Rosenbaum E, Zahurak M, Sinibaldi V, et al. Marimastat in the treatment of patients with biochemically relapsed prostate cancer: a prospective randomized double-blind, phase I/II trial. Clinical Cancer Research 2005; 11(12):4437-4443.
14. Smith MR, Manola J, Kaufman DS, et al. Rosiglitazone versus placebo for men with prostate carcinoma and a rising serum prostate-specific antigen level after radical prostatectomy and/or radiation therapy. Cancer 2004; 101(7):1569-1574.
15. Lin AM, Rini BI, Weinberg V, et al. A phase II trial of imatinibmesylate in patients with biochemical relapse of prostate cancer after definitive local therapy. BJUI 2006; 98:763-769.

Version 4.0: October 8, 2014

16. Lin J, Zahurak M, Beer TM, et al. A non-comparative randomized phase II study of 2 doses of ATN-224, a copper/zinc superoxide dismutase inhibitor, in patients with biochemically recurrent hormone-naïve prostate cancer. *Urologic Oncology: Seminars and Original Investigations*. Article in press. Accepted for Publication 23 April 2011.
17. De Beule K. Itraconazole: pharmacology, clinical experience and future development. *International Journal of Antimicrobial Agents* 1996; 6:175-181.
18. Phillips P, Graybill JR, Fetchick R, and Dunn JR. Adrenal response to corticotropin during therapy with itraconazole. *Antimicrobial Agents and Chemotherapy* 1987; 31(4):647-649.
19. Antonarakis ES, Heath EI, Smith DC, et al. A noncomparative randomized phase II study of two dose levels of itraconazole in men with metastatic castration-resistant prostate cancer (mCRPC): a DOD/PCCTC trial. *J ClinOncol* 2011;29 (suppl; abstr 4532)
20. Small EJ, Halabi S, Dawson NA, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). *J ClinOncol* 2004;22(6):1025-1033.
21. Ryan CJ, Halabi S, Ou S-S, et al. Adrenal androgen levels as predictors of outcome in prostate cancer patients treated with ketoconazole plus antiandrogen withdrawal: results from a Cancer and Leukemia Group B study. *Clin Cancer Research* 2007; 13(7):2030-2037.
22. Chong CR, Xu J, Lu J, Bhat S, Sullivan Jr. DJ, and Liu JO. Inhibition of angiogenesis by the antifungal drug itraconazole. *ACS Chemical Biology* 2007; 2(4): 263-270.
23. Aftab BT, Dobromilskaya I, Liu JO, and Rudin CM. Itraconazole inhibits angiogenesis and tumor growth in non-small cell lung cancer. *Cancer Research* 2011; 71(21): 6764-72.
24. Taipale J and Beachy PA. The Hedgehog and Wnt signaling pathways in cancer. *Nature* 2001; 411:349-354.
25. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the Hedgehog pathway in advanced basal-cell carcinoma. *New Engl J of Med* 2009; 361:1164-1172.
26. Low JA and de Sauvage FJ. Clinical experience with Hedgehog pathway inhibitors. *J ClinOncol* 2010; 28(36):5321-5326.
27. Karhadkar SS, Bova SG, Abdallah N, et al. Hedgehog signaling in prostate regeneration, neoplasia and metastasis. *Nature* 2004; 431:707-712.
28. Sheng T, Li C, Zhang X, et al. Activation of the Hedgehog pathway in advanced prostate cancer. *Molecular Cancer* 2004;3:29.
29. Kim J, Tang JY, Gong R, et al. Itraconazole, a commonly used antifungal that inhibits Hedgehog pathway activity and cancer growth. *Cancer Cell* 2010; 17:388-399.
30. Kim D, Kim J, Spaunhurst K, et al. An open-label, exploratory phase II study of oral itraconazole for the treatment of basal cell carcinoma. Abstract # LB-223. AACR Annual Meeting April 2012; Chicago, IL, USA.
31. Antonarakis ES, Heath EI, Smith DC, et al. A randomized phase 2 study of two doses of itraconazole in men with metastatic castration-resistant prostate cancer (mCRPC). Abstract # LB-224. AACR Annual Meeting April 2012; Chicago, IL, USA.
32. Tucker RM, Haq Y, Denning DW, and Stevens DA. Adverse events associated with itraconazole in 189 patients on chronic therapy. *Journal of Antimicrobial Chemotherapy* 1990; 26:561-566.
33. Sharkey PK, Rinaldi MG, Dunn JF, Hardin TC, Fetchick RJ, and Graybill JR. High-dose itraconazole in the treatment of severe mycoses. *Antimicrobial Agents and Chemotherapy* 1991; 35(4):707-713.
34. Pont A, Graybill JR, Craven PC, et al. High-dose ketoconazole therapy and adrenal and testicular function in humans. *Archives of Internal Medicine* 1984; 144:2150-2153.

Version 4.0: October 8, 2014

35. Stewart AJ, Scher HI, Chen MH, et al. Prostate-specific antigen nadir and cancer-specific mortality following hormonal therapy for prostate-specific antigen failure. *J Clin Oncol* 2005; 23(27):6556-6560.
36. Scher HI, Eisenberger M, D'Amico AV, et al. Eligibility and outcomes reporting guidelines for clinical trials for patients in the state of a rising prostate-specific antigen: recommendations from the Prostate-Specific Antigen Working Group. *J Clin Oncol* 2004; 22(3):537-556.
37. Antonarakis ES, Zahurak ML, Lin J, et al. Changes in PSA kinetics predict metastasis-free survival in men with PSA-recurrent prostate cancer treated with nonhormonal agents. *Cancer* 2012;118:1533-1542.
38. D'Amico AV, Moul J, Carroll PR, et al. Prostate specific antigen doubling time as a surrogate end point for prostate cancer specific mortality following radical prostatectomy or radiation therapy. *J Urol* 2005; 172(5): S42-S47.
39. Zelefsky MJ, Ben-Porat L, Scher HI, et al. Outcome predictors for the increasing PSA state after definitive external-beam radiotherapy for prostate cancer. *J Clin Oncol*, 2005; 23(4):826-831.
40. Roach M 3rd, Hanks G, Thames H Jr, et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. *Int J Radiat Oncol Biol Phys*. 2006; 65(4):965-74.
41. Yu EY, Gulati R, Telesca D, et al. Duration of first off-treatment interval is prognostic for time to castration resistance and death in men with biochemical relapse of prostate cancer treated on a prospective trial of intermittent androgen deprivation. *J Clin Oncol* 2010; 28(16):2668-2673.
42. Ryan C, Li J, Kheoh T, et al. Baseline serum adrenal androgens are prognostic and predictive of overall survival (OS) in patients (pts) with metastatic castrate-resistant prostate cancer (mCRPC): Results of the COU-AA-301 phase 3 randomized trial. Abstract # LB-434. AACR Annual Meeting 2012; Chicago, IL, USA.
43. Aggarwal R, Weinberg V, Small EJ, Oh W, Rushakoff R, and Ryan CJ. The mechanism of action of estrogen in castration-resistant prostate cancer: clues from hormone levels. *Clinical Genitourinary Cancer* 2009; 7(3):E71-E76.

Appendix 1. List of Prohibited Medications and Supplements While Patients Are Taking Itraconazole

Abiraterone acetate
Amiodarone
Astemizole
Atorvastatin
Bicalutamide
Chemotherapy
Cisapride
Cyproterone acetate
Diethylstilbestrol
Degarelix
Dofetilide
Dutasteride
Enzalutamide (MDV3100)
Eplerenone
Ergot alkaloids
Estrogens
Felodipine
Finasteride
Fluconazole
Flutamide
GM-CSF
Goserelin
Ketoconazole
Levomethadyl
Leuprolide acetate
Lovastatin
Megestrol
Methadone
Midazolam
Milk thistle
Nilutamide
Nisoldipine
Other investigational agents
PC-SPES Pimozide
Pomegranate extract
Pomegranate juice
Progesterones
Quinidine
Radiation therapy
Saw palmetto
Simvastatin
Sipuleucel-T
Spironolactone
Steroids at an equivalent dose of prednisone 5 mg per day or higher

Phase II Study of Itraconazole in Biochemical Relapse

Version 4.0: October 8, 2014

Terfenadine

Triazolam

Appendix 2. Potential Drug-Drug Interactions

CYP3A4 Inductors (drugs that decrease the bioavailability of itraconazole)

I. Aminoglutethimide	7. Nafcillin	13. Rifabutin
2. Barbiturates	8. Nevirapine	14. Rifampin
3. Carbamazepine	9. Oxcarbazepine	15. St John's wort
4. Efavirenz	10. Phenobarbital	16. Troglitazone
5. Glucocorticoids	II. Phenytoin	
6. Modafinil	12. Pioglitazone	

CYP3A4 Inhibitors (drugs that increase the bioavailability of itraconazole)

I. Amiodarone *	9. Grapefruit juice	17. Ritonavir
2. Aprepitant	10. Indinavir	18. Saquinavir
3. Cimetidine	II. Isoniazid	19. Telithromycin
4. Clarithromycin	12. Ketoconazole	20. Troleandomycin
5. Diltiazem	13. Mibepradil	21. Veraparnil
6. Erythromycin	14. Nefazodone	22. Voriconazole
7. Fluconazole	15. Nelfinavir	
8. Fluvoxamine	16. Nortloxacin	

CYP3A4 Substrates (drugs whose bioavailability may increase with itraconazole use)

I. Alprazolam	19. Erythromycin	37. Quinidine •
2. Amiodarone *	20. Felodipine	38. Quinine
3. Amlodipine	21. Fentanyl	39. Ritonavir
4. Aripiprazole	22. Glucocorticoids	40. Saquinavir
5. Astemizole •	23. Haloperidol	41. Sildenafil
6. Atorvastatin •	24. Inlatinib	42. Simvastatin •
7. Buspirone	25. Indinavir	43. Sirolimus
8. C Worbenirantine	26. Levacetylmetadol •	44. Tacrolimus
9. Cilostazol	27. Lovastatin •	45. Tamoxifen
10. Cisapride •	28. Methadone	46. Telithromycin
II. Clarithromycin	29. Midazolam *	47. Terfenadine •
12. Cyclosporine	30. Mirtazapine	48. Trazodone
13. Diazepam	31. Nateglinide	49. Triazolam *
14. Digoxin	32. Nifedipine	50. Veraparnil
15. Diltiazem	33. Nisoldipine	51. Vinca alkaloids
16. Disopyrarnide	34. Nitrendipine	52. Warfarin
17. Dofetilide •	35. Oral hypoglycemics	
18. Ergot alkaloids *	36. Pimozide •	

- These drugs are absolutely *contraindicated* in patients taking concurrent itraconazole. The remainder maybe used with caution.

Appendix 3. Skin Biopsy Procedure and Specimen Handling Shipping Instructions for Serum and Tissue Specimens

There are three categories of specimens for this trial which will be shipped to a central processing site: skin biopsies, archived primary prostate cancer tissue (radical prostatectomy or biopsy specimens), and peripheral blood collected for serum drug levels. Serum hormone levels will be analyzed locally at each participating institution.

I. Skin Biopsy Procedure, Processing, and Shipping Instructions

For patients enrolled at UCSF, the skin biopsies will occur at the Helen Diller Family Comprehensive Cancer Center. Skin biopsies will be obtained twice for each patient who signs additional consent to participate in this correlative study: (1) pre-study (within 6 weeks of Day 1 of protocol therapy) and (2) at 28 days (+/- 7 days) of protocol therapy.

Specimen Requirements: A 3 mm skin punch biopsy (to the level of the subcutaneous tissue) should be obtained from an area of non-scalp skin with hairs and/or hair follicles. The upper back and arms are the preferred biopsy sites. Subsequent biopsies should be taken from the same general area of the body. Skin biopsies should be performed according to standard local protocols. One example is the following: the biopsy site should be cleaned and prepped using sterile technique, and anesthetized with 1-2% lidocaine with epinephrine. The biopsy should be performed using a sterile 3 mm punch biopsy tool. The biopsy site may be closed with steri-strips, or with one or two sutures, and a sterile dressing may be applied. The skin biopsy sample should be placed immediately into a 5 ml Tissue Protect Tube containing RNAlater solution (Ambion, catalog # AM7023). Care must be taken to ensure that the skin sample is fully submerged in RNAlater. RNAse Zap spray should *not* be used on gloves or the work surface during skin biopsy collection.

Processing Instructions: The sample should be kept at 4°C overnight (for ≥ 12 hours) so that RNAlater can seep into the tissue. The sample should then be frozen at -20°C or colder until the time of transport, and should be shipped to the analyzing lab on dry ice (see below). The sample is stable for 5 days at room temperature, for one month at 4°C, and for one year at -20°C. Once received by the analyzing lab, the sample should be stored at -20°C or colder until the time of RNA extraction.

Comments: The date and time of collection should be recorded on the Biomarkers Worksheet. Tissue Protect Tubes should be labeled with patients' initials, identification number, biopsy site, date, time, study number, and protocol day.

Shipping Instructions: Skin *GLI1*mRNA analysis will be performed at Johns Hopkins in the research laboratory of SushantKachhap, PhD, under the direction of David M. Berman, MD,

PhD. Skin biopsies arriving from outside institutions should be shipped on dry ice (a minimum of 5 kg) using overnight shipping to Kai Pollard in the Specimen Accessioning Core (SAC) laboratory at Johns Hopkins, at the address below. Overnight shipments should occur on Monday through Thursday, except when the following day is a holiday. A fax or call should be placed to the SAC laboratory prior to shipment, providing the shipment tracking information.

Kai Pollard
Specimen Accessioning Core (SAC)
Johns Hopkins Hospital
Attn: Itraconazole Study Samples
417 North Caroline Street, Room 305
Baltimore, MD 21231
Phone: (410) 502-3672 or (410) 502-0586
Pager: (410) 283-2148
Fax: (410) 614-2688
Email: mkai1@jhmi.edu

II. Archived Primary Prostate Cancer Specimens

Availability of archived primary prostate cancer tissue (prior biopsy or prostatectomy specimens) is not a requirement for study entry but is encouraged. Archived primary prostate cancer tissue will be sent to Dr. Christopher Sweeney at the Dana-Farber Cancer Institute for mRNA expression and immunohistochemical analysis as outlined in Section 9. (Tissue specimens will be returned to submitting site.)

Shipping Address:
Dana-Farber Cancer Institute
Attn: Dr. Edward Stack
450 Brookline Avenue, JF-215
Boston, MA, 02215
Phone: 617-582-7536
Fax: 617-582-8750

III. Processing and Shipping of Serum Drug Levels

Patients will have about 5 ml of blood collected into tubes containing K2-EDTA as an anticoagulant (lavender top). Following extraction, blood will be immediately placed on ice or refrigerated until processed. The actual day and time of collection will be recorded. Plasma will be separated from whole blood as soon as possible following extraction. This will be done by centrifuging whole blood at 1000 x g for 10 minutes at 4°C. Plasma will then be transferred to a screw-cap polypropylene cryotube for determination of itraconazole and hydroxyitraconazole concentrations. The polypropylene tube will be labeled with patient's initials, study number, day, time, and protocol day and then frozen at -70°C until the time of analysis.

Pharmacokinetic studies will be performed in the Analytical Pharmacology Core Laboratory at Johns Hopkins, under the direction of Michelle Rudek, PharmD, PhD.

Michelle A. Rudek, PharmD, PhD
Analytical Pharmacology Core Laboratory
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
1650 Orleans Street, CRB1 - Room 184
Baltimore, MD 21231
Phone: (410) 955-1129 or (410) 502-7192
Fax: (410) 502-0895
Email: mrudek2@jhmi.edu

Samples arriving from outside institutions should be shipped on dry ice (a minimum of 5 kg) using overnight shipping to Kai Pollard in the Specimen Accessioning Core (SAC) laboratory at Johns Hopkins, at the address below. Overnight shipments should occur on Monday through Thursday, except when the following day is a holiday. A fax or call should be placed to the SAC laboratory prior to shipment, providing the shipment tracking information.

Kai Pollard
Specimen Accessioning Core (SAC)
Johns Hopkins Hospital
Attn: Itraconazole Study Samples
417 North Caroline Street, Room 305
Baltimore, MD 21231
Phone: (410) 502-3672 or (410) 502-0586
Pager: (410) 283-2148
Fax: (410) 614-2688
Email: mkai1@jhmi.edu

IV. Processing and Shipping of Serum Hormone Levels

Peripheral blood for measurement of serum testosterone, cortisol, aldosterone, deoxycorticosterone, 11-deoxycortisol, adrenocorticotropic hormone (ACTH), dihydroepiandrosterone-sulfate (DHEA-S), and androstenedione will be collected at each site according to the instructions outlined below, and shipped to UCSF for analysis. Specimens should be shipped within 1 month of date of collection.

Peripheral blood collection for banking of peripheral blood mononuclear cells, plasma, and serum is optional. Specimens will be banked at UCSF-HDFCCC Tissue Bank.

A. Patient Confidentiality

Prior to the date of collection of each sample to be shipped to UCSF, the local CRC should notify the lead center CRC that specimen is to be collected at the patient's next visit. The lead center CRC will then assign unique specimen log numbers to be written on the specimen vials; this will ensure patient confidentiality.

The UCSF-HDFCCC Tissue Bank will serve as a repository for banking human samples. Specimen and data registries will be kept by the UCSF-HDFCCC. This registry will have a coordinated database to protect patient confidentiality and safety. The samples will receive a patient-insensitive identifier and the link to patient identity will be kept in a locked file with access only by the director of the Tissue Bank. Other investigators will have access to the samples only through established Tissue Core procedures. That is, investigators must submit a written request to use the stored samples as part of a CHR-approved protocol that is reviewed by the Tissue Core committee. This review limits the testing that can be done on these samples. Patients have the right at any time to request that all remaining samples be destroyed. The patient or relatives may be contacted about future, additional research on stored samples, if necessary. Additional written consent will be required if additional samples are to be taken.

B. Serum Hormones

At baseline, cycle 2 day 1, cycle 4 day 1, and at disease progression, **nine** mL of whole blood will be collected in two red top tubes (no gel) and centrifuged at 3000 rpm for 10 minutes. The serum will be decanted into three 2mL polypropylene screw cap (leak-proof) vials that have been properly labeled with the unique specimen log numbers provided and stored at -80°C until shipment to UCSF.

At baseline, cycle 2 day 1, cycle 4 day 1, and at disease progression, **twelve** mL of whole blood will be collected in two gold top tubes and centrifuged at 3000 rpm for 10 minutes. The serum will be decanted into four 2mL polypropylene screw cap (leak-proof) vials that have been properly labeled with the unique specimen log numbers provided and stored at -80°C until shipment to UCSF.

At baseline, cycle 2 day 1, cycle 4 day 1, and at disease progression, **three** mL of whole blood will be collected in a pre-chilled lavender top tube at centrifuged at 3000 rpm for 10 minutes. The plasma will be decanted into one 2mL polypropylene screw cap (leak-proof) vial that has been properly labeled with the unique specimen log numbers provided and stored at -80°C until shipment to UCSF.

C. Banking of Peripheral Blood Mononuclear Cells, Plasma, and Serum (Optional)

At baseline, on cycle 2 day 1, cycle 4 day 1, and disease progression, twenty mL of whole blood will be collected in one yellow-top serum separator tube and one lavender top tube (plasma) and

centrifuged at 3000 rpm for 10 minutes. The serum and plasma will be decanted into two 2mL polypropylene screw cap (leak-proof) vials (four total vials) that have been properly labeled with the unique specimen log numbers provided and stored at -80°C until shipment to UCSF.

At baseline, twelve mL of whole blood in 2 plastic EDTA tubes will also collected and freshly frozen at -80°C until shipment to UCSF.

IV. Shipping Instructions

Specimen may be batch-shipped on dry ice to the lead center (UCSF-HDFCCC) by overnight delivery Mondays-Thursdays. The local coordinator should notify the lead center coordinator prior to specimen shipment.

V. Local Storage and Confidentiality

Samples will be cataloged individually in password protected tissue database. Patient identifiers will be removed from this database and substituted with study ID numbers. Annotations for individual tissue/blood samples will be made for processing.

Appendix 4. Multicenter Institutional Studies

4.1 Data and Safety Monitoring Plan for Multicenter Institutional Study (Phase 2 or 3 Institutional Study)

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all HDFCCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data
- Review of suspected adverse reactions considered “serious”
- Monthly monitoring (depending on study accrual)
- Minimum of a yearly regulatory audit

Monitoring and Reporting Guidelines

All institutional Phase 2 or 3 therapeutic studies are designated with a moderate risk assessment. The data is monitored every six months, with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate quarterly conference calls with the participating sites to communicate the review of adverse events, safety data, and other study matters.

The Principal Investigator at the UCSF Coordinating Center will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating sites. The Study Chair will conduct continuous review of data and subject safety and discuss each subject’s treatment at monthly UCSF Site Committee meetings. The discussions are documented in the UCSF Site Committee meeting minutes.

Multicenter communication

The UCSF Coordinating Center provides administration, data management, and organizational support for the participating sites in the conduct of a multicenter clinical trial. The UCSF Coordinating Center will also coordinate, at minimum, monthly conference calls with the participating sites at the completion of each cohort or more frequently as needed to discuss risk assessment. The following issues will be discussed as appropriate:

- Enrollment information
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

Adverse events reporting to the DSMC will include reports from both the UCSF Coordinating Center, as well as the participating sites. The DSMC will be responsible for monitoring all data entered in OnCore® at the UCSF Coordinating Center and the participating sites. The data (i.e. copies of source documents) from the participating sites will be sent electronically or faxed over to the UCSF Coordinating Center prior to the monitoring visits in order for the DSMC to monitor the participating site’s compliance with the protocol, patient safety, and to verify data entry.

Adverse Event Review and Monitoring

Adverse Event Monitoring

All Grade 3-5 Adverse Events, whether or not unexpected, and whether or not considered to be associated with the use of study drug, will be entered into OnCore®, UCSF's Clinical Trial Management System.

All Grade 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the UCSF Site Committee meetings. All clinically significant adverse events must be reported to the UCSF Coordinating Center by the participating sites within 10 business days of becoming aware of the event or during the next scheduled quarterly conference call, whichever is sooner. The UCSF Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s) from the UCSF Coordinating Center and the participating sites.

In addition, all suspected adverse reactions considered "serious" must be entered in OnCore® and reported to the UCSF Coordinating Center within 1 business day. The suspected adverse reactions considered "serious" will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at the DSMC meeting, which take place every six (6) weeks.

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and is determined to be related either to the investigational drug or any research related procedure, the Study Chair at the UCSF Coordinating Center or the assigned designee must be notified within 1 business day from the participating site(s) and the Study Chair must then notify the DSMC Chair or qualified alternate within 1 business day of this notification. The contact may be by phone or e-mail.

Increase in Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert), the Study Chair at the UCSF Coordinating Center is responsible for notifying the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Study Chair stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Manager must be notified within 1 business day via e-mail. The DSMC must receive a formal letter within 10 business days and the CHR must be notified.

Data and Safety Monitoring Committee Contacts:

DSMC Chair:	Alan Venook, MD	DSMC Monitors
Phone:	415-353-2745	Box 1297
Email:	venook@cc.ucsf.edu	UCSF Helen Diller Family
Address:	Box 1705 UCSF San Francisco, CA 94115	Comprehensive Cancer Center San Francisco, CA 94115

* DSMP approved by NCI 09/February2012

4.2 UCSF Policy/Procedure for Required Regulatory Documents for a UCSF Multicenter Investigator-Initiated Oncology Clinical Trials with an Investigator held Investigational New Drug (IND)

Purpose

This policy defines the required Regulatory Documents for Single Site and Multicenter Investigator Initiated Oncology Clinical Trials at the Helen Diller Family Comprehensive Cancer Center (HDFCCC) where the Principal Investigator (PI) holds the IND.

Background

The International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guidelines define Essential Regulatory Documents as those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of data produced. These documents serve to demonstrate compliance with standards of GCP and with all applicable regulatory requirements. Filing essential documents in a timely manner can greatly assist in the successful management of a clinical trial.

The Regulatory Documents will consist of electronic files in both iMedRIS and OnCore[®], as well as paper files in the Regulatory Binders for both the Coordinating Site and the Participating Site(s) in the HDFCCC Investigator Initiated Oncology Clinical Trials.

Procedures

1. HDFCCC Essential Regulatory Documents

Documents Filed in iMedRIS:

- CHR approvals for initial submission of application, all modifications, and continuing annual renewals
- Current and prior approved protocol versions with signed protocol signature page(s)
- Committee for Human Research (CHR) approval letters and Informed Consent Form(s) (ICF)
- Current and prior versions of the Investigator Brochure (IB).
- Serious Adverse Event Reporting
- Protocol Violations and Single Patient Exception (SPE) Reports to CHR with supporting fax documentation

Documents Filed in OnCore[®]:

- Package Insert (if the study drug is commercial) or Investigator Brochure
- Protocol Review Committee (PRC) approved protocols, protocol amendments and Summary of Changes (SOC)
- Patient handouts
- Screening/enrollment log
- Data and Safety Monitoring Committee (DSMC) monitoring reports
- OnCore[®] Case Report Form (CRF) completion manual

Documents Filed in Regulatory Binder:

- Completed Food and Drug Administration (FDA) 1572 document with Principal Investigator's signature
- For all Principal Investigators and Sub-Investigators listed on the FDA 1572, will need Financial Disclosure Forms, CVs, MD Licenses, Drug Enforcement Agency (DEA) Licenses, and Staff Training Documents (i.e. Collaborative Institute Training Initiative (CITI), etc.)
- Site Initiation Visit (SIV) minutes and correspondence with participating site(s).
- As applicable, approvals for Biosafety Committee, Radiation Committee, and Infusion Center
- Serious Adverse Event (SAE) reports to CHR and sponsor.
- MedWatch reporting to FDA and sponsor
- Delegation of Authority Form
- Drug Destruction Standard Operating Procedure (SOP)
- For all laboratories listed on the FDA 1572, will need CLIA certifications, CAP certifications, lab licenses, CVs of Lab Directors, and laboratory reference ranges

2. Additional Essential Documents for Multicenter Trials for the Coordinating Center (filed in Regulatory Binder or OnCore)

- Institutional Review Board (IRB) approval letters, IRB roster, Informed Consent Form (ICF), and Health Insurance Portability and Accountability Act (HIPAA) Consent Form for the Participating Site(s)
- For all Principal Investigators and Sub-Investigators listed on the 1572 at the Participating Site(s) – Financial Disclosure Forms, CVs, MD Licenses, and Staff Training documents (i.e. Collaborative Institute Training Initiative (CITI), etc.) (for Investigational New Drug Application)
- Site Initiation Visit (SIV) minutes and correspondence with Participating Site(s).
- As applicable, approvals for Biosafety Committee, Radiation Committee, and Infusion Center for the Participating Site(s)
- Protocol Violations and Single Patient Exception (SPE) reports to IRB with supporting fax documentation for Participating Site(s)
- Drug Destruction Standard Operating Procedure (SOP) for the Participating Site(s)
- Data and Safety Monitoring Committee (DSMC) monitoring reports for the Participating Site(s)
- For all laboratories listed on FDA 1572, will need CLIA certifications, CAP certifications, lab licenses, CVs of Lab Directors, and laboratory reference ranges for the Participating Site(s)
- Copy of the Data and Safety Monitoring Plan (DSMP) Monitoring Plan for all participating site(s) in Multicenter studies or Contract Research Organization (CRO) Monitoring Plan (if an outside CRO is used for the study)
- Serious Adverse Event (SAE) forms submitted to both the IRB and the sponsor for the Participating Site(s)

4.3 Required Regulatory Documents for Sub-sites Participating in a UCSF Investigator Initiated Multicenter Trial (Checklist)

Directions:

- 1) Fax the documents listed below to the UCSF Coordinating center at 415-514-6955 or
- 2) Scan the documents and upload to OnCore® and create a Note to File for the on-site Regulatory binder to indicate where these documents may be found

1572

PI and Sub investigators:

- CV and Medical license
- Financial disclosure form
- NIH or CITI human subject protection training certification
- Laboratories
- CLIA and CAP
- CV of Lab Director and Lab Licenses
- Laboratory reference ranges

Local Institutional Review Board

- IRB Approval letter
- Reviewed/Approved documents
 - Protocol version date: _____
 - Informed consent version date: _____
 - Investigator Brochure version date: _____
 - HIPAA
- Current IRB Roster

Other

- Delegation of Authority Log
- Include NIH or CITI human subject protection training certificates or GCP training certification
- Pharmacy
 - Drug destruction SOP and Policy
- Protocol signature page
- Executed sub contract

27.apr.2012