A Phase II Study of Oral Calcitriol

in Combination with Ketoconazole in Castration Resistant Prostate Cancer, Progressing despite Primary ADT and Abiraterone

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PROTOCOL SYNOPSIS

A Phase I/II Study of Oral Calcitriol in Combination with Ketoconazole in Castration Resistant Prostate Cancer Following Progression on Abiraterone

Number of centers: 1

Study period		**Phase of development**
Estimated date of first patient enrolled	Sept 2016	II
Estimated date of last patient completed	Sept 2017	

Objectives

Primary Objective

1. To estimate the PSA response rate with the use of ketoconazole/hydrocortisone (400mg QD + hydrocortisone 20mg AM, 10 mg PM) + calcitriol (10mcg QD X3 weekly) among men with CRPC in whom disease has progressed despite abiraterone

Secondary Objectives

1. Describe objective tumor responses to the combination of oral calcitriol and ketoconazole and hydrocortisone-among patients with measurable disease using modified RECIST 1.1 criteria.
2. Determine toxicities, and tolerability of oral calcitriol combination with daily oral ketoconazole, and hydrocortisone in this patient population.

Trial Design

2. A single arm phase II trial of ketoconazole (400mg QD + hydrocortisone 20mg AM, 10 mg PM) + calcitriol (10mcg QD X3 weekly) among men with CRPC in whom disease has progressed despite abiraterone

Background and Rationale

In vitro, calcitriol has both antiproliferative and differentiating effects. Growth inhibition following exposure to calcitriol has been demonstrated in melanoma, breast, colon, prostate, osteosarcoma, and leukemia cell lines, and in xenografts of human colorectal carcinoma and melanoma in immunosuppressed mice. *In vitro* and *in vivo* maximum tumor response requires high calcitriol concentrations (>10 nM). These concentrations are not achievable with daily administration of calcitriol because dose-limiting hypercalcemia develops. Treatment with

calcitriol on an intermittent basis (once weekly or daily x 3/ week) is better tolerated than daily treatment and results in peak blood concentrations 1/5-1/10 those needed for activity *in vivo*. Dexamethasone and other glucocorticoids inhibit calcium absorption by the intestine and increase calcium excretion by the kidneys, thus ameliorating the hypercalcemic effects of calcitriol. Furthermore, when combined with calcitriol, glucocorticoids enhance vitamin-D receptor expression, cell cycle arrest, apoptosis and antitumor effects.

One of the postulated mechanisms of resistance to calcitriol antitumor effects is intratumoral catabolism of calcitriol; the primary catabolic pathway is by 24-hydroxylation, mediated by 24 hydroxylase (CYP 24). *In vitro* and more recently *in vivo* CYP 24 inhibition by the non-specific cytochrome P450 inhibitor, ketoconazole, has been shown substantially to potentiate the antitumor activity of calcitriol in LNCaP, PC-3 and SCC models.

Both ketoconazole and calcitriol are agents with definite antitumor activities in preclinical models. These agents are relatively well tolerated with acceptable toxicity profiles. Both ketoconazole and calcitriol have been evaluated clinically as single agents or in combination therapies. Ketoconazole in combination with glucocorticoids (the latter required because high dose ketoconazole can cause hypoadrenalism through inhibition of adrenal steroidogenesis) has shown definite antitumor activity manifested as prolonged stabilization and objective responses in patients with androgen independent prostate cancer (AIPC). Clinical data suggest efficacy of ketoconazole primarily at high dose, 400 mg TID. Calcitriol, on the other hand, has not shown significant antitumor activity when evaluated clinically as a single agent. It has been postulated that the reason for lack of responses with single-agent calcitriol is due to failure to achieve and maintain adequate serum concentrations for prolonged periods without experiencing dose-limiting hypercalcemia; in addition, the potential for CYP24 inhibition to enhance intratumoral and intracellular calcitriol exposure is a potential advantage of this approach. When given intermittently, such as on a weekly basis or on 3 consecutive days a week, calcitriol serum concentrations ranging between 1 nM and 5 nM are achieved. Higher concentration of calcitriol could not be achieved with oral supplementation of liquid or pill formulations.

Recent preclinical data suggest synergistic antitumor effect by combining calcitriol and ketoconazole. Recent work (Feldman and Peehl, unpublished data: Johnson CS, Yu WD, Muindi J and Trump DL) supports a synergistic tumor inhibitory effect from combining calcitriol and ketoconazole. Ketoconazole is utilized to block androgen biosynthesis by inhibiting P450 enzymes involved in steroidogenesis and in addition, blocks other P450 enzymes such as 24 hydroxylase (CYP24) involved in the catabolism of calcitriol. Calcitriol-mediated *in vitro* anti-tumor activity is significantly enhanced by ketoconazole in primary cultures of human prostatic cancer cells. A significant increase in anti-proliferative activity is observed in human lung tumor cells treated with calcitriol and ketoconazole as compared to either agent alone after 72 hr as measured in an MTT assay. In addition in prostate cancer models in vivo and in vitro, ketoconazole and calcitriol are substantially more active than either agent alone. (See full protocol introduction)

Patient Population and Inclusion/Exclusion Criteria

Inclusion Criteria

For inclusion in the trial, a patient must fulfill all of the following criteria:

1. >=18 years of age. The effects of ketoconazole and high-dose calcitriol have not been studied adequately in patients <18 years of age and prostate cancer has not been described in children.
2. Histologically or cytologically confirmed adenocarcinoma consistent clinically with androgen Independent prostate cancer
3. Measurable disease with elevated PSA or evaluable disease (PSA elevation will constitute evaluable disease).
4. No cytotoxic chemotherapy for extensive disease prior to study entry will be allowed; given the recent data regarding the role of docetaxel + ADT in patients beginning ADT for advanced disease, such "adjuvant chemotherapy will be allowed (no more than 6 cycles) retinoids, vitamin D analogues, PPARγ agonists or antagonists, antiandrogens, progestational agents, estrogens, PC-SPES, LHRH-analogues, vaccines, cytokines will not be considered "cytotoxics." Patients who have previously received ketoconazole + glucocorticoids will **NOT** be eligible for this trial.
5. Patients who have received antiandrogens or progestational agents as therapy for prostate cancer must discontinue therapy and demonstrate a rising PSA ≥ 28 days following discontinuation (antiandrogren withdrawal – AAW) (≥42 days for bicalutamide or nilutamide). Patients who receive megestrol acetate as therapy for "hot flashes" at a dose of ≤40mg per day may continue this therapy during this trial. The dose of the megestrol acetate should not be changed during protocol treatment. Patients undergoing androgen deprivation using LHRH analogues must continue such agents or undergo orchiectomy to maintain castrate levels of testosterone.
6. Patients must have prostate cancer that is advanced or recurrent.
7. Patients should not have received any chemotherapy or investigational agents for at least 28 days before entering the study.
8. Eastern Clinical Oncology Group performance status 0 or 1
9. Life expectancy >3 months.
10. Patients must have normal organ and marrow function as defined below: patients may be transfused to meet these parameters at the physician's discretion.
    - leukocytes: ≥3,000/µl
    - hemoglobin: ≥ 8 g/dl
    - absolute neutrophil count (ANC):≥1,500/µl
    - platelets: ≥75,000/µl
    - total bilirubin: within normal institutional limit
    - AST/ALT: ≤2.5 X institutional upper limit of normal
    - creatinine: ≤ 2mg/dL
    - calcium: not above normal institutional limit
11. Patients should be able to receive oral medications.
12. Patients with brain metastases which are stable and have been treated with surgery and/or irradiation will be eligible for this trial. Stable is defined as being asymptomatic

and requiring no anticonvulsants or corticosteroids for >/= 28 days prior to the initiation of study therapy.

13. The effects of high-dose calcitriol and ketoconazole on the developing human fetus are unknown. For this reason and because these agents as well are known to be teratogenic, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform the treating physician immediately.
14. Ability to understand and the willingness to sign a written informed consent document.
15. Progressive disease must have occurred on abiraterone within the prior 12 months and patient must not have received treatment with enzalutamide.

Men of all ethnic groups are eligible for this trial. Efforts will be made to include minority groups and all representative ethnicities and races in the community.

Exclusion Criteria

Any of the following is a criterion for exclusion from the trial:

1. Known severe hypersensitivity to ketoconazole, calcitriol or any of the excipients of these products.
2. History of allergic reactions attributed to compounds of similar chemical or biologic composition to calcitriol, ketoconazole, or other agents used in study.
3. Evidence of any other significant clinical disorder or laboratory finding that makes it undesirable for the patient to participate in the trial.
4. History of kidney, ureteral, or bladder stones within the last 5 years
5. Heart failure or significant heart disease including significant arrhythmias, myocardial infarction within the last 3 months, unstable angina, documented ejection fraction <30%, or current digoxin therapy.
6. Thiazide therapy within 7 days from entering the study.
7. Requirement for concurrent systemic glucocorticoid therapy at greater than physiologic replacement doses
8. Unwillingness to stop calcium or vitamin D supplementation, including when part of a multivitamin. As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g., unstable or uncompensated respiratory, cardiac, hepatic, or renal disease) or intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
9. Human immunodeficiency virus-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible PK interactions with ketoconazole or other agents administered during the study. Appropriate studies will be undertaken in patients receiving combination anti-retroviral therapy when indicated.
11. Concomitant use of phenytoin, carbamazepine, barbiturates, rifampicin, phenobarbital, or St John's wort, alfentanil, alfuzosin, almotriptan, alprazolam, amiodarone, amitriptyline, amprenavir, aprepitant, aripiprazole, bepridil, bortezomib, bosentan, budesonide, buprenorphine, buspirone, carbamazepine, cilostazol, cisapride, cyclosporine, delaviridine, didanosine, digoxin, disopyramidedofetilide, donepezil, eletriptan, elperenone, fluticasone,

fosamprenavir, galantamine, systemic griseofulvins, indinavir, levobupivacaine, lopinavir, midazolam, mifepristone, modafinil, nateglinide, nefazadone, nelfinavir, oxcarbazepine, pimozide, quetiapine, quinidine, repaglinide, rifabutin, rifampins, rifapentine, ritonavir, saquinavir, sildenafil, sirolimus, tacrolimus, tadalafil, tolterodine, theophyllines, tolterodine, trazolam, valdecoxib, vardenafil, ziprasidone, zanisamide, statins, with the exception of pravastatin (Pravachol) or other "statins" which are not metabolized by or induce CYP3A4, calcium channel blockers, and macrolides or other agents that will be significantly perturbed in a clinically important way by the P450 inhibitory properties of ketoconazole

12. Concomitant use of proton pump inhibitors or H2 blockers
13. Treatment with a non-approved or investigational drug or agent within 28 days before day 1 of trial treatment.
14. Any unresolved chronic toxicity greater then CTC Grade 2 from previous anticancer therapy.
15. Incomplete healing from previous oncologic treatments or other major surgery.
16. Inability to swallow oral capsules.
17. Patients on digoxin will be excluded from this study.

Products Dosage and Mode of Administration

- Ketoconazole, 200 mg tablets, 2 tablets orally TID
- Calcitriol (0.5 mcg caplets) given orally QD X3 consecutive days every week
- Hydrocortisone 20mg AM, 10mg PM orally starting in the evening before the first dose of Calcitriol.

Number of Patients and Duration of Treatment

Number of patients expected to be enrolled each month: 2-3 patients
Total number of patients expected to enroll: 15 patients
Treatment will continue as long as there is absence of prohibitive toxicities and lack of clinical or radiological progression.

Estimated Time to Complete Accrual: 1 year

Statistical Analysis:

It is highly likely that the antitumor activity of ketoconazole and abiraterone in prostate cancer depend substantially on the ability of both agents to inhibit CYP 17A1 and disrupt androgen synthesis. We and others have shown that antitumor effects of abiraterone are substantially reduced in patients in whom ketoconazole has previously failed to be effective. In addition, enzalutamide, whose mechanism is not CYP17A1 dependent, does inhibit CRPC through disruption of androgen signaling. There are essentially no data regarding the success of ketoconazole therapy after abiraterone “failure”. If ketoconazole and abiraterone share the same mechanism of action one would expect the same low PSA response rate in men with CRPC who receive (I) ketoconazole → abiraterone and those who receive (II) abiraterone → ketoconazole. If the response rate among men treated with sequence (II) substantially exceeds the PSA RR of 10% we saw with sequence (I), this would suggest additional effects of

ketoconazole. Further, the hypothesis of this trial is that ketoconazole also inhibits CYP24A1, the predominant vitamin D metabolizing enzyme and hence potentiates the antitumor effect of calcitriol (1,25 dihydroxycholecalciferol, 1,25 vitamin D_3) - in a synergistic manner.

This study proposes to treat men in whom abiraterone has proven to be ineffective within 12 months of entry onto this portion of this study. Based on the above, a PSA response rate of 10% or less for this regimen following abiraterone failure would be considered to have too little activity to be of clinical significance in this patient population, whereas a response rate of 40% or more would be of interest. We utilize a flexible design (Chen TT, NG T. Optimal flexible designs in phase II clinical trials. *Stat Med.* 1998;17:2301-2312.) that permits the actual number of evaluable patients in each stage to vary slightly in order to accommodate the challenges of accruing precise numbers of evaluable patients. In the first stage, we target an accrual of 9 eligible and evaluable patients but permit accrual to range from 7 to 11 patients. If more than 0 patients respond and medical judgment indicates, accrual to the second stage of the trial will be initiated; otherwise, the study will be stopped and the treatment regimen will be classified as clinically uninteresting. If the study advances to the second stage, then an overall study accrual of 15 eligible and evaluable patients will be targeted but will be permitted to range from 13 to 17 patients. If more than 2 out of 13-14 patients respond or 3 out of 15-17 patients respond then the regimen will be considered worthy for additional investigation. If the true response rate is 10% (H_0), these decision rules limit the average probability of designating the treatment as active to 10% and the average probability of stopping after completing only the first stage of accrual is 39%. On the other hand, if the true response rate is 40% (H_1) then the average probability of correctly classifying the treatment as active is 93%. These are average probabilities computed from the individual probabilities averaged over all permitted accrual combinations and assuming each combination is equally likely. Limited investigations have indicated that the type I and type II errors are fairly insensitive to variations in the true probability distribution of accrual combinations.

Stage of Accrual	Targeted Cumulative Accrual	Limits of Actual Accrual	Max number of responses to reject H_1
1	9	7-11	0/(7-11)
2	15	13-17	2/(13-14), 3/(15-17)

Entry criteria for this addendum phase will be identical to the ongoing trial except that progressive disease will be required to have occurred on abiraterone within the prior 12 months. Patient may also not have received enzalutamide in order to, as much as possible, “standardize” prior manipulation of the androgen axis.

Schedule

Calcitriol 10mcg QD X3, weekly + Ketoconazole 400mg po TID and Hydrocortisone 20mg AM, 10mg PM

Day	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Calcitriol		C	C	C					C	C	C					C	C	C					C	C	C				
Ketoco Nazole		K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K
Hydro Cortisone	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

K = ketoconazole 400 mg TID (1200mg/day).
C = Calcitriol.
H =Hydrocortisone 20mg AM, 10mg PM (starting in the evening before the first dose of calcitriol)

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
ALT	alanine transaminase
ANC	absolute neutrophil count
AST	aspartate transaminase
AUC	area under the curve
BUN	blood urea nitrogen
CR	complete response
CRFs	case report forms
CSR	clinical study report
CTC	common toxicity criteria
DCR	disease control rate
DLT	dose-limiting toxicity
DSMB	data and safety monitoring board
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	gastrointestinal
HRPC	hormone-refractory prostate cancer
IC_{50}	inhibitory concentration 50%
ILD	interstitial lung disease
INR	international normalized ratio
IRB	institutional review board
IV	intravenous
LCS	lung cancer symptom
LHRH	luteinizing hormone-releasing hormone
MAPK	mitogen-activated protein kinase
MTD	maximum tolerated dose
NCI	National Cancer Institute
NSCLC	non-small cell lung cancer
OAE	other significant adverse event
PARP	poly (ADP-ribose) polymerase
PD	progressive disease
p-EGFR	phosphorylated-EGFR
PK	pharmacokinetic
p.o.	given by mouth
PPARγ	peroxisome proliferators-activated receptor-gamma
PR	partial response
PSA	prostate-specific antigen
PVC	polyvinyl chloride
QOD	every other day
RPCI	Roswell Park Cancer Institute
RR	response rate
SAE	serious adverse event
SCC	squamous cell carcinoma

SD	stable disease
TCF	T-cell transcription factor
TGF	transdermal growth factor
VDR	vitamin D receptor
WOCBP	women of childbearing potential
WNY	Western New York
ZD1839	IRESSA®

1. OBJECTIVES

Primary Objective

To estimate the PSA response rate with the use of ketoconazole (400mg QD + hydrocortisone 20mg AM, 10 mg PM) + calcitriol (10mcg QD X3 weekly) among men with CRPC in whom disease has progressed despite abiraterone

Secondary Objectives

Describe objective tumor responses to the combination of oral calcitriol and ketoconazole and hydrocortisone-among patients with measurable disease using modified RECIST1.1 criteria.

Determine toxicities, and tolerability of oral calcitriol combination with daily oral ketoconazole, and hydrocortisone in this patient population.

2. BACKGROUND

2.1 Calcitriol (Rocaltrol® for Oral Formulations)

The clinically required vitamin D supply in humans depends mainly on exposure to ultraviolet (UV) light of the sun for conversion of 7-dehydrocholesterol in the skin to vitamin D_3 (cholecalciferol). Vitamin D_3 must be metabolically activated in the liver and the kidney before it is fully activated as a regulator of calcium and phosphorous metabolism in target tissues. The initial transformation of vitamin D_3 is catalyzed by 25-hydroxylase enzyme present in the liver, and the product of the reaction is 25-hydroxyvitamin D_3 [25-(OH)D_3]. Hydroxylation of 25-(OH)D_3 occurs in the mitochondria of kidney tissue (alpha-OHase), to produce 1,25-$(OH)_2D_3$ (Calcitriol), the active form of vitamin D_3. Physiologic daily production is normally 0.5 to 1.0 mcg and is usually higher during periods of active bone synthesis.

Epidemiologic evidence suggests that low calcitriol may play an important role in the development of colon and prostate cancer. An inverse relationship between incidence and UV light exposure has been reported for these neoplasms. UV exposure is the major route of metabolic activation of calcitriol, and increasing risk of malignancy is associated with decreasing serum levels of calcitriol (prostate) and its metabolic precursor 25-(OH)D_3 (colon). There is also evidence that dietary supplementation with calcitriol can reduce the incidence of colon cancer (1-5).

2.2 Preclinical Studies

Studies in our laboratory demonstrate that vitamin D (1,25 dihydroxycholecalciferol or calcitriol), a central factor in bone and mineral metabolism (1,2), has significant antitumor activity *in vitro* and *in vivo* in murine squamous cell carcinoma (SCC), human xenograft prostatic adenocarcinoma (PC-3) and rat metastatic prostatic adenocarcinoma Dunning (MLL) model systems (3-6). Calcitriol induces G_0/G_1 arrest, modulates $p27^{Kipl}$ and $p21^{WafI/Cipl}$, the cyclin dependent kinase (cdk) inhibitors implicated in G_1 arrest (4,7,8) and induces cleavage of caspase 3, PARP and the mitogen-activated protein kinase (MEK) in a caspase-dependent manner (5,9). Calcitriol also decreases phospho-Erk (P-Erk) and phospho-Akt (P-Akt), kinases

that regulate cell survival pathways and up-regulates the pro-apoptotic signaling molecule, MEKK-1 (10).

Calcitriol significantly enhances the *in vitro* and *in vivo* antitumor efficacy of the platinum analogues and taxanes (6,7,11,12). Enhancement of drug-mediated apoptosis is associated with an increase in PARP, MEK and caspase3-cleavage, the expression of p73 and MEKK-1 and a decrease in P-Erk and P-Akt (6,12). Based on these pre-clinical data, we performed two phase I clinical trials of calcitriol with either carboplatin or paclitaxel and administered higher doses of calcitriol than previously reported, without toxicity (13). Pharmacokinetic (pk) data following oral administration indicate that the serum calcitriol AUC is not proportional to calcitriol dose suggesting a decrease in bioavailability and calcitriol exposure (14). Oral administration of calcitriol at the highest dose studied results in an AUC in man (7.5ng/ml.hr) that is significantly lower than the AUC in mice (38.1ng/ml.hr) at doses that correlate with a significant antitumor effect *in vivo* (15).

Glucocorticoids enhance calcitriol-mediated activities pre-clinically (*in vitro* and *in vivo*) and clinically. We have demonstrated that dexamethasone (dex) significantly potentiates the antitumor effect of calcitriol and decreases calcitriol-induced hypercalcemia (16,17). Both *in vitro* and *in vivo*, dex increases vitamin D receptor (VDR) ligand binding in the tumor while decreasing binding in intestinal mucosa (16), the site of calcium absorption (18). P-Erk and P-Akt are also decreased with calcitriol/dex, as compared to either agent alone (17). When the dex is added to calcitriol and a number of cytotoxic drugs, a greater antitumor effect is observed than with each drug alone or any two drug combination (19-21). Dex enhances VDRE transcriptional activity through a potential effect on coactivator stimulation of transcription. We have examined whether there are differences among various glucocorticoid preparations in potentiating calcitriol in vitro or in vivo effects. No convincing differences between dex, prednisone or hydrocortisone have been detected at equivalent glucocorticoid doses. In a phase II trial in androgen independent prostate cancer (AIPC) oral calcitriol (12 μg/day QDx3, weekly) and dex (4 mg QDx4, weekly), we saw a 50% reduction in prostate specific antigen (PSA) in 28% of the patients, no hypercalcemia (22) and molecular changes in peripheral blood monocytes (PBM) similar to those observed in cell lines. Antitumor effects were also noted following calcitriol/dex in men with localized disease with a rising PSA following prostatectomy or irradiation. These trials utilized oral calcitriol – a route of administration where drug exposure and bioavailability may be an issue and limit response (14). Induction of CYP24, the enzyme primarily responsible for calcitriol catabolism (23), may be a factor in bioavailability and calcitriol exposure; therefore, studies were initiated to investigate the effect of ketoconazole, a CYP24 inhibitor (24,25), on *in vitro* and *in vivo* anti-tumor effects of calcitriol/dex in the PC-3 prostate tumor model. Ketoconazole enhanced the anti-tumor activity of calcitriol/dex and decreased CYP24 activity.

2.3 Clinical Trials

Since 1999, we initiated six clinical trials with one detailed below. The first trial was a phase I study to evaluate the pharmacokinetics and MTD of calcitriol following subcutaneous (sc) QOD administration (93). Thirty-six patients were entered at doses ranging from 2 μg to 10μg QOD; dose-limiting toxicity (hypercalcemia) occurred in 3 of 3 patients entered at the 10 μg QOD dose. Hypercalciuria occurred at all dose levels examined. No other toxicity was seen.

Serum calcitriol levels by radioimmunoassay revealed a decrease in concentration-time curves on the seventh day compared to the first day of therapy. A dose dependent increase in peak serum level and estimated area under

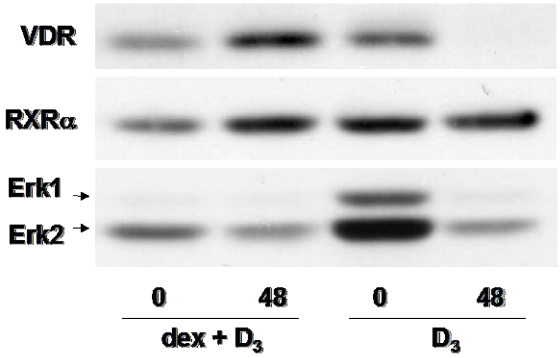

Fig 1. Representative Western blot analysis of monocytes isolated from patients treated with calcitriol alone (8μg) or calcitriol (8μg) plus dex (4mg) after 48hr

the curve (AUC) were seen; the maximum serum levels occurred at the 10 μg QOD dose: 288 ± 74 pg/mL and 321 ± 36 pg/mL days 1and 7 respectively. The normal range of calcitriol serum concentrations using this assay is 16-56 pg/mL. Serum calcitriol levels were maintained at near peak concentrations for at least 8 hr following sc. injection. This study indicates that substantial doses of calcitriol can be administered via this route with tolerable toxicity.

2.3.1 Calcitriol + glucocorticoids: trials in prostate cancer. As our preclinical data indicated, dex potentiates calcitriol antitumor effects and blocks hypercalcemia. As a result, we initiated a phase II study of calcitriol and dex in AIPC (22). Calcitriol and dex were administered according to the following schedule: calcitriol 8μg Monday, Tuesday and Wednesday (MTW) weekly X4, then if no toxicity was seen the dose was escalated to 10μg MTW for one month. If no toxicity occurred the dose of calcitriol was increased to 12μg MTW weekly for the duration of the study. Dex was administered orally 4mg Sunday MTW each week. Forty-three patients were treated and 35 received 12μg MTW $\geq$ 1 month; no patient required dose reduction because of hypercalcemia. The only calcitriol related toxicity in this trial was the development in 2 patients of urinary tract stones. All patients underwent pretreatment and Q3 month renal ultrasound to monitor for nephrolithiasis. Thirty-five patients were evaluable and 28% of the 35 evaluable patients experienced a 50% reduction in PSA; patients with bone pain at study entry –experienced pain relief, eighty percent of patients experienced a slowing in the rate of PSA rise and 34% have had stable disease or decrease in PSA (>50% reduction). Time to a 50% decrease in PSA was 23-143 days with a mean of 65 days and the duration of response was 159-411 days with a mean of 270 days (9 months). Our studies indicate that modification in the schedule and route of administration of calcitriol and dex permit dose escalation of this agent; as described below, a bioavailability concern emerges with the oral route of administration.

We examined VDR expression in monocytes of patients treated with dex and/or calcitriol. Patients received either dex (4mgQDx3) and/or calcitriol (8μg QDx2). In monocytes from patients treated with calcitriol, VDR expression was suppressed 48hr after therapy, whereas in patients treated with calcitriol plus dex, VDR expression was increased (Fig 1). RXR expression was unchanged by calcitriol alone but increased following dex/calcitriol. P-Erk was

decreased from both treatment groups. These results demonstrate that modulation of VDR, RXR and P-Erk can be observed in the monocytes of patients treated with calcitriol +/- dex.

Peripheral blood monocytes were also examined for changes in calcitriol oxidation activity (CYP24) in patients treated with calcitriol alone. As shown in Fig 2 from a representative patient from one of the phase I trials, CYP24 activity peaked 6hr after administration of oral calcitriol (12μg) and returned to lower than baseline activity by 24hr. In AIPC patients treated with dex and calcitriol in the phase II trial, CYP24 levels were 1.0± 0.9 fmol/2hr /10^6 (mean±SD from 8 patients) after the first dose of dex and 2.4±1.1 fmol/2hr/10^6 after calcitriol with dex. These results demonstrate that PBM can be utilized to determine CYP24 expression in patients treated with calcitriol.

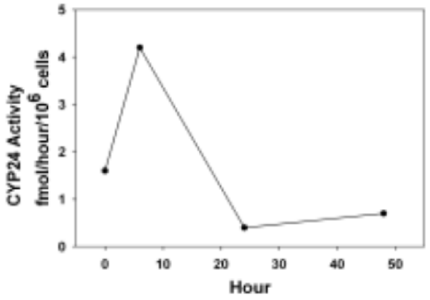

Fig 2. CYP24 levels in PBM isolated from patients treated with calcitriol alone (12μg) at 0, 6, 24 and 48hr after treatment.

We also initiated a phase I study of calcitriol and dex in men with AIPC to determine the effect of escalating doses of calcitriol alone and in combination with dex in this patient population. In this trial, patients were treated first with calcitriol alone at escalating doses and then calcitriol in combination with dex. Patients were treated with calcitriol QDx3 at doses 21-36 μg. This study accrued 22 patients and was closed due to the bioavailability issue with oral administration (see below).

2.3.2 Clinical trials with calcitriol in combination with cytotoxic agents. We conducted two phase I trials of calcitriol + cytotoxic drugs; calcitriol/carboplatin and calcitriol/paclitaxel (21). Patients with advanced cancer were treated with carboplatin (AUC=5) Q28 days + escalating doses of calcitriol QDX3 Q28 days. Calcitriol starting dose was 4μg QDX3. Studies were designed such that in each patient, carboplatin was given on day 1 before calcitriol in one of the first two cycles of treatment and on day 3 after two days of high dose calcitriol on the other. Dose–limiting toxicity was not encountered in this trial. The AUC of carboplatin was higher in <u>each</u> patient following calcitriol than before calcitriol (mean AUC = 7.6 μg/ml.hr ± 1.8, carboplatin day 3 [DDDC] vs. AUC = 6.6μg/ml.hr ± 1.4, carboplatin day 1 [CDDD], p= 0.04). This increase in AUC was observed with no change with increasing calcitriol dose. While no dose limiting toxicity was seen, myelosuppression (%change in platelet count) following the sequence carboplatin→calcitriol was less than that following calcitriol →carboplatin, consistent with the change in AUC. No clinically detectable renal impairment has been seen with either sequence. In another trial, patients with advanced cancer were treated with paclitaxel (80mg/m^2 weekly x 6) + escalating doses of calcitriol, QDX3 weeklyX6. The starting dose of calcitriol was 4μg po QDX3, weekly and we entered patients through the 38μg dose level where it appears that we reached saturable concentrations at 16-20μg (see pharmacokinetic data below (14). No dose limiting toxicity was encountered. No

changes in peak concentration, AUC or T 1/2 were noted. To investigate the issue of bioavailability of calcitriol, patients were given escalating doses of calcitriol starting at 14μg using a liquid formulation of calcitriol with potentially greater bioavailability.

2.3.3 Calcitriol plasma pharmacokinetics. Pharmacokinetic studies were required in at least 2 of 3 patients at each dose level of the calcitriol/paclitaxel clinical trial and were performed in 26 of the 36 patients; six patients at the highest dose level (38 μg) underwent pharmacokinetic studies (14). Baseline plasma calcitriol concentrations of the 26 cancer patients resulted in a median concentration of 26 pg/ml (range 13-81). The normal range for this assay is 16-74pg/ml. Serum calcitriol concentrations higher than baseline occurred within an hour of oral calcitriol administration. A scatter plot of the maximum concentration of calcitriol (Cmax) for each patient studied at each dose level is portrayed in Fig3A. As shown in Fig3B, baseline-subtracted serum calcitriol $AUC_{0->24hr}$ (area under the concentration-time curve for the 24 hour period after calcitriol administration) is plotted against dose. A fit to the Michaelis Menten function ($AUC = a \times dose/(1 + b \times dose)$ indicates that $AUC_{0->24hr}$ is not proportional to dose ($a = 540 \pm 140$ pg-hr/ml-μg; if AUC were proportional to dose, b would equal 0).

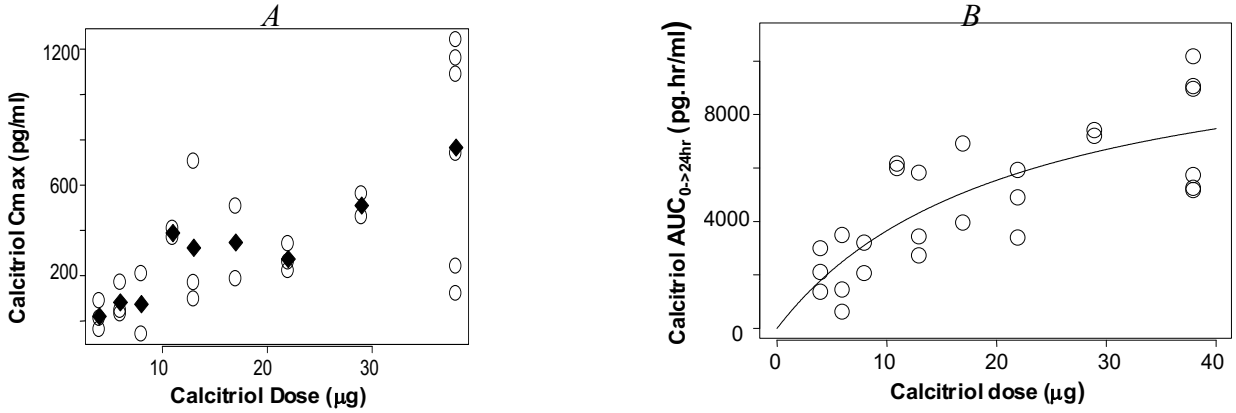

Fig 3. **(A)** Scatter plot of the maximum serum calcitriol concentration (Cmax) vs. calcitriol doses. Closed symbols represent mean values at each dose level. **(B)** Baseline-subtracted serum calcitriol $AUC_{0->24hr}$ (area under the concentration-time curve for the 24 hour period after calcitriol administration) plotted against dose, and a fit of the Michaelis-Menten function, p-value of 0.0014. The effect of the nonlinearity over the range of doses studied is large: the fit value of $AUC_{0->24hr}$ at 38 μg was only 4 times that at 4 μg, instead of the 9.5 times expected for a proportional relationship. However, no deviation from linearity can be detected up to a dose of 17 μg (p=0.4). In addition, there is insufficient evidence for an association between serum calcium and dose. No patient became hypercalcemic.

Clinical studies to date have predominantly utilized oral administration of capsules at 0.5 μg/capsule. At doses of 38 μg, the patients took 72 gel capsules at one time. To investigate decreased bioavailability and lack of absorption due to the mass of gel caps in the stomach, we utilized a liquid formulation of calcitriol in palm oil (1 μg/ml) which was tasteless and easy to swallow. No significant difference was observed in Cmax or AUC in patients given liquid or capsules at various doses.

2.4 Effect of ketoconazole on calcitriol-mediated anti-tumor activity. Ketoconazole is utilized to block androgen biosynthesis by inhibiting P450 enzymes involved in steroidogenesis (24,25) and in addition, blocks other P450 enzymes such as 24 hydroxylase (CYP24) involved in the catabolism of calcitriol (23). Calcitriol-mediated *in vitro* anti-tumor activity is significantly enhanced by ketoconazole in primary cultures of human prostatic cancer cells (61). A significant increase in anti-proliferative activity is observed in human lung tumor cells treated with calcitriol and ketoconazole as compared to either agent alone after 72 hr as measured in an MTT assay (data not shown). To examine the effect of ketoconazole on calcitriol anti-tumor activities with and without dex, we utilized the PC-3 prostate model system and treated PC-3 cells *in vitro* with varying concentrations of calcitriol and constant concentrations of ketoconazole and dex as described previously for three drug combinations (91). As shown in Fig 4A as the dose of calcitriol increases with a constant ratio of dex, ketoconazole significantly enhanced the growth inhibition observed with calcitriol alone, calcitriol/dex or calcitriol/ketoconazole. Likewise, a similar effect was observed when the dose of ketoconazole is increased with a constant dose of calcitriol/dex. Median dose effect analysis (92) was used to evaluate the nature of the interaction between calcitriol/dex and ketoconazole. PC-3 cells were treated with calcitriol/dex, ketoconazole alone or each of the two/three drug combinations. After incubation for 48hr (24hr pre-treatment dex groups), dose-effect data were used to derive a combination index (CI) as described previously (6,21). CIs < 1.0 were observed from a fraction affected of less than 0.2-0.9 indicating that the interaction between these agents was strongly synergistic (Fig 4A .)

To determine whether an increase in clonogenic cell kill *in vivo* was associated with inhibition of tumor cell growth, PC-3 mice were treated with various combinations of calcitriol (50mg/kg/day QDx3, weekly), dex (9μg QDx3, weekly) and/or ketoconazole (50mg/kg/day QDx3, weekly). 24 hr after the last dose of drug from a three week treatment period, animals were taken, tumors removed, dissociated and plated in the in vivo excision clonogenic assay as described previously (7). As shown in Fig 5A, treatment with calcitriol/dex/ketoconazole resulted in a significantly greater decrease in survival fraction as compared any other treatment group. We have shown previously that a significant effect by excision clonogenic assay correlates with a decrease in tumor volume in tumor bearing mice (6, 7, 11, and 16).

A. B.

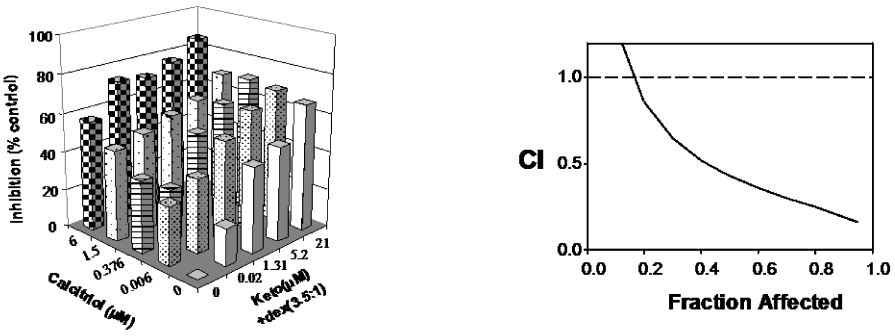

Figure 4 A,B. Effect of ketoconazole on calcitriol/dex-mediated anti-tumor activity in PC-3 cells. (A) PC-3 were pretreated with dex for 24hr (500nM) and varying concentrations of calcitriol and /or ketoconazole and after 48hr, growth inhibition measured using the MTT assay

(6). (B) Combination Index (CI) of calcitriol/dex/ketoconazole by median dose effect analysis (92). A CI<1.0 is synergistic.

To determine whether these anti-tumor effects resulted in a significant enhancement of calcitriol/dex-mediated anti-tumor activity in tumor bearing PC-3 nude mice, animals were treated as described above and monitored for tumor size. As shown in Fig 6B, significantly greater anti-tumor activity was observed when animals were treated with the three-drug combination (calcitriol/dex/ketoconazole) as compared to any single drug or two drug combinations.

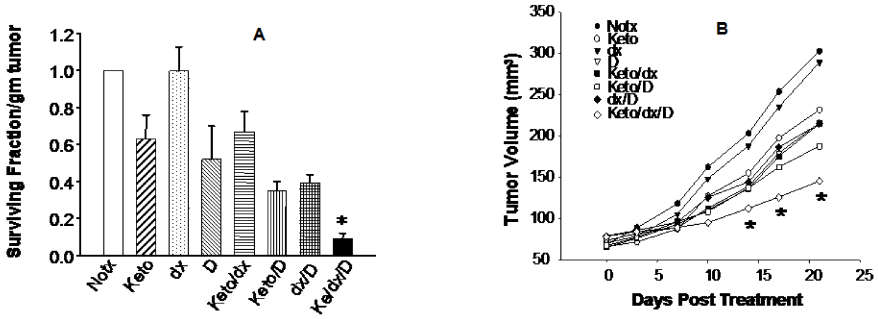

Fig 5 A, B. Ketoconazole increases calcitriol/dex antitumor activity *in vivo*. Tumor bearing PC-3 were treated with various combinations of either calcitriol (50mg/kg/day QDx3, weekly), dex (9μg QDx3, weekly) and/or ketoconazole (50mg/kg/day QDx3, weekly) and were treated for three weeks. (A) *In vivo* clonogenic assay where animals were treated either with calcitriol (D) QDx2, dex (dx) QDx3 and /or ketoconazole (Keto) QDx3. ∗significantly different than animals treated with calcitriol/dex (the most active regime), $p< 0.01$. (B) Animals with palpable PC-3 tumors were treated as described above in A and monitored for change in tumor volume ∗significantly different from calcitriol/ketoconazole or calcitriol/dex treated animals, $p<0.01$.

1.5 Effect on CYP24 Activity

To determine the effect of calcitriol alone or in combination with ketoconazole on CYP24 activity, which could limit calcitriol mediated anti-tumor activity, PC-3 tumor cells were treated with 10 and 100nM of calcitriol and examined for inducible CY24 activity. As shown in Fig 6A, CYP24 was induced in a dose-dependent manner using 25-OH-D_3 as substrate in PC-3 cells. The important peaks from this chromatogram are **a:** 25-(OH)-24-oxo- D_3, **b:** 23,25-$(OH)_2$-24-oxo- D_3 and **c:** 24,25-$(OH)_2$-D_3. An increase is observed in **a**, **b** and **c** which demonstrates induction of CYP24. When PC-3 cells were treated with calcitriol and ketoconazole (Fig 6B), a decrease is observed in peaks **a, b** and **c**, demonstrating a significant inhibition of CYP24 activity in these cells (Fig 6C).

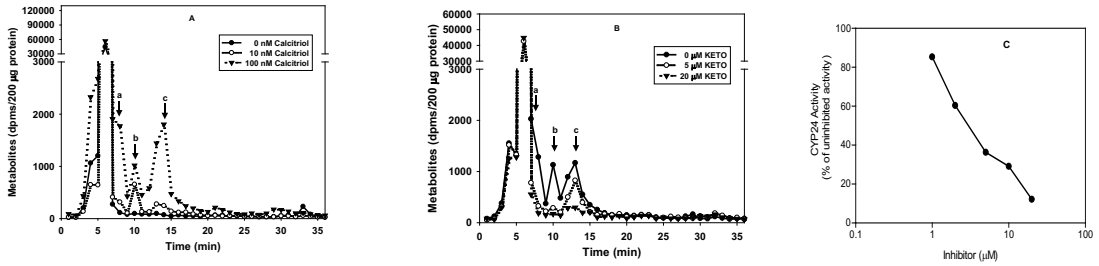

Fig 6 A, B, C. CYP24 activity of PC-3 treated with calcitriol +/- ketoconazole (A) Dose response for calcitriol inducible CYP24 of PC-3 cells either untreated (●), 10nM (□), or 100nM (▼) of calcitriol (B) Effect of various doses of ketoconazole (0-20µM) on calcitriol (100nM) inducible CYP24 levels (C) % inhibition of CYP24 by ketoconazole in PC-3 cells.

Peripheral blood monocytes were also examined for changes in calcitriol oxidation activity (CYP24) in patients treated with calcitriol alone. (see above)

2.4 Summary and Rationale for Calcitriol Starting Dose

These data point out that high dose calcitriol is well tolerated when given weekly or daily up to 3 consecutive days. Daily and every-other-day regimens of calcitriol are associated with substantial hypercalcemia which limits dose escalation.

These clinical and preclinical data have led us to conclude that the oral route of administration of calcitriol is not the optimal route of administration through which to attempt to achieve the exposures in animals that are required for optimal antitumor activity because:

1. interpatient variation in systemic exposure is substantial
2. exposure-dose relationships are nonlinear and unpredictable at large oral doses

Therefore we have initiated two clinical trials to establish the feasibility, toxicity pattern and pharmacokinetics of intravenous calcitriol administered either QD X2 monthly + docetaxel on day 2 or weekly + gefitinib. Three patients have been entered on these trials and the following data suggest that at the initial dose level (10 mcg) and the second dose level pharmacokinetics of oral and IV calcitriol are not dramatically different:

Route/No. subjects/formulation	Dose	Cmax (pg/ml)	AUC (pg.hr/ml)
po, N=7 ,caplet	**11mcg**	**437 +/- 180**	**5751+/-1563**
po, N=2, caplet	**11mcg**	**588 +/- 280**	**6942+/-250**
po, N=3, caplet	**13mcg**	**523 +/- 332**	**4557+/-1659**
po, N=4, liquid	**13mcg**	**692 +/- 497**	**7167 +/-2734**
Iv, N=3	**10mcg**	**438 +/- 30**	**4000, 4131**
Iv, N=2	**15mcg**	**860 ±.270**	**6290 ± 330**

These data suggest that the starting dose for this trial of oral calcitriol + ketoconazole is a safe starting dose; 3x this dose has been given safely**.** AUC and Cmax following doses in the range 10mcg-15mcg are similar following iv and oral administration. Since 38 mcg of calcitriol has been given QD X3, weekly without toxicity, we estimate that a starting dose of 10 mcg calcitriol will be a safe starting dose: a dose that is safe and non-toxic when given iv 15mcg achieves serum levels comparable to oral administration at 13 mcg and is 1/4 the dose that is safe and non-toxic when given orally weekly

These preclinical data lend a strong rationale to explore clinically the combination of ketoconazole and calcitriol. To achieve calcitriol serum concentrations that are proven to have antitumor activity and to maximize intratumoral calcitriol content through inhibition of catabolism by ketoconazole calcitriol will be administered orally. This phase I dose escalation study will determine the MTD of po calcitriol in combination with hydrocortisone 20mg q AM, 10mg q PM and ketoconazole 400 mg po TID.

Recent preclinical data suggest a potential synergistic antitumor effect by combining calcitriol and ketoconazole. Synergistic antitumor activity was seen with calcitriol concentrations of 10 nM. This has been delineated in detail above. This synergism forms the rationale for combining theses 2 agents in clinical studies. This phase I study will determine the feasibility, safety, and the MTD of a combination of calcitriol and hydrocortisone (30 mg total daily dose) with ketoconazole (400 mg TID) This study will determine a recommended dose of the combination for further investigation in phase II efficacy trials.
The rationale for this study can be summarized by the following:

1. Both calcitriol and ketoconazole have antitumor activity in AIPC
2. Calcitriol and ketoconazole show synergistic antitumor activity in prostate models
3. A mechanism of calcitriol resistance is catabolism mediated via CYP 24 activity
4. Ketoconazole inhibits CYP 24 activity
5. Higher calcitriol dosages are associated with improved antitumor activity and synergy. Adequate concentrations of calcitriol (>10 nM or higher) are not achieved with currently available oral formulations, hence the need to explore IV dosing or other approaches-such as inhibiting calcitriol catabolism- to enhance calcitriol exposure

Glucocorticoids have shown protective effects against vitamin D induced hypercalcemia and exert a significant degree of pre-clinical antitumor synergy when combined with calcitriol.

3.0 PATIENT SELECTION

Patients will be enrolled from the urologic oncology practices at ISCI. No additional recruitment procedures will be used.

Screening for eligibility consists of standard evaluation for castration resistant prostate cancer (CRPC). No such data will be recorded until after consent is given. Screening will be done by the patient's urologic oncology specialist and Clinical Research Services personnel. Consent will be presented to the patient when CRPC is recognized and initial inquiry from the patient's physician is answered favorably by the patient. There is no time table/limit for consent though 1-2 weeks is typically sufficient for completion of consent process which is followed by initiation of research-specific screening procedure.

3.1 Inclusion Criteria

For inclusion in the trial, a patient must fulfill all of the following criteria:

1. >=18 years of age. The effects of ketoconazole and high-dose calcitriol have not been studied adequately in patients <18 years of age and prostate cancer has not been described in children.

2. Histologically or cytologically confirmed adenocarcinoma consistent clinically with androgen Independent prostate cancer
3. Measurable disease with elevated PSA or evaluable disease (PSA elevation will constitute evaluable disease).
4. No cytotoxic chemotherapy for extensive disease prior to study entry will be allowed; given the recent data regarding the role of docetaxel + ADT in patients beginning ADT for advanced disease, such "adjuvant chemotherapy will be allowed (no more than 6 cycles) retinoids, vitamin D analogues, PPARγ agonists or antagonists, antiandrogens, progestational agents, estrogens, PC-SPES, LHRH-analogues, vaccines, cytokines will not be considered "cytotoxics." Patients who have previously received ketoconazole + glucocorticoids will **NOT** be eligible for this trial.
5. Patients who have received antiandrogens or progestational agents as therapy for prostate cancer must discontinue therapy and demonstrate a rising PSA $\geq$ 28 days following discontinuation (antiandrogren withdrawal – AAW) ($\geq$42 days for bicalutamide or nilutamide). Patients who receive megestrol acetate as therapy for "hot flashes" at a dose of $\leq$40mg per day may continue this therapy during this trial. The dose of the megestrol acetate should not be changed during protocol treatment. Patients undergoing androgen deprivation using LHRH analogues must continue such agents or undergo orchiectomy to maintain castrate levels of testosterone.
6. Patients must have prostate cancer that is advanced or recurrent.
7. Patients should not have received any chemotherapy or investigational agents for at least 28 days before entering the study.
8. Eastern Clinical Oncology Group performance status 0 or 1
9. Life expectancy >3 months.
10. Patients must have normal organ and marrow function as defined below: patients may be transfused to meet withese parameters at the physician's discretion.
    - leukocytes: $\geq$3,000/μl
    - hemoglobin: $\geq$ 8 g/dl
    - absolute neutrophil count (ANC):$\geq$1,500/μl
    - platelets: $\geq$75,000/μl
    - total bilirubin: within normal institutional limit
    - AST/ALT: $\leq$2.5 X institutional upper limit of normal
    - creatinine: $\leq$ 2mg/dL
    - calcium: not above normal institutional limit
11. Patients should be able to receive oral medications.
12. Patients with brain metastases which are stable and have been treated with surgery and/or irradiation will be eligible for this trial. Stable is defined as being asymptomatic and requiring no anticonvulsants or corticosteroids for >/= 28 days prior to the initiation of study therapy.
13. The effects of high-dose calcitriol and ketoconazole on the developing human fetus are unknown. For this reason and because these agents as well are known to be teratogenic, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform the treating physician immediately.
14. Ability to understand and the willingness to sign a written informed consent document.

15. Progressive disease must have occurred on abiraterone within the prior 12 months and patient must not have received treatment with enzalutamide.

Men of all ethnic groups are eligible for this trial. Efforts will be made to include minority groups and all representative ethnicities and races in the community.

3.2 Exclusion Criteria

Any of the following is a criterion for exclusion from the trial:

1. Known severe hypersensitivity to ketoconazole, calcitriol or any of the excipients of these products.
2. History of allergic reactions attributed to compounds of similar chemical or biologic composition to calcitriol, ketoconazole, or other agents used in study.
3. Evidence of any other significant clinical disorder or laboratory finding that makes it undesirable for the patient to participate in the trial.
4. History of kidney, ureteral, or bladder stones within the last 5 years
5. Heart failure or significant heart disease including significant arrhythmias, myocardial infarction within the last 3 months, unstable angina, documented ejection fraction <30%, or current digoxin therapy.
6. Thiazide therapy within 7 days from entering the study.
7. Requirement for concurrent systemic glucocorticoid therapy at greater than physiologic replacement doses
8. Unwillingness to stop calcium or vitamin D supplementation including when part of a multivitamin.
9. As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g., unstable or uncompensated respiratory, cardiac, hepatic, or renal disease) or intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10. Human immunodeficiency virus-positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible PK interactions with ketoconazole or other agents administered during the study. Appropriate studies will be undertaken in patients receiving combination anti-retroviral therapy when indicated.
11. Concomitant use of phenytoin, carbamazepine, barbiturates, rifampicin, phenobarbital, or St John's wort, alfentanil, alfuzosin, almotriptan, alprazolam, amiodarone, amitriptyline, amprenavir, aprepitant, aripiprazole, bepridil, bortezomib, bosentan, budesonide, buprenorphine, buspirone, carbamazepine, cilostazol, cisapride, cyclosporine, delaviridine, didanosine, digoxin, disopyramidedofetilide, donepezil, eletriptan, elperenone, fluticasone, fosamprenavir, galantamine, systemic griseofulvins, indinavir, levobupivacaine, lopinavir, midazolam, mifepristone, modafinil, nateglinide, nefazadone, nelfinavir, oxcarbazepine, pimozide, quetiapine, quinidine, repaglinide, rifabutin, rifampins, rifapentine, ritonavir, saquinavir, sildenafil, sirolimus, tacrolimus, tadalafil, tolterodine, theophyllines, tolterodine, trazolam, valdecoxib, vardenafil, ziprasidone, zanisamide, statins, with the exception of pravastatin (Pravachol) or other "statins" which are not metabolized by or induce CYP3A4, calcium channel blockers, and macrolides or other agents that will be significantly perturbed in a clinically important way by the P450 inhibitory properties of ketoconazole

12. Concomitant use of proton pump inhibitors or H2 blockers
13. Treatment with a non-approved or investigational drug or agent within 28 days before day 1 of trial treatment.
14. Any unresolved chronic toxicity greater then CTC Grade 2 from previous anticancer therapy.
15. Incomplete healing from previous oncologic treatments or other major surgery.
16. Inability to swallow oral capsules.
17. Patients on digoxin will be excluded from this study.

3.3 Inclusion of Minorities

Only men are eligible for this trial. Different races and ethnicities and minorities will be sought for participation in this study.

3.4 Restrictions

Men enrolled in this study must practice acceptable methods of birth control to prevent pregnancy.

4.0 TREATMENT PLAN

4.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported AEs and potential risks for calcitriol and ketoconazole are described below. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Calcitriol will be administered orally as 0.5 mcg caplets. Patients will be instructed to take calcitriol over a period $\leq$ 15 minutes. Ketoconazole will be administered orally TID. The dose of ketoconazole will be at a standard dose, 400 mg TID. Hydrocortisone will be administered orally 20mg in the AM and 10mg PM starting the night prior to the first dose of calcitriol. The scheduling is detailed below.

Calcitriol will be given orally daily 10 mcg x 3 consecutive days per week starting on day 1 subsequently. On Day 1, ketoconazole will be started orally at 400 mg TID (Table 1). Hydrocortisone 20 mg every morning and 10 mg every evening will be given starting the evening before the first dose of calcitriol.

4.2 Treatment Schedule

Table 1

Calcitriol 10mcg QD X3, weekly + Ketoconazole 400mg po TID and Hydrocortisone 20mg AM, 10mg PM

Day	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Calcitriol		C	C	C					C	C	C					C	C	C					C	C	C				
Ketoco Nazole		K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K
Hydro Cortisone	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

K = ketoconazole 400 mg TID (1200mg/day).
C = Calcitriol.
H =Hydrocortisone 20mg AM, 10mg PM (starting in the evening before the first dose of calcitriol)

4.1 Supportive Care Guidelines

Patients should receive full supportive care, including antiemetics, antibiotics, transfusions of blood and blood products, etc., when appropriate. Whenever any medications are administered, the reason(s) for treatment, dosage and date of treatment must be recorded on the case report forms (CRFs).

Erythropoietin alpha can be administered concurrently.

4.2 Duration of Experimental Therapy

Experimental therapy consisting of calcitriol, hydrocortisone and ketoconazole will continue in the absence of disease progression and absence of unacceptable/ unmanageable drug-related AEs.

There will be no rest period between cycles. Cycle 2 will start on day 29 in the absence of dose delays.

Treatment will continue unless 1 of the following is satisfied:

- Disease progression (see section 6.3)
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- For up to one year if none of the above and if the treatment is still working at one year patients may discuss with the treating provider whether continuing calcitriol treatment is recommended but will not receive calcitriol as part of this study.

5.0 DOSING DELAYS/DOSE MODIFICATIONS

5.1 Ketoconazole

Dose interruptions should be the first approach to managing toxicity. Repeat dose interruptions are allowed as required. No dose reduction is allowed.

5.2 Dose Interruption for Non-Hematopoeitic Toxicity

In the event of CTC Grade 3 or 4 non-hematopoeitic AE(s) that the investigator considers due to suspected disease progression, re-evaluation of tumor status is indicated, irrespective of scheduled clinic visits.

CTC Grade 3 or 4 believed to be drug related will be considered DLTs and will result in taking patients off study. One exception will be in the case of CTC Grade 3 diarrhea or Grade 3 nausea that is not attributed to hypercalcemia. In those situations ketoconazole may be resumed after a period of interruption that does not exceed 2 weeks with full antiemetics or antidiarrheal medication. In the event that these toxicities recur at grade 3 or greater, despite adequate supportive medications, this will be a DLT and the patients will be taken off study.

Ketoconazole may also be interrupted for a period that does not exceed 2 weeks in the event of symptomatic intolerable Grade 2 rash. At a minimum, reassessment of toxicity should be done twice weekly and more frequently if clinically indicated. When the AE decreases in severity to CTC Grade 1, the patient may resume the assigned dose.

5.3 Nausea and/or Vomiting

In patients who have emesis and are unable to retain ketoconazole, every attempt should be made to obtain control of nausea and vomiting. The dose of ketoconazole may be repeated if emesis occurs within 30 minutes of taking the tablets.

5.4 Diarrhea

Diarrhea can be debilitating and on rare occasions is potentially life-threatening. Guidelines developed by an American Society of Clinical Oncology panel for treating chemotherapy-induced diarrhea are abstracted below.

Pharmacological approaches include the following:

- Diarrhea has been successfully managed with anti-diarrheal agents such as loperamide. Loperamide administered as an initial 4-mg dose followed by 2-mg doses every 4 hours. This dose and regimen is moderately effective.
- Clonidine, non-steroidal inflammatory drugs, and the serotonin antagonist cryoheptadine have been shown to be effective in controlling diarrhea associated with inflammation of the bowel.

The synthetic octapeptide octreotide has been shown to be effective in the control of diarrhea induced by fluoropyrimidine-based chemotherapy regimens when administered as an escalating dose by continuous infusion or subcutaneous injection. Octreotide can be administered at doses ranging from 100 micrograms twice daily to 500 micrograms 3 times daily, with a MTD of 2000 micrograms 3 times daily in a 5-day regimen.

In the event of:

- **CTC Grade 1 diarrhea.** No specific supportive care is usually needed or indicated.
- **CTC Grade 2 or 3 diarrhea.** If this occurs and immediate supportive care measures are begun (e.g. loperamide) ketoconazole should be discontinued for up to a maximum of 14 days, until resolution or the diarrhea has decreased in severity to CTC Grade 1.
- **CTC Grade 4 diarrhea will be declared a DLT**
- If a Grade 4 diarrhea is associated with hemodynamic collapse, the investigator should report it as an SAE and remove the patient from the trial.

5.5 Other Concomitant Therapy with Ketoconazole

If surgery is considered necessary for the patient, whenever possible at least 7 days should elapse after the last dose of ketoconazole before surgery is performed. Treatment may be restarted after adequate wound healing.

No concomitant use of the following drugs is allowed: phenytoin, carbamazepine, rifampicin, barbiturates, or St John's wort as these drugs induce CYP3A4 and may alter levels of ketoconazole and/or calcitriol.

In addition patients cannot receive the following agents while on study since ketoconazole is known to impair the metabolism of these drugs. Patients on these agents may enter trial if the agent of concern can be discontinued or an alternative drug can be utilized: alfentanil, alfuzosin, almotriptan, alprazolam, amiodarone, amitriptyline, amprenavir, aprepitant, aripiprazole, bepridil, bortezomib, bosentan, budesonide, buprenorphine, buspirone, carbamazepine, cilostazol, cisapride, cyclosporine, delaviridine, didanosine, digoxin, disopyramidedofetilide, donepezil, eletriptan, elperenone, fluticasone, fosamprenavir, galantamine, systemic griseofulvins, indinavir, levobupivacaine, lopinavir, midazolam, mifepristone, modafinil, nateglinide, nefazadone, nelfinavir, oxcarbazepine, pimozide, quetiapine, quinidine, repaglinide, rifabutin, rifampins, rifapentine, ritonavir, saquinavir, sildenafil, sirolimus, tacrolimus, tadalafil, tolterodine, theophyllines, tolterodine, trazolam, valdecoxib, vardenafil, ziprasidone, zanisamide

Ketoconazole requires acidity for dissolution and absorption. Patients taking antacid should wait 2 hours before taking ketoconazole. Patients may not take a proton pump inhibitor or H2 blockers while on ketoconazole.

Patients receiving oral hypoglycemic agents should be monitored carefully for hypoglycemia since the clearance of these agents may be reduced by ketoconazole.

International normalized ratio (INR) elevations, and/or bleeding events have been reported in some patients taking warfarin. Patients taking warfarin should be monitored regularly for changes in prothrombin time or INR.

5.6 Skin Adverse Events

A variety of agents can be used to manage skin rashes. These include mild-to-moderate strength steroid creams, topical or systemic antibiotics, topical or systemic antihistamines, and occasionally retinoid creams.

5.7 Hypercalcemia

A possible side effect of calcitriol is hypercalcemia; therefore all calcium levels used for the determination of toxicity will be corrected for serum albuim levels using the following formula:

$$Ca^{+2}_{cor} = Ca^{+2}_{ser} + (0.8)(4.0 - Alb_{ser})$$

Patients developing:

a) Corrected serum calcium $\geq$12 mg/dl persisting $\geq$7 days or
b) Any corrected calcium $\geq$13 mg/dl with symptoms secondary to hypercalcemia or
c) Any corrected serum calcium $\geq$14 mg/dl

Calcitriol will be stopped and calcium level will be repeated in 1 week. Treatment will resume when calcium level returns to $\leq$ 10.5 mg/dl. Recovery from hypercalcemia must occur within 2

weeks to continuation on study. If this toxicity recurs, the patient will be taken off study. Further therapy off study would then be at the discretion of the treating physician.

5.8 Renal Dysfunction

In the event where creatinine levels increase ≥2 mg/dL, calcitriol therapy will be withheld until creatinine decreases <2.0 mg/dL. Sustained increases (>72 hours) in creatinine of ≥2.5mg/dL will constitute a DLT and will result in removal from study even in the absence of hypercalcemia

5.9 Other Concomitant Therapy with Calcitriol

Caution should be exercised when calcitriol is co-administered with calcium supplements as this might enhance hypercalcemia. All patients will be instructed to discontinue calcium supplementation. Thiazides decrease urinary excretion of calcium and will be a contraindication to this treatment.

Since hypercalcemia increases the risk of arrhythmias patients on digoxin will not be allowed on study.

5.10 Criteria for Discontinuation

Patients may be discontinued from trial treatment and assessments at any time at the discretion of the investigator(s). Specific reasons for discontinuing a patient from this trial are the following:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AE
- Patient develops kidney stones
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

PSA progression is an increase of 50% above the nadir at a minimum of 5 ng/mL. (i.e. PSA must rise a minimum of 5 units)

If that criterion is reached patients will be declared to have PSA Progressive Disease (PD), **but**:

a. If radiographs do not confirm PD
b. PSA and symptoms are stable or improved from baseline
c. Patient is in agreement

Investigator will have the discretion to continue investigational therapy until:

PSA achieves a level of >50% of baseline
or
Radiographic or symptomatic PD
or
Patient wish to discontinue

The two options for categorizing withdrawal are either progressive disease (PD) or an AE (>1AE may be documented as a reason for withdrawal). Only 1 event will be captured as the cause of death. Note that death is an outcome and not an AE.

All trial treatment-related toxicities and SAEs must be followed up until resolution.

All patients who have new or worsening CTC Grade 3 or 4 laboratory values at the time of withdrawal must have further tests performed, and the results must be recorded appropriately. Until the laboratory values have returned to CTC Grade 1 or 2, unless these values are not likely to improve because of the underlying disease. In these cases, the investigators must record their opinions in the patients' medical records. Laboratory abnormalities should **not** be reported as AEs unless any criterion for a SAE is fulfilled, the laboratory abnormality causes the patient to discontinue from the study, or the investigator insists the abnormality should be reported as an AE.

At withdrawal all ongoing study-related toxicities and SAEs must be followed until resolution, unless in the investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease.

After withdrawal from treatment, patients must be followed up for existing AEs for 30 calendar days after administration of the last dose of trial drug. All AEs occurring during that period must be reported to the U.S. Food and Drug Administration and must be followed up until resolved, unless in the investigator's opinion the condition is unlikely to resolve because of the patient's underlying disease.

6.0 AGENT FORMULATION AND PROCUREMENT

6.1 Ketoconazole

Ketoconazole will be obtained from commercial sources and provided by third party or patient resources.

Ketoconazole Formulation

Treatment	Strength	Description	Daily dose	Tablets per dose
Ketoconazole	200-mg tablet	Ketoconazole is a white to slightly beige, odorless powder, soluble in acids, with a molecular weight of 531.44.	1200 mg	2 tablets TID

Ketoconazole is available under the brand name Nizoral^{R}. It is available generically as ketoconazole.

Ketoconazole treatment will be taken 3 times per day (e.g. with meals), every day about the same time. It can be taken with or without food. If a patient forgets to take a dose, he should take the last missed dose as soon as they remember. However, do not double dose if it is time for the next dose.

6.1.1 Storage
Ketoconazole should be stored at room temperature between 15 and 30□C (59 and 86□F). Protect from moisture.

6.2 Calcitriol
Calcitriol will be provided to the study subjects by study staff. 0.5 mcg caplets will be employed. Our ISCI investigational pharmacist will order the calcitriol and distribute it as investigational for this study. The pharmacist will follow all GCP for tracking, maintaining and distributing the calcitriol as investigational in this study.

6.2.1 Storage
Please refer to the Complete Product Information.

6.3 Hydrocortisone
Hydrocortisone is available commercially in 5, 10, 25 and 50 mg tablets and will be obtained from commercial sources and provided by third party or patient resources

Please refer to the complete product information.

6.4 Expected Toxicities

6.4.1 Calcitriol
Commonly seen side effects with high dose calcitriol include hypercalcemia, hypercalciuria, and hyperphosphatemia. Clinical toxicity has been mostly linked to the level and duration of associated hypercalcemia. Early signs of hypercalcemia include weakness, headache, somnolence, nausea, vomiting, dry mouth, constipation, muscle pain, bone pain, metallic taste, and anorexia.

Late signs include polyuria, polydipsia, anorexia, weight loss, nocturia, conjunctivitis (calcific), pancreatitis, photophobia, rhinorrhea, pruritis, hyperthermia, decreased libido, elevated blood urea nitrogen (BUN), albuminuria, hypercholesterolemia, elevated aspartate transaminase (AST) and alanine transaminase (ALT), ectopic calcification, nephrocalcinosis, hypertension, cardiac arrhythmias, dystrophy, sensory disturbances, dehydration, apathy, arrested growth, urinary tract infections, and, rarely, overt psychosis. Chronic hypercalcemia has also been associated with an increase in creatinine levels in patients with normal baseline renal function.

Hypersensitivity reactions to calcitriol are rare but have been reported. One case of allergic reaction and another case of erythema multiforme have been confirmed by rechallenge (Rocaltrol® Complete Product Information).

6.4.2 Ketoconazole
Ketoconazole is one of a series of imidazoles which are active antifungal agents; ketoconazole at a dose of 200mg daily is widely used in the therapy of cutaneous, esophageal and urinary tract fungal infections and at 400mg TID is frequently prescribed in the therapy of androgen independent prostate cancer (with glucocorticoids to prevent adrenal insufficiency)

Ketoconazole mechanism of antifungal and perhaps antitumor action depends on its ability to inhibit numerous cytochrome P450 enzymes, disrupting membrane lipid structure as well as modifying the activity of a number of other intracellular compounds: vitamin D, retinoids. Ketoconazole has demonstrated a broad range of antitumor effects in preclinical systems (in vitro), but has not been widely tested in the active therapy of any cancer other than prostate cancer, where its activity was originally believed to depend on disruption of androgen signaling. Ketoconazole is generally a well tolerated agent. The major toxicities are GI (nausea, vomiting, hepatotoxicity) constitutional toxicities (headache, dizziness, rash, lethargy, dry skin, pruritis, somnolence, diarrhea) and rare myelosuppression and anaphylaxis.

6.4.3 Hydrocortisone

Adverse reactions to hydrocortisone are those associated with glucocorticoid and mineralocorticoid use. Most occur with prolonged and high dose therapy and are somewhat reduced in patients receiving alternate day therapy where suppression of endogenous adrenal steroid production is reduced. The hydrocortisone dose in this trial is intended as glucocorticoid replacement and is anticipated to have limited potential to cause any of the following side effects: electrolyte disturbances, fluid retention, and exacerbation of congestive heart failure increased risk of infection (esp. mucocutaneous candidiasis), polyuria, fluid retention, “moon faces”, centripetal obesity, mood alterations (depression or anxiety). Long term use may cause myopathy, osteoporosis, impaired wound healing, fragile skin, and Cushingoid habitus. Patients with mild glucose intolerance may develop frank diabetes, and glaucoma can be precipitated in patients with increased intraocular pressure. Mood disturbances and increased appetite are also commonly reported. This drug must not be abruptly discontinued following prolonged usage since adrenal insufficiency is common.

7.0 STUDY CALENDAR

Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy.
Radiographs must be done within 6 weeks of entering the study.

		Cycle 1				Cycle 2				Cycle 3				Cycles 4+	
	Pre-Study (-28 to -1 days)	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**Wk 6**	**Wk 7**	**Wk 8**	**Wk 9**	**Wk 10**	**Wk 11**	**Wk 12**		**Off Study**
Ketoconazole		X	X	X	X	X	X	X	X	X	X	X	X	Weekly	
Calcitriol		X	X	X	X	X	X	X	X	X	X	X	X	Weekly	
Hydrocortisone		X	X	X	X	X	X	X	X	X	X	X	X	Weekly	
Informed consent	X														
Demographics	X														
Concurrent meds	X					X				X				Every 4 wks	
Medical history	X													Every 4 wks	X
Physical exam, Vital Signs, Weight	X					X				X				Every 4 wks	X
CBC w/diff, plts	X					X				X				Every 4 wks	X
Serum chemistry[a]	X					X				X				Every 4 wks	X
Adverse event evaluation						X				X				Every 4 wks	
Performance status	X					X				X				Every 4 wks	X
PSA	X					X				X				Every 4 wks	
Tumor measurements	X	Tumor measurements are repeated every 4-12 weeks (measurable disease). Documentation (radiologic) must be provided for patients removed from study for progressive disease.													X[c]
Radiologic evaluation [b]	X	Radiologic measurements should be performed every 12 weeks from the start of calcitriol													X[c]

Ketoconazole: 400 mg given TID starting D 1 (except if participating in the PK portion begin day 4)
Calcitriol: 10 mcg; Calcitriol will be given for 3 consecutive days starting the 1st day of each week, day 1,2,3,8,9,10,15,16,17
Hydrocortisone: will be given 20mg AM, 10mg PM in the evening starting the day before the first dose of calcitriol

a-–Alkaline phosphatase, total bilirubin, LDH, total protein, SGOT(AST), SGPT(ALT) will be obtained monthly. Albumin, Bicarbonate, BUN, calcium, chloride, creatinine, glucose, total protein, and phosphorous will be obtained monthly.

b- Every12 weeks in patients with measurable disease. Every 12 weeks in the absence of measurable disease.

c- If partial or complete response are assessed, repeat in 1 month for confirmation. Longer intervals as determined by the study protocol may also be appropriate.

8.0 MEASUREMENT OF EFFECT

8.1 RECIST

The modified Response Evaluation Criteria in Solid Tumors (RECIST) and Recommended NCI PSA Response Criteria will be used for this trial for objective tumor response assessment. The primary response objective of this trial is the PSA response rate. In patients with measurable disease CR and PR will be assessed by RECIST criteria. Measurable response is a seconary objective of the phase II portion of this study.

Overall responses for all possible combinations of tumor responses in target and nontarget lesions with or without the appearance of new lesions*

Target lesions	Nontarget lesions	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; and PD = progressive disease. See Appendix B for more details.

Responses should be confirmed at a follow-up visit at least 4 weeks later

8.2 PSA Response

PSA will be used as an indicator of response in all disease categories.
Changes in PSA will be assessed utilizing the following guidelines:

Assessment of response requires two PSA values ≥4 weeks apart.
PSA must be assessed >4 weeks after prostatic biopsy.

Complete response:
Normalization (< 4 ng/ml (non prostatectomy) < 0.2ng/ml (prostatectomy) of PSA on three successive determinations.

Partial response:
>50% decrease in PSA, persistent for ≥4 weeks and without clinical or radiological evidence of disease progression during this time period.

Progression: Increase in PSA to ≥50% above baseline, if maintained ≥4 weeks.

Relapse from response or progression:
>50% increase above the nadir at a minimum of 5 ng/mL (i.e. PSA must rise a minimum of 5 units).

If that criterion is reached patients will be declared to have PSA Progressive Disease (PD), **but**:

a. If radiographs do not confirm PD
b. PSA and symptoms are stable or improved from baseline
c. Patient is in agreement

Investigator will have the discretion to continue investigational therapy until:

PSA achieves a level of >50% of baseline
or
Radiographic or symptomatic PD
or
Patient wish to discontinue

9.0 SAFETY EVALUATION

9.1 Adverse Events

9.1.1 Definitions

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

9.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

9.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

11.1.1.3 Abnormal Normal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

11.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

9.1.2 Grading and Relationship to drug

The descriptions and grading scales found in the CTEP Version 4.3of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 3 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 3.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs administered to the participant.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions or concomitant drugs.
- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the participant's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

9.1.3 Reporting Adverse Events

Table 1 Guidelines for Routine Adverse Event Reporting for Phase 1 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Table 2 Guidelines for Routine Adverse Event Reporting for Pilot, Phase 2, and Phase 3 Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible, will be reported. New information will be reported after it is received.

9.2 Serious Adverse Events

11.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participant, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does **NOT** include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.1.4 Reporting Serious Adverse Events

All new SAEs occurring from the date the participant signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. During the screening period, only SAEs related to study procedures need to be reported. The SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAEs occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention should be reported.

SAEs identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 10.0 for details on reporting Unanticipated Problems

9.1.5 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.0 Unanticipated Problems

10.1.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  b) The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed serious

10.1.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Unanticipated Problem Form will be submitted to the CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

11.0 DATA SAFETY AND MONITORING

The Principal Investigator, Sub-Investigator, and Clinical Research Coordinators shall meet on a monthly basis to review toxicities and follow up on results of patients enrolled on the study.

The adequacy of the data and safety monitoring plan will be reviewed by the Inova Schar Cancer Institute's Protocol Review and Monitoring Committee (PRMC). The PRMC shall also determine the risk level of the proposed trial, which in turn determines the frequency of review by the Data and Safety Monitoring Committee (DSMC).

All Severe Adverse Events (SAEs) are required to be reported to the appropriate Institutional Review Board (IRB). Based on SAEs, the IRB retains the authority to close the study to further accrual pending more detailed reporting and/or modifications to further reduce risk and maximize the safety of participating patients.

Progress on the trial and the toxicities experienced will be reviewed by the DSMC. The DSMC shall review trial progress, protocol adherence, data management, and patient safety parameters, including rates of adverse events and serious adverse events, on a scheduled determined by the trial's risk level. They shall also include reviews performed by selected reviewers and auditors. Results from these DSMC reviews and the Committee's recommendations shall be forward to the Principal Investigator, the Research Oversight Committee, and the appropriate Institutional Review Board.

If the DSMC recommends a study change for patient safety or efficacy reasons, or that accrual be halted, the PI will be notified immediately, as will the PRMC and IRB.

In the unlikely event that the trial PI does not concur with the DSMC, the ISCI CEO/ Director must be informed of the reason for the disagreement. The trial PI, DSMC Chair, and ISCI Director will be responsible for reaching a mutually acceptable decision about the study. Confidentiality must be preserved during these discussions. However, in some cases, relevant data may be shared with other selected trial investigators and staff to seek advice to assist in reaching a mutually acceptable decision.

12.0 STATISTICAL CONSIDERATIONS

It is highly likely that the antitumor activity of ketoconazole and abiraterone in prostate cancer depend substantially on the ability of both agents to inhibit CYP 17A1 and disrupt androgen synthesis. We and others have shown that antitumor effects of abiraterone are substantially reduced in patients in whom ketoconazole has previously failed to be effective. In addition, enzalutamide, whose mechanism is not CYP17A1 dependent, does inhibit CRPC through disruption of androgen signalingThere are essentially no data regarding the success of ketoconazole therapy after abiraterone "failure". If ketoconazole and abiraterone share the same mechanism of action one would expect the same low PSA response rate in men with CRPC who receive (I) ketoconazole → abiraterone and those who receive (II) abiraterone → ketoconazole. If the response rate among men treated with sequence (II) substantially exceeds the PSA RR of 10% we saw with sequence (I), this would suggest additional effects of ketoconazole. Further, the hypothesis of RPCI I68905 is that ketoconazole also inhibits CYP24A1, the predominant vitamin D metabolizing enzyme and hence potentiates the antitumor effect of calcitriol (1,25 dihydroxycholecalciferol, 1,25 vitamin D_3) - in a synergistic manner.

This study proposes to treat men in whom abiraterone has proven to be ineffective within 12 months of entry onto this portion of this study. Based on the above, a PSA response rate of 10% or less for this regimen following abiraterone failure would be considered to have too little activity to be of clinical significance in this patient population, whereas a response rate of 40% or more would be of interest. We utilize a flexible design (Chen TT, NG T. Optimal flexible designs in phase II clinical trials. *Stat Med.* 1998;17:2301-2312.) that permits the actual number of evaluable patients in each stage to vary slightly in order to accommodate the challenges of accruing precise numbers of evaluable patients. In the first stage, we target an accrual of 9 eligible and evaluable patients but permit accrual to range from 7 to 11 patients. If more than 0 patients respond and medical judgment indicates, accrual to the second stage of the trial will be initiated; otherwise, the study will be stopped and the treatment regimen will be classified as clinically uninteresting. If the study advances to the second stage, then an overall study accrual of 15 eligible and evaluable patients will be targeted but will be permitted to range from 13 to 17 patients. If more than 2 out of 13-14 patients respond or 3 out of 15-17 patients respond then the regimen will be considered worthy for additional investigation. If the true response rate is 10% (H_0), these decision rules limit the average probability of designating the treatment as active to 10% and the average probability of stopping after completing only the first stage of accrual is 39%. On the other hand, if the true response rate is 40% (H_1) then the average probability of correctly classifying the treatment as active is 93%. These are average probabilities computed from the individual probabilities averaged over all permitted accrual combinations and assuming each combination is equally likely. Limited investigations have indicated that the type I and type II errors are fairly insensitive to variations in the true probability distribution of accrual combinations.

Stage of Accrual	Targeted Cumulative Accrual	Limits of Actual Accrual	Max number of responses to reject H_1
1	9	7-11	0/(7-11)
2	15	13-17	2/(13-14), 3/(15-17)

Entry criteria for this addendum phase will be identical to the ongoing trial except that progressive disease will be required to have occurred on abiraterone within the prior 12 months. Patient may also not have received enzalutamide in order to, as much as possible, “standardize” prior manipulation of the androgen axis.

12.1 Stratification Factors

No stratifications are planned in either phase of the study.

12.2 Analysis of Secondary Endpoints

Toxicity will be graded according to the NCI CTC version 4.03 and relationship to study drug will be assessed. Toxicities will be tabulated by dose of calcitriol. All patients who receive study drug will be considered evaluable for toxicity.

Objective tumor response will be judged by monthly physical exam and radiographic evaluation which will be done q12 weeks. Patients will be considered evaluable for tumor response if they have at least two post-baseline tumor assessments at least 4 weeks apart, received study medication for 8 weeks or if they have evidence of disease progression. Analysis of the correlative studies in section 8.0 will be descriptive.

13.0 TRIAL MANAGEMENT

13.1 Audits and Inspections

Audits and data management will be overseen by the ISCI CTO

13.2 Changes to the Protocol

If it is necessary for the trial protocol to be amended, the amendment or a new version of the trial protocol must be notified to or approved by the IRB. If a protocol amendment requires a change to theInformed Consent Form the IRB will be notified. Approval of the revised Written Informed Consent Form by the IRB is required before the revised form is used.

13.3 Ethics

The principal investigator is responsible for providing the IRB with reports of any SAEs from any other trial conducted with the investigational product. The trial will be performed in accordance with Good Clinical Practice.

Amendments, reporting deviations, AE reports will be reported to the Institutional Review Board.

13.4 Emergency Procedures

13.4.1 Procedures in Case of a Medical Emergency

The principal investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study.

13.4.2 Procedures in Case of Pregnancy

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

13.5 Data Accessibility

Data are stored in locked files in Clinical Research Services (CRS), access to which is limited to authorized CRS personnel only. Research data are also maintained in the ISCI clinical trials data base and secured, password protected and not connected to a network.

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APPENDIX A

Performance Status Criteria

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

APPENDIX B

Response Criteria

1 Introduction

The introduction explores the definitions, assumptions, and purposes of tumor response criteria**.** Below, guidelines that are offered may lead to more uniform reporting of outcomes of clinical trials. Note that, although single investigational agents are discussed, the principles are the same for drug combinations, non-investigational agents, or approaches that do not involve drugs.

Tumor response associated with the administration of anticancer agents can be evaluated for at least three important purposes that are conceptually distinct:

- Tumor response as a prospective end point in early clinical trials. In this situation, objective tumor response is employed to determine whether the agent/regimen demonstrates sufficiently encouraging results to warrant further testing. These trials are typically Phase II trials of investigational agents/regimens (see Section 1.2), and it is for use in this precise context that these guidelines have been developed.

- Tumor response as a prospective end point in more definitive clinical trials designed to provide an estimate of benefit for a specific cohort of patients. These trials are often randomized comparative trials or single-arm comparisons of combinations of agents with historical control subjects. In this setting, objective tumor response is used as a surrogate end point for other measures of clinical benefit, including time to event (death or disease progression) and symptom control (see Section 1.3).

- Tumor response as a guide for the clinician and patient or study subject in decisions about continuation of current therapy. This purpose is applicable both to clinical trials and to routine practice (see Section 1.1), but use in the context of decisions regarding continuation of therapy is not the primary focus of this document.

However, in day-to-day usage, the distinction among these uses of the term "tumor response" can easily be missed, unless an effort is made to be explicit. When these differences are ignored, inappropriate methodology may be used and incorrect conclusions may result.

1.1 Response outcomes in daily clinical practice of oncology

The evaluation of tumor response in the daily clinical practice of oncology may not be performed according to predefined criteria. It may, rather, be based on a subjective medical judgment that results from clinical and laboratory data that are used to assess the treatment benefit for the patient. The defined criteria developed further in this document are not necessarily applicable or complete in such a context. It might be appropriate to

make a distinction between "clinical improvement" and "objective tumor response" in routine patient management outside the context of a clinical trial.

1.2 Response outcomes in uncontrolled trials as a guide to further testing of a new therapy

"Observed response rate" is often employed in single-arm studies as a "screen" for new anticancer agents that warrant further testing. Related outcomes, such as response duration or proportion of patients with complete response, are sometimes employed in a similar fashion. The utilization of a response rate in this way is not encumbered by an implied assumption about the therapeutic benefit of such responses but rather implies some degree of biologic antitumor activity of the investigated agent.

For certain types of agents (ie, cytotoxic drugs and hormones), experience has demonstrated that objective antitumor responses observed at a rate higher than would have been expected to occur spontaneously can be useful in selecting anticancer agents for further study. Some agents selected in this way have eventually proven to be clinically useful. Furthermore, criteria for "screening" new agents in this way can be modified by accumulated experience and eventually validated in terms of the efficiency by which agents so screened are shown to be of clinical value by later, more definitive, trials.

In most circumstances, however, a new agent achieving a response rate determined *a priori* to be sufficiently interesting to warrant further testing may not prove to be an effective treatment for the studied disease in subsequent randomized Phase III trials. Random variables and selection biases, both known and unknown, can have an overwhelming effect in small, uncontrolled trials. These trials are an efficient and economic step for initial evaluation of the activity of a new agent or combination in a given disease setting. However, many such trials are performed, and the proportion that will provide false-positive results is necessarily substantial. In many circumstances, it would be appropriate to perform a second small confirmatory trial before initiating large resource-intensive Phase III trials.

Sometimes, several new therapeutic approaches are studied in a randomized Phase II trial. The purpose of randomization in this setting, as in Phase III studies, is to minimize the impact of random imbalances in prognostic variables. However, randomized Phase II studies are, by definition, not intended to provide an adequately powered comparison between arms (regimens). Rather, the goal is simply to identify one or more arms for further testing, and the sample size is chosen so to provide reasonable confidence that a truly inferior arm is not likely to be selected. Therefore, reporting the results of such randomized Phase II trials should not imply statistical comparisons between treatment arms.

1.3 Response outcomes in clinical trials as a surrogate for palliative effect

1.3.1 Use in nonrandomized clinical trials

The only circumstance in which objective responses in a nonrandomized trial can permit a tentative assumption of a palliative effect (ie, beyond a purely clinical measure of benefit)

is when there is an actual or implied comparison with historical series of similar patients. This assumption is strongest when the prospectively determined statistical analysis plan provides for matching of relevant prognostic variables between case subjects and a defined series of control subjects. Otherwise, there must be, at the very least, prospectively determined statistical criteria that provide a very strong justification for assumptions about the response rate that would have been expected in the appropriate "control" population (untreated or treated with conventional therapy, as fits the clinical setting). However, even under these circumstances, a high rate of observed objective response does not constitute proof or confirmation of clinical therapeutic benefit. Because of unavoidable and non-quantifiable biases inherent in nonrandomized trials, proof of benefit still requires eventual confirmation in a prospectively randomized, controlled trial of adequate size. The appropriate end points of therapeutic benefit for such a trial are survival, progression-free survival, or symptom control (including quality of life).

1.3.2 Use in randomized trials

Even in the context of prospectively randomized Phase III comparative trials, "observed response rate" should not be the sole, or major, end point. The trial should be large enough that differences in response rate can be validated by association with more definitive end points reflecting therapeutic benefit, such as survival, progression-free survival, reduction in symptoms, or improvement (or maintenance) of quality of life.

2 MEASURABILITY OF TUMOR LESIONS AT BASELINE

2.1 Definitions

At baseline, tumor lesions will be categorized as follows: measurable (lesions that can be accurately measured in at least one dimension [longest diameter to be recorded] as 20 mm with conventional techniques or as 10 mm with spiral CT scan [*see* Section 2.2]) or nonmeasurable (all other lesions, including small lesions [longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan] and truly non-measurable lesions).

The term "evaluable" in reference to measurability is not recommended and will not be used because it does not provide additional meaning or accuracy.

All measurements should be recorded in metric notation by use of a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of treatment.

Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

(*Note:* Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable, and the conditions under which such lesions should be considered must be defined in the protocol when appropriate.)

2.2 Specifications by methods of measurements

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

2.2.1 Clinical examination

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

2.2.2 Chest x-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

2.2.3 Computed tomography (CT) and magnetic resonance imaging (MRI)

CT and MRI are the best currently available and most reproducible methods for measuring target lesions selected for response assessment. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed by use of a 5-mm contiguous reconstruction algorithm; this specification applies to the tumors of the chest, abdomen, and pelvis, while head and neck tumors and those of the extremities usually require specific protocols.

2.2.4 Ultrasound

When the primary end point of the study is objective response evaluation, ultrasound should not be used to measure tumor lesions that are clinically not easily accessible. It may be used as a possible alternative to clinical measurements for superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. Ultrasound might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

2.2.5 Endoscopy and laparoscopy

The utilization of these techniques for objective tumor evaluation has not yet been fully or widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may be available only in some centers. Therefore, utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete histopathologic response when biopsy specimens are obtained.

2.2.6 Tumor markers

PSA will be used as a measure of response in this trial.
PSA will be used as an indicator of response in all disease categories.
Changes in PSA will be assessed utilizing the following guidelines:

Assessment of response requires two PSA values ≥4 weeks apart.
PSA must be assessed >4 weeks after prostatic biopsy.

Complete response:
Normalization (<4 ng/ml [non prostatectomy], <0.2ng/ml [Prostatectomy] of PSA on three successive determinations.

Partial response:
>50% decrease in PSA, persistent for ≥4 weeks.

Progression:
Increase in PSA to ≥50% above baseline, if maintained ≥4 weeks.

Relapse from response or progression:
PSA progression is an increase of 50% above the nadir at a minimum of 5 ng/mL (i.e. PSA must rise a minimum of 5 units)

If that criterion is reached patients will be declared to have PSA Progressive Disease (PD), **but**:

a. If radiographs do not confirm PD
b. PSA and symptoms are stable or improved from baseline
c. Patient is in agreement

Investigator will have the discretion to continue investigational therapy until:
PSA achieves a level of >50% of baseline
or
Radiographic or symptomatic PD
or
Patient wish to discontinue

Progression of disease will be defined as any progression as assessed by criteria for measurable disease, evaluable disease, osseous disease, or performance status.

2.2.7 Cytology and histology

Cytologic and histologic techniques can be used to differentiate between partial response and complete response in rare cases (eg, after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors). Cytologic confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required when the measurable tumor has met criteria for response or stable disease. Under such circumstances, the cytologic examination of the fluid collected will permit differentiation between response or stable disease (an effusion may

be a side effect of the treatment) and progressive disease (if the neoplastic origin of the fluid is confirmed). New techniques designed to better establish objective tumor response will be integrated into these criteria when they are fully validated; they will be used in the context of tumor response evaluation.

3 TUMOR RESPONSE EVALUATIONS

3.1 Baseline evaluation

3.1.1 Assessment of overall tumor burden and measurable disease

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary end point. Measurable disease is defined by the presence of at least one measurable lesion (as defined in Section 2.1). If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

3.1.2 Baseline documentation of "target" and "nontarget" lesions

All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum longest diameter. The baseline sum of the longest diameter will be used as the reference by which to characterize the objective tumor response.

All other lesions (or sites of disease) should be identified as nontarget lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

3.2 Response criteria

3.2.1 Evaluation of target lesions

This section provides the definitions of the criteria used to determine objective tumor response for target lesions. The criteria have been adapted from the original WHO Handbook, taking into account the measurement of the longest diameter only for all target lesions: complete response—the disappearance of all target lesions; partial response—at least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter; progressive disease—at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions; stable disease—neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started.

3.2.2 Evaluation of nontarget lesions

This section provides the definitions of the criteria used to determine the objective tumor response for nontarget lesions: complete response—the disappearance of all nontarget lesions and normalization of tumor marker level; incomplete response/stable disease—the persistence of one or more nontarget lesion(s) and/or the maintenance of tumor marker level above the normal limits; and progressive disease—the appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions.
(*Note:* Although a clear progression of "nontarget" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later by the review panel [or study chair]).

3.2.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (*see* Section 3.3.1). Table 1 provides overall responses for all possible combinations of tumor responses in target and nontarget lesions with or without the appearance of new lesions.
Notes:

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

- Conditions that may define early progression, early death, and nonevaluability are study specific and should be clearly defined in each protocol (depending on treatment duration and treatment periodicity).

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

3.2.4 Frequency of tumor re-evaluation

Frequency of tumor re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of Phase II studies where the beneficial effect of therapy is not known, follow-up of every other cycle (ie, 6-8 weeks) seems reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances.
After the end of the treatment, the need for repetitive tumor evaluations depends on whether the Phase II trial has, as a goal, the response rate or the time to an event (disease progression/death). If time to an event is the main end point of the study, then routine re-evaluation is warranted of those patients who went off the study for reasons other than the

expected event at frequencies to be determined by the protocol. Intervals between evaluations twice as long as on study are often used, but no strict rule can be made.

Table 1 Overall responses for all possible combinations of tumor responses in target and nontarget lesions with or without the appearance of new lesions*

Target lesions	Nontarget lesions	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; and PD = progressive disease. *See* text for more details.

3.3 Confirmatory measurement/duration of response

3.3.1 Confirmation

The main goal of confirmation of objective response in clinical trials is to avoid overestimating the response rate observed. This aspect of response evaluation is particularly important in nonrandomized trials where response is the primary end point. In this setting, to be assigned a status of partial response or complete response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol (*see* Section 3.3.3).

(*Note:* Repeat studies to confirm changes in tumor size may not always be feasible or may not be part of the standard practice in protocols where progression-free survival and overall survival are the key end points. In such cases, patients will not have "confirmed response." This distinction should be made clear when reporting the outcome of such studies.)

3.3.2 Duration of overall response

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

measurement criteria are first met for complete response until the first date that recurrent disease is objectively documented.

3.3.3 Duration of stable disease

Stable disease is measured from the start of the treatment until the response criteria for disease progression is met (taking as reference the smallest measurements recorded since the treatment started). The clinical relevance of the duration of stable disease varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of stable disease. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

(*Note:* The duration of response or stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency that should take into account many parameters, including disease types and stages, treatment periodicity, and standard practice. However, these limitations to the precision of the measured end point should be taken into account if comparisons among trials are to be made.)

3.4 Progression-free survival/time to progression

This document focuses primarily on the use of objective response end points. In some circumstances (eg, brain tumors or investigation of noncytoreductive anticancer agents), response evaluation may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases, progression-free survival/time to progression can be considered valuable alternatives to provide an initial estimate of biologic effect of new agents that may work by a noncytotoxic mechanism. It is clear though that, in an uncontrolled trial proposing to utilize progression-free survival/time to progression, it will be necessary to document with care the basis for estimating what magnitude of progression-free survival/time to progression would be expected in the absence of a treatment effect. It is also recommended that the analysis be quite conservative in recognition of the likelihood of confounding biases, e.g., with regard to selection and ascertainment. Uncontrolled trials using progression-free survival or time to progression as a primary end point should be considered on a case-by-case basis, and the methodology to be applied should be thoroughly described in the protocol.

4 RESPONSE REVIEW

For trials where the response rate is the primary end point, it is strongly recommended that all responses are reviewed by an expert or experts independent of the study at the study's completion. Simultaneous review of the patients' files and radiologic images is the best approach.

(*Note:* When a review of the radiologic images is to take place, it is also recommended that images be free of marks that might obscure the lesions or bias the evaluation of the reviewer[s]).

5 REPORTING OF RESULTS

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). (*Note:* By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.)

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients.

Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should be provided.