An Open-Label, Parallel, Phase II Study of Single-Agent Oral ESK981 in Men with Metastatic Castration-Resistant Prostate Cancer (mCRPC)

Prostate Cancer Clinical Trials Consortium, LLC (PCCTC)

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CONFIDENTIALITY STATEMENT

The information in this document is provided to you as an investigator, potential investigator, consultant, or contractor, for review by you, your staff, and the appropriate Institutional Review Board or Ethics Committee. By accepting this document, you agree that the information contained herein will not be disclosed to others without written authorization from the lead site/sponsor, except to the extent necessary to initiate the study or conduct study-related activities.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing Esanik Therapeutics with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted good clinical practice (GCP) principles and to abide by the terms of this protocol.

Principal Investigator Signature: _____

Principal Investigator Print: _____

Date: _____

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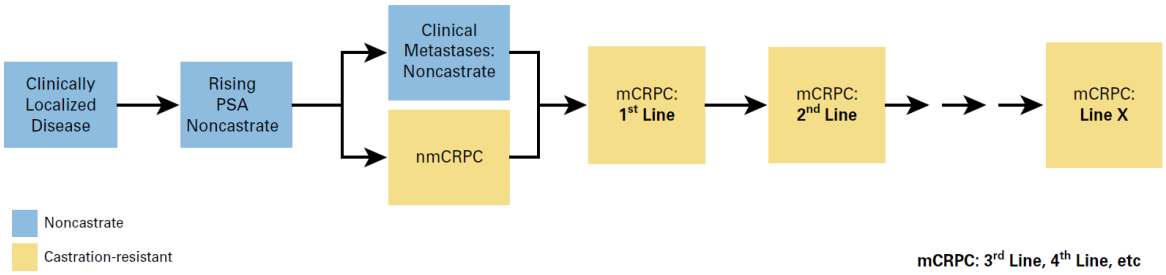
1. INTRODUCTION

1.1 Disease Background

Prostate cancer is the second leading cause of cancer deaths in men. According to American Cancer Society estimates, in 2016 as many as 180,890 American men will be diagnosed with prostate cancer, and nearly 26,120 will die of the disease.[2] Yet, localized prostate carcinoma is often curable, and even metastatic disease frequently responds to treatment.

The course of prostate cancer from diagnosis to death is best categorized as a series of clinical states defined by the status of the primary tumor, the presence or absence of distant disease (metastatic versus non-metastatic), testosterone levels, and the lines of therapy a patient has received (Figure 1).[3] These clinical states involve the complex interplay of a network of signaling molecules that collectively promote net cell proliferation relative to cell death. The clinical states model aligns with the indications and uses of currently approved drugs and provides a framework for a decision tree that closely follows contemporary clinical practice.

Figure 1. Clinical states of prostate cancer



Although advanced prostate cancer usually responds to therapies that suppress androgen-axis signaling, resistance inevitably develops, leading to the emergence of castration-resistant prostate cancer (CRPC). Importantly, the clinical efficacy of novel therapies targeting androgen receptor (AR) signaling, such as abiraterone and enzalutamide, have confirmed that most CRPC remains AR signaling intact.[4-6] Since resistance to these therapies inevitably develops, approaches to improve the duration of response and address the key pathways of resistance are very much needed.

Despite promising early phase clinical trial results,[7] a recently presented Phase III trial evaluating the multi-tyrosine kinase inhibitor (MTKI) cabozantinib in CRPC did not meet its primary survival endpoint.[8] Although the AR signaling status was unknown in trial participants, the majority of men with CRPC retain active AR signaling.[9,10] Our preclinical study provided a mechanistic basis and rationale of combining cabozantibib with enzalutamide to co-target the MET kinase and AR respectively and, furthermore, provided a potential explanation for the failure of cabozantinib monotherapy in the pivotal Phase III study.[11]

In a search for alternate MTKIs that could be re-positioned for the treatment of metastatic CRPC (mCRPC), we synthesized and evaluated ESK981 amongst a panel of other MTKIs. Interestingly, as our preliminary data will highlight, ESK981 exhibited nanomolar activity in the inhibition of prostate cancer cell line growth relative to other MTKIs tested including cabozantinib and crizotinib (both of which have been evaluated clinically in mCRPC). These early results piqued our further interest in deciphering the molecular basis for the log-fold increased sensitivity to ESK981 relative to other MTKIs in prostate cancer cells.

ESK981 is an orally active multiplex kinase inhibitor with favorable safety, pharmaceutical, ADME (absorption, distribution, metabolism, and excretion), and pharmacokinetic profiles. Clinical testing demonstrated linear dose-related oral exposure in humans with 8-15 h half-life and a high level of patients achieving stable disease at doses at or right below the expected Phase II dose. Our observation of a direct activity of ESK981 on prostate cancer cell autophagy coupled with its anti-angiogenesis activities provides a compelling rationale to investigate this MTKI in the treatment of mCRPC patients.

1.2 Treatment Background

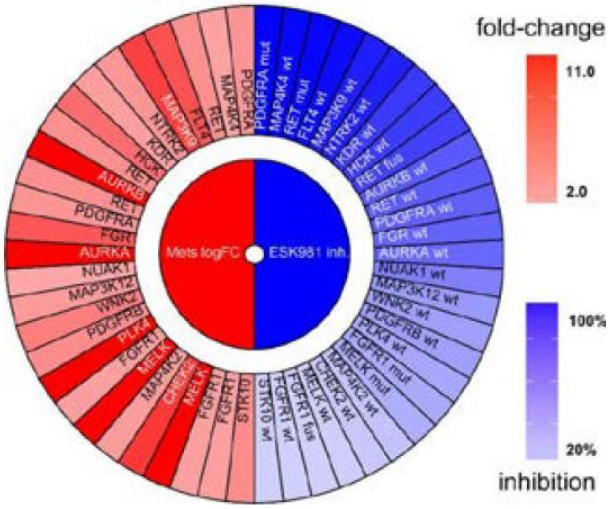


Figure 2. Kinases overexpressed in mCRPC (in red) and their *in vitro* inhibition with 25 nM ESK981 (blue).
Log-fold change of kinases in mCRPC relative to benign prostate assessed from a compendia of RNA-seq data. Rank ordered inhibition profile of 25 nM ESK981 determined in *in vitro* kinase assays. Intensity of color is associated with log-fold change or

ESK981, formerly known as CEP-11981, is a novel oral MTKI originally developed by Cephalon[12] and currently being developed by Esanik Therapeutics. The compound was initially identified as an oral angiogenesis inhibitor targeting several important pathways involved in the angiogenic response, but without the off-target activities of other TKIs, like sunitinib and sorafenib, resulting in adverse events. ESK981 has potent activity in kinases implicated in angiogenesis including vascular endothelial growth factor receptor 1 (VEGFR-1), VEGFR-2, and Tie-2, (IC50 of 3, 4, 22nM, respectively). ESK981 treatment potently inhibited HUVEC capillary tube formation and aortic ring explant angiogenesis *in vitro*, and inflammation-induced angiogenesis in Tucker mice and several VEGF-induced angiogenesis models *in vivo*. ESK981 was tested in a dose-escalating Phase I clinical trial to determine its pharmacokinetics and pharmacodynamics in patients with advanced, relapsed, or refractory solid tumors.[13] ESK981 was acceptably tolerated up to a dose of 97.4 mg/m^2 (maximum tolerated dose) that was determined to be the recommended Phase II dose. Most adverse events were Grade 1 or Grade 2 and included fatigue, nausea, diarrhea, decreased appetite, abdominal pain, back pain, vomiting, constipation, headache, dizziness, and dyspnea. Grade 3/4 neutropenia was observed in the highest-dose cohorts. More importantly, up to 85% of patients achieved stable disease when measured at ≥6 weeks in cohorts receiving ≥73.0 mg/m^2.

After Cephalon was acquired by TEVA, further development of the compound was halted due to TEVA's decision to discontinue internal oncology drug development and focus on other therapeutic areas. Due to the exciting data seen in pre-clinical prostate cancer models highlighted in this proposal, Esanik Therapeutics (in which Dr. Chinnaiyan serves on the Scientific Advisory Board) acquired the rights to develop ESK981 in prostate cancer and other indications. To better understand the mechanism of action in prostate cancer, the kinase screen was repeated with modern kinase assays and lower drug concentrations, identifying novel mutant oncogenic kinases inhibited by ESK981 at single digit nanomolar potency. The new screen also confirmed the potency of ESK981 against TKs involved in angiogenesis, as well as a number of other kinases involved in cancer processes including PDGFRA, PDGFRB, RET, FGFR1, FGFR2, AURKA, and AURKB, among others (IC50 of 43, 12, 9, 92, 80, 12, and 17nM, respectively). We carried out a preliminary analysis of the top kinases overexpressed (by RNA-seq) in mCRPC and kinases most inhibited by ESK981 to provide leads to identify the kinase target in mCRPC (Figure 2). Importantly, as our preliminary data will cover, ESK981, at nanomolar levels, induces robust vacuolization associated with activation of the autophagy pathway suggesting that a kinase involved in this pathway was being inhibited. Other MTKIs, such as cabozantib and crizotinib, did not have this autophagic vacuolization effect even at high micromolar concentrations. *In vitro* data also suggested combined effects with enzalutamide and continued sensitivity in enzalutamide-resistant cell lines. Interestingly, in addition to AR+ prostate cancer cell lines, AR- prostate cancer cell lines were similarly sensitive to ESK981 intimating its potential use in small cell/neuroendocrine variants of prostate cancer. *In vivo*, ESK981 monotherapy inhibited castrated VCaP xenograft growth in a dose-dependent fashion. Tumors from mice treated with ESK981 displayed vacuolization associated with autophagy as well as marked decreases in proliferation (by Ki67 staining).

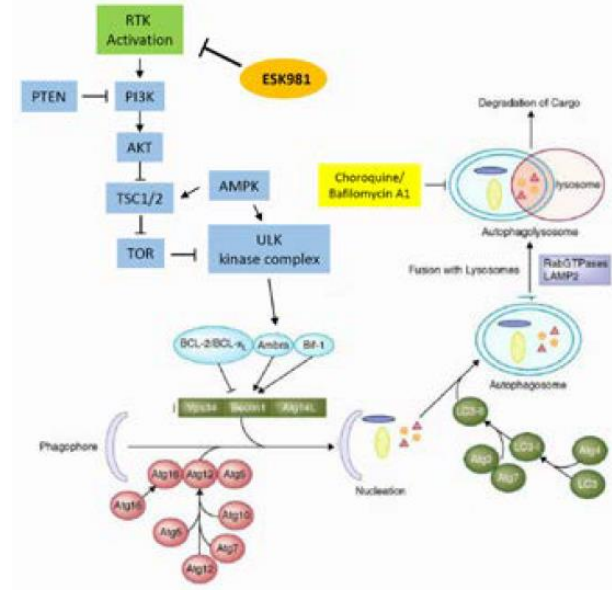


Figure 3. Overview of the autophagy pathway
Adapted from Yang et al.[1] The ULK kinase pathway is the key proximal kinase complex that controls autophagy. It can be regulated by a number of up-stream kinases including receptor tyrosine kinase (RTKs), PI3K, AKT, and TOR. The beclin1 complex is a mediator of autophagy. LC3 processing is a key step in autophagosome maturation. Chloroquine and Bafilomycin A1 block late stage autophagy by blocking lysosomal fusion. ESK981 presumably activates autophagy in CRPC by inhibiting an upstream TK.

Interestingly, autophagy is an evolutionarily conserved and orderly process of degradation and destruction of cellular components (Figure 3). As part of this process, double membraned vesicles known as autophagosomes are formed by engulfing cytoplasmic constituents, which then fuse with lysosomes to initiate degradation and recycling.[1] Exaggerated activation of the autophagic process can be observed as gross vacuolization of cells. Monoallelic loss of beclin-1, a gene critical for the autophagy pathway, has been found in 40 to 75% of human breast, prostate, and ovarian cancers.[1,14] Thus, autophagy has been considered an important tumor suppressive pathway and sustained activation of autophagy in tumor cells leads to apoptosis. A number of approaches to regulate the autophagy pathway are being explored clinically in the treatment of cancer.[1]

1.2.1 Description and mechanism of action

Nonclinical activity

ESK981 is an orally active inhibitor of a number of receptor tyrosine kinases, specifically human TIE-2, VEGFR-1 and VEGFR-2, and FGFR-1, with concentrations resulting in 50% inhibition of activity (IC_{50}) of 22±6 nM, 4±1 nM, 3±1 nM, and 13±2 nM, respectively, in enzyme-based assays. These kinase targets have been shown to have essential and nonredundant roles in tumor angiogenesis and vascular maintenance, and ESK981 is designed to inhibit tumor growth by blocking these processes. ESK981 shows comparable activity against a number of other kinases including tropomyosin receptor kinase (TRK) A (3 nM), TRKB (5 nM), receptor tyrosine kinase (RET) (5 nM), B lymphocyte kinase (BLK) (8 nM), hemopoietic cell kinase (HCK) (13 nM), lymphocyte-specific protein tyrosine kinase (LCK) (12 nM), TGF-ß activated kinase 1 (TAK1) (14 nM), and mammalian STE20-like kinase 2 (MST2) (21 nM). Some of these kinases are also known to be involved in tumor growth and survival. ESK981 demonstrates a significant concentration-related activity in a range of experimental systems including VEGF-A, -C, -D-induced, Angiopoietin-1-induced, and fibroblast growth factor-2 (FGF2)-induced human and murine endothelial cell proliferation, chemotaxis, migration, and survival *in vitro*; and microvessel outgrowth and branching in primary rat aortic ring explant cultures *ex vivo*.

Preclinical studies

Significant oral anti-angiogenic efficacy has been observed in VEGF-A-induced, tumor-induced and inflammation-induced neo-vascularization models in rodents. Sustained dose-related anti-tumor activity has been demonstrated with ESK981 *in vitro* across a panel of human and murine tumor cell lines. Significant dose-related and exposure driven *in vivo* anti-tumor efficacy is observed with ESK981 in multiple subcutaneous (melanoma, glioblastoma, breast carcinoma, prostate carcinoma) and orthotopic human and rodent solid (colon carcinoma, renal carcinoma, and glioblastoma) and hematologic (acute leukemia) tumor xenograft models in normal and immunocompromised hosts. Depending upon tumor models with differing dosing regimens, ESK981 exhibits dose-related tumor growth inhibitory and anti-angiogenic effects as well as sustained partial and complete tumor regressions when administered as monotherapy. Specific intermittent oral dosing schedules (drug dosing/drug holidays) of ESK981 using once daily (qd) and twice daily (bid) dosing regimens demonstrate significant anti-tumor efficacy (tumor growth inhibition and partial and complete regressions). In both solid and hematologic tumor models, however, qd and bid continuous administration are optimal. ESK981 is generally well tolerated when administered chronically with cytotoxic agents (e.g., temozolomide [TMZ]) and the combination of ESK981 with TMZ conferred a significant benefit on median survival of orthotopic human glioblastoma-bearing animals relative to that achieved with TMZ alone. In a series of pharmacologic anti-tumor and anti-angiogenic studies conducted in various murine strains, the maximum tolerated dose of ESK981 is 60 mg/kg/day or higher in syngeneic, nude, and severe immunocompromised mice (SCID) mice, and 30 mg/kg/day or higher in nonobese diabetic/severe immunocompromised mice (NOD-SCID) severely immunocompromised tumor-bearing mice.

Phase I open-label, dose-escalating study pharmacokinetics

ESK981 was tested in an open-label dose-escalating Phase 1 clinical trial to determine its pharmacokinetics (PK) and pharmacodynamics (PD) in patients with advanced, relapsed, or refractory solid tumors (Study C11981/1047/ON/US, NCT00875264).[13] After daily oral

administration of ESK981 to cancer patients, the median t_{max} values for ESK981 were typically in the range of 2 to 4 hr. (Table 1 and Table *2*); however, for most of the dose groups there appeared to be considerable inter-patient variability in this pharmacokinetic parameter. The reason(s) for the large inter-patient variability in t_{max} is (are) not presently known, but may be related to the fed/fasted state of the patients or other unknown factors which could potentially influence drug absorption (e.g., disease state, concomitant medications).

Table 1. Mean (SD) Pharmacokinetic Parameters for SK981 in Cancer Patients on Day 1 of Daily Oral Administration of ESK981

Dose (mg/m^2)	C_{max} (ng/mL)	t_{max} [a] (hr)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	R_{pred}
3.0 (n=3)	13.7 (6.1)	6.0 [2.0-6.1]	127 (16)	151 (36)	8.0 (2.8)	1.2 (0.2)
4.2 (n=3)	16.8 (4.9)	4.0 [1.0-6.0]	119 (14)	136 (6)	7.8 (3.7)	1.2 (0.1)
5.9 (n=5)	26.0 (6.5)	2.1 [1.5-4.0]	195 (67)	210 (78)	5.9 (1.1)	1.1 (0.1)
11.8 (n=3)	84.9 (23.0)	1.6 [1.5-3.0]	517 (126)	569 (196)	6.0 (3.7)	1.1 (0.1)
19.7 (n=3)	135.7 (76.5)	4.0 [1.0-4.3]	874 (358)	928 (408)	5.1 (1.3)	1.0 (0.1)
29.6 (n=4)	266.9 (182.0)	2.3 [1.0-3.1]	2376 (2064)	3195 (3199)	8.5 (5.5)	1.2 (0.2)
41.4 (n=3)	351.9 (94.4)	2.0 [1.0-4.0]	2820 (2070)	3572 (3177)	8.1 (4.5)	1.2 (0.2)
55.0 (n=4)[b]	272.6 (127.5)	3.0 [3.0-3.2]	2274 (666)	2675 (508)	8.9 (2.5)	1.2 (0.1)
73.0 (n=3)	444.2 (149.4)	4.0 [3.0-4.1]	3939 (1966)	4659 (2634)	8.1 (2.2)	1.2 (0.1)
97.4 (n=9)	526.4 (185.7)	4.0 [2.0-6.0]	5320 (1459)	6915 (2115)	10.3 (4.6)	1.3 (0.3)
126.6 (n=2)	751.2 (780.5, 721.9)	5.0 [8.0, 2.0]	5767[d]	NC	NC	NC
Overall Mean[c]	-	-	-	-	8.0 (3.7)	1.2 (0.2)

[a] Values presented for t_{max} are the median [range]; [b]Excludes data from patient 1019 due to receipt of improper dose of study medication; [c] Overall mean for all dose groups; [d] n=1.
NC: Not calculable.
$R_{pred} = AUC_{0-\infty}/AUC_{0-24}$.

Table 2. Mean (SD) Pharmacokinetic Parameters for ESK981 in Cancer Patients on Day 15 of Daily Oral Administration of ESK981

Dose (mg/m^2)	C_{max} (ng/mL)	t_{max} [a] (hr)	AUC_{τ} (ng•hr/mL)	$t_{1/2}$ (hr)	R_{obs}	R_{ss}
3.0 (n=3)	16.1 (2.3)	2.9 [1.5-4.0]	165 (83)	8.0 (2.9)	1.3 (0.6)	1.1 (0.5)
4.2 (n=3)	24.3 (9.2)	3.9 [1.5-4.0]	202 (47)	9.9 (4.6)	1.8 (0.6)	1.5 (0.4)
5.9 (n=5)	32.4 (8.4)	3.0 [1.5-6.0]	278 (69)	6.6 (1.5)	1.5 (0.6)	1.4 (0.6)
11.8 (n=3)	98.1 (5.6)	2.0 [1.6-2.1]	764 (128)	12.3 (9.7)	1.5 (0.2)	1.4 (0.3)
19.7 (n=3)	128.5 (98.4)	4.0 [1.5-4.0]	1102 (735)	5.4 (1.2)	1.2 (0.3)	1.1 (0.2)
29.6 (n=4)	218.8 (130.3)	4.5 [2.0-8.0]	2924 (2940)	8.7 (5.7)	1.1 (0.2)	0.9 (0.1)
41.4 (n=3)	412.6 (93.9)	2.1 [1.7-3.1]	3256 (1816)	10.3 (6.3)	1.3 (0.5)	1.1 (0.5)
55.0 (n=4)[b]	320.7 (75.1)	5.2 [3.0-8.0]	4313 (1091)	12.8 (4.3)	1.9 (0.2)	1.6 (0.1)
73.0 (n=2)	713.0 (734.5, 691.6)	3.3 [3.5, 3.1]	9429 (12253, 6605)	13.9 (20.6, 7.2)	1.9 (2.0, 1.8)	1.7 (1.6, 1.7)
97.4 (n=7)	7431.3 (336.7)	2.7 [1.5-6.0]	6623 (3283)[d]	11.0 (6.2)[d]	1.5 (0.9)[d]	1.2 (0.7)[d]
126.6 (n=1)	1481.5	8.0	23565	14.7	NC	NC
Overall Mean[d]	-	-	-	9.9 (5.3)	1.5 (0.5)	1.3 (0.5)

[a] Values presented for t_{max} are the median [range]; [b]Excludes data from patient 1019 due to receipt of improper dose of study medication; [c] Overall mean for all dose groups; [d] n=6.
$R_{obs} = AUC_{\tau}/AUC_{0-24,\ day\ 1}$.
$R_{ss} = AUC_{\tau}/AUC_{0-\infty,\ day\ 1}$.

After reaching peak plasma levels, ESK981 declined in a bi-phasic manner that was characterized by an initial phase of drug distribution and a slower terminal elimination phase. For some profiles, however, only the later phase was evident due to what appeared to be a more prolonged period of drug absorption. The mean $t_{1/2}$ of the terminal elimination phase was generally in the range of approximately 8 to 10 hr; however, the

values tended to be slightly larger after multiple doses at the higher dose levels (i.e., doses ≥55 mg/m^2) (Table 1 and Table 2).

The systemic exposures (i.e., C_{max} and AUC) of patients to ESK981 generally increased with increasing dose across the dose range evaluated on both days 1 and 15 (Table 1 and Table 2). There were some dose groups for which the mean values of C_{max} and AUC did not show an increase relative to the values from the preceding 1 or 2 dose levels; however, this result was not surprising given the large inter-patient variability and the fact that most dose increments were less than 40%. Nonetheless, the overall results obtained are not suggestive of the presence of any obvious non-linearity in absorption and/or elimination of ESK981 in this dose range.

After once-daily administration of ESK981 at doses ranging from 3.0 to 126.6 mg/m^2, there was a small to moderate amount of accumulation of the compound in plasma at each dose level. The overall mean observed accumulation ratio (R_{obs}) for ESK981 was 1.5 (range = 1.1 to 1.9), which was slightly larger than the value predicted from the single-dose data (overall mean R_{pred} = 1.2; range = 1.0 to 1.3) (Table 1 and Table 2). Although the mean R_{obs} was slightly larger than the R_{pred}, this is not believed to be a strong indication of the presence of any time-dependent pharmacokinetic processes.

1.2.2 Clinical safety and efficacy

Adverse events

All 43 patients experienced ≥1 adverse event, and 38 patients (88.3 %) were deemed to have had adverse events possibly, probably, or definitely related to study drug. The most frequently reported adverse event of any grade was fatigue (n= 22, or 51 %). Other frequently reported adverse events (≥20 % of patients) were nausea (47 %), diarrhea (33 %), decreased appetite (33 %), abdominal pain (30 %), back pain (28 %), vomiting (28 %), constipation (28 %), headache (28 %), dizziness (28 %), and dyspnea (23 %). These adverse events were reported at a similar frequency between dosage cohorts, and no relationship with dose was evident. Most adverse events were Grade 1 or Grade 2.

Grade 3 or 4 adverse events occurred in 16 (37 %) patients across dosing cohorts (14 [32.6 %] Grade 3 and 2 [4.7 %] Grade 4). Treatment-related Grade 3 or 4 adverse events were most frequent in the 97.4 mg/m^2 cohort. Grade 3 or 4 laboratory hematologic toxicities were reported in 8 (18.6 %) patients across dosage cohorts. The most common Grade 3 or 4 laboratory hematologic toxicity was lymphopenia, which occurred in 8 patients and across dosage cohorts (5.9, 29.6, 55.0, 97.4, and 126.6 mg/m^2). Grade 4 leukopenia occurred in 1 patient in the 126.6 mg/m^2 cohort. Grade 3 or Grade 4 neutropenia also occurred in 2 patients in the 97.4 mg/m^2 cohort (Grade 3) and in 1 patient in the 126.6 mg/m^2 cohort (Grade 4).

Serious adverse events occurred in 12 patients; most were deemed unlikely or not related to ESK981. Three patients (1 patient in the 97.4 mg/m^2 cohort and 2 patients in the 126.6 mg/m^2 cohort) experienced serious adverse events that were categorized as possibly or definitely related to ESK981: pyrexia, hemolytic anemia, hyperbilirubinemia, dyspnea, neutropenia, ECG change, and chest discomfort. No deaths occurred during the study.

One patient with an unrelated serious adverse event was a 76-year-old white man with metastatic prostate cancer. He started open-label treatment with CEP-11981 on 21 January 2010. He had a history of being heavily pretreated with bicalutamide, leuprolide, ketoconazole, GVAX vaccine, CNTO, docetaxel, cyclophosphamide, mitoxantrone, and several other agents in clinical trials. Relevant medical history included lumbar

laminectomies, osteoarthritis, lower back pain, pelvic pain, and intermittent right hand cramping. On 04 February 2010 (day 15), the patient had an elevated creatine phosphokinase laboratory value of 241 U/L (normal range, 25-195 U/L); study drug was interrupted from 05-10 February 2010. The laboratory value was back to normal at 165 U/L on 09 March 2010 (day 48).

The last dose of study drug was administered on 23 February 2010 (day 34). On 25 February 2010 (day 36), the patient experienced serious adverse events of back pain, musculoskeletal pain, and abdominal pain, all Grade 3 in severity. Pain reduction was achieved by treatment with oxycodone and intravenous dexamethasone and morphine. The patient was hospitalized for pain control and a magnetic resonance imaging (MRI) scan of the thoracic spine to rule out cord compression. The MRI scan was negative for cord compression. All 3 serious adverse events resolved on day 37, with residual effects, and the patient was discharged from the hospital. The investigator considered the serious adverse events of back pain, musculoskeletal pain, and abdominal pain unlikely related to the study drug. The patient was withdrawn from the study on 09 March 2010 (day 48) because of disease progression.

Another patient with experienced pyrexia, hemolytic anemia, and hyperbilirubinemia. The patient was a 26 year-old African American woman with advanced lung cancer. The patient started open-label treatment with CEP-11981 on 25 August 2010. Relevant medical history included migraine headaches, cough, nausea, shortness of breath, and fatigue. Concurrent medications included oxycodone, lorazepam, guaifenesin, and oxycocet. On 30 August 2010 (day 6), the patient was hospitalized for Grade 3 serious adverse events of pyrexia (102°F), hyperbilirubinemia, and hemolytic anemia. She also had a urinary tract infection, pain in bilateral lower extremities, blurry vision, sinus tachycardia, lethargy and thrush. The study drug was held during admission. The patient's laboratory tests performed on days 6 and 7 showed an elevated bilirubin at 5.2 mg/dL and hemoglobin at 9.6 g/dL, a concern for hemolytic anemia; the patient's haptoglobin was 315 mg/dL.

Blood cultures were negative, but the urine culture was positive for *E. coli*. The patient was treated with ciprofloxacin for the urinary tract infection and fluconazole for thrush. On 02 September 2010 (day 9), the patient's blood lactate dehydrogenase (LDH) was 618 U/L. The events of pyrexia and hyperbilirubinemia resolved by day 10, with a temperature of 97.1°F, total bilirubin of 1.3 mg/dL and a hemoglobin of 10.6 mg/dL. The patient did not require a transfusion, and her bilirubin, hemoglobin, and hematocrit resolved during hospitalization. The patient was discharged from the hospital on day 9 (data on file). The investigator considered the serious events of pyrexia, hemolytic anemia, and hyperbilirubinemia as possibly related to the study drug. On 08 September 2010 (day 15), the patient had Grade 3 serious adverse events of nausea and vomiting that required hospitalization. An x-ray of the kidneys, ureters, and bladder was negative for obstruction or ileus, but indicated constipation. Her constipation was treated with docusate/senna, which improved the nausea and vomiting. The investigator considered the serious adverse events of nausea and vomiting, which resolved on 10 September 2010 (day 17), to be not related to the study drug. The last dose of study drug administered was on 29 August 2010 (day 5). The patient was withdrawn from the study on 08 September 2010 (day 15) because of the serious adverse events of pyrexia, hemolytic anemia, and hyperbilirubinemia.

Another patient experienced dyspnea, electrocardiogram changes, chest discomfort. The patient was a 57 year-old white woman with colorectal cancer. She started open-label treatment with CEP-11981 on 08 December 2010. On 15 December 2010 (day 8), the

patient experienced serious adverse events of dyspnea (Grade 2), electrocardiogram (EKG) change (Grade 3), and chest discomfort (Grade 2). The EKG showed a t-wave inversion that was not present on previous EKGs. She was subsequently hospitalized for cardiac workup (data on file). The last dose of study drug was administered on 14 December 2010 (day 7). The events of EKG change and chest discomfort resolved with no residual effects on 20 December 2010 (day 13). The event of dyspnea did not resolve. On 29 December 2010 (day 22), the patient had a nonserious adverse event of Grade 1 chest discomfort that did not resolve and was withdrawn from the study because of the nonserious chest discomfort and serious adverse events of dyspnea and EKG change. The investigator considered the serious adverse events of dyspnea and EKG change and both the serious and nonserious chest discomfort to be possibly related to the study drug.

In the Phase I study, 19/43 patients received more than 28 days (range 33-504 days) of treatment. Table 3 provides the patient's dose level, number of days on treatment, and adverse events reported. There were no Grade 4 toxicities in this group. Six patients had at least one Grade 3 toxicity.

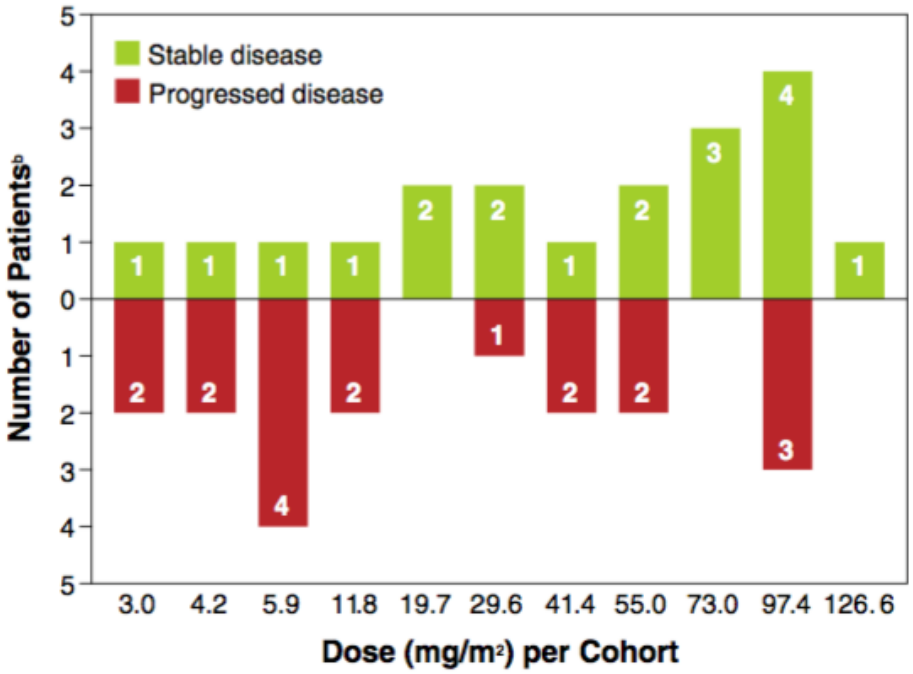
Table 3. Adverse Events Occurring On or After Day 28 in Patients that Received more than 28 days of Treatment

Pts treated >27 days	Days on Treatment	Dose (mg/m2)	Grade 1	Grade 2	Grade 3
001002/K-M	110	3.0	Dry skin, Nausea, Sinus headache, Gamma-glutamyltransferase increased, Abdominal tenderness, Hypercalcaemia, Blood lactate dehydrogenase increased	Hyperglycaemia, Vulvovaginal pruritus, Fatigue	Abdominal pain
001001/RAM	322	3.0	Rhinitis, Wheezing, Hypoalbuminaemia, Nasopharyngitis, Sinus headache, Gamma-glutamyltransferase increased, Dyspnoea, Nausea, Oedema peripheral, Hypokalaemia, Burning sensation, Hyperglycaemia, Headache, Hypercholesterolaemia, Blood creatine phosphokinase increased, Vomiting, Pain in extremity, Hypoglycaemia, Blood creatinine increased, Back pain	Abdominal pain, Fatigue	
002003/CEC	69	4.2	Hyperkalaemia, Wheezing, Dyspnoea, Oral discomfort, Blood alkaline phosphatase increased, Constipation, Decreased appetite, Tachycardia, Oedema peripheral, Hyperglycaemia		
002005/DNH	70	5.9	Gamma-glutamyltransferase increased, Hypoglycaemia, Dyspepsia, Incontinence, Dizziness, Bradycardia, Decreased appetite, Hyperglycaemia, Nausea, Chest discomfort, Asthenia, Hyperhidrosis	Fatigue, Nocturia	Hyperkalaemia
002008/DLH	70	11.8	Groin Pain, Decreased appetite, Nasopharyngitis, Myalgia, Hyperuricaemia, Urinary incontinence, Haemoglobin decreased	Hyperglycaemia, Neuropathy peripheral, Tongue ulceration	
002009/RMH	70	19.7	Hot flush, Gastrooesophageal reflux disease, Rhinorrhoea, Fatigue, Eye pain		
001015/SRG	110	19.7	Hyperglycaemia, Upper respiratory tract infection, Hyponatraemia, Oedema peripheral, Erythema, Cough, Dyspnoea, International normalised ratio increased, Proteinuria	Ureteric obstruction, Abdominal pain, Proteinuria	
001017/D-F	41	29.6	None (All AEs had onset before day 28),		
003002/MAR	90	29.6	Decreased appetite	Anaemia, Myocardial ischaemia	
002010/EJB	217	29.6	Hypoaesthesia, Lip pain, Colonic polyp, Dry skin, Nausea, Decreased appetite, Asthenia, Constipation	Cough	
002011/KJD	111	41.4	Dyspnoea, Musculoskeletal chest pain, Constipation, Bladder pain, Hot flush, Abdominal pain upper	Night sweats, Upper respiratory tract infection	
002014/CSS	33	55.0	Decreased appetite	Fatigue, Oedema peripheral	Back pain, Musculoskeletal pain, Abdominal pain, Scrotal oedema
001018/ADM	152	55.0	Insomnia, Upper respiratory tract infection, Flushing, Lymph node pain, Skin nodule, Contusion, Stomatitis, Blister, Seasonal allergy, Neutropenia, Thrombocytopenia, Upper respiratory tract infection, Hyperuricaemia, Abdominal pain, Decreased appetite, Mood swings	Pain in extremity, Presyncope, Mucosal inflammation, Blood bilirubin increased	
003003/MLK	504	55.0	Hiccups, Joint injury, Pain in extremity, Cheilitis, Stomatitis, Wheezing, Urinary retention, Incision site pain, Muscular weakness, Vision blurred, Vomiting, Headache, Erythema, Neuropathy peripheral, Chills, Rash macular, Stasis dermatitis, Rheumatoid arthritis,	Joint effusion, Hypotension, Oedema peripheral, Anaemia, Weight increased, Urinary tract infection	Somnolence, Arthralgia, Anxiety, Impaired healing, Cellulitis, Oedema peripheral,
001021/JBR	172	73.0	Chest pain, Muscle spasms, Abdominal pain, Pharyngitis streptococcal, Stomatitis, Sexual dysfunction, Dysuria, Pyrexia, Abdominal pain upper, Rales	Back Pain, Abdominal distension, Urinary tract infection, Weight descreased, Tachycardia, Dehydration, Back pain, Flank pain, Oedema peripheral, Vomiting	Headache, Neck pain, Diarrhea, Decreased appetite, Anemia, Musculoskeletal pain,
001022/EDM	65	97.4	Chest pain, Pain in extremity		
001025/VGY	76	97.4	Neuropathy peripheral, Flank pain, Contusion, Insomnia		
002017/RWF	127	97.4	Rhinorrhoea, Pulmonary mass, Diarrhoea, Dysphonia		
002018/CRC	196	97.4	Musculoskeletal pain, Diarrhoea, Nasopharyngitis, Nausea, Dizziness, Onychoclasis, Abdominal pain, Vomiting, Dysphonia, Sinus congestion, Abdominal distension, Flatulence, Neuropathy peripheral, Oropharyngeal pain, Respiratory tract congestion, Decreased appetite, Diarrhoea, Insomnia	Cough, Pneumonia	Chronic obstructive pulmonary disease

Tumor response

Out of 43 patients who received ≥1 dose of study drug, 37 patients were evaluated for tumor response. Although no enrolled patients had complete or partial response according to RECIST criteria, 19 of 37 (51 %) patients evaluated for tumor response had stable disease at ≥6 weeks; 18 had disease progression. The frequency of stable disease (defined as <30 % decrease and <20 % increase in the sum of the longest diameter of the target lesions) was higher in cohorts receiving doses ≥73.0 mg/m² (8 of 14 [57.1 %] patients) compared with cohorts receiving ≤55.0 mg/m² (11 of 29 [37.9 %] patients) (Figure 4).

Figure 4.
Best overall tumor response per cohort[a] at >/= 6 weeks. Depicts tumor response, with bars above the x-axis indicating patients (n = 19) who achieved stable disease and bars below the x-axis indicating patients (n = 18) with disease progression. [a]No patient achieved complete or partial response. [b]Six patients were not evaluable at dosages of 19.7, 29.6, 55.0, and 126.6 mg/m^2 (n = 1, each); 97.4 mg/m^2 (n = 2)

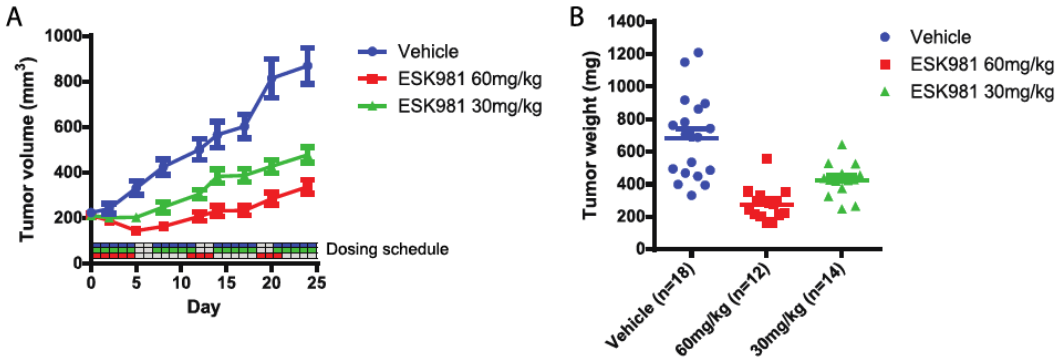


1.3 Rationale

ESK981 has cleared a dose-escalating Phase I clinical trial and demonstrated favorable pharmacokinetics and pharmacodynamics in patients with advanced, relapsed, or refractory solid tumors with up to 85% of patients achieving stable disease at doses ≥73.0 mg/m². In our preliminary studies, ESK981 appears to induce robust vacuolization associated with activation of the autophagy pathway in prostate cancer *in vitro* and *in vivo* and demonstrated dose-dependent inhibition of castrated VCaP xenograft growth (Figure 5).

Figure 5.

ESK981 inhibits castrated VCaP tumor growth. Castrated VCaP-bearing mice received vehicle or ESK981 (60 or 30mg/kg) as indicated. A, Tumor volume was measured twice per week over a 25-day course. B, Tumor weight was measured for each group after surgical resection.



Thus, we are conducting a focused open-label Phase II study of ESK981 in men with mCRPC. The study will evaluate men with mCRPC who have progressed on enzalutamide and/or abiraterone acetate. Consenting patients will undergo a biopsy at baseline and after 8 weeks of treatment to assess gene expression and biomarkers. The primary objective will be to determine the PSA ≥ 50% response rate (PSA50) from baseline using the Prostate Cancer Working Group 3 (PCWG3) criteria to ESK981 as a single agent.

Currently, CRPC patients receive either enzalutamide or abiraterone acetate as front-line treatment for mCRPC without visceral metastases. If a patient has metastatic disease with visceral metastases he may also receive chemotherapy such as docetaxel. This study explores if ESK981, administered 5 days on and 2 days off can induce a high PSA response rate and/or if AR expression is induced or enhanced from baseline following a standard front-line treatment to determine the PSA50 response rate to ESK981.

1.4 Rationale for Correlative Studies

1.4.1 Autophagy induction

ESK981 demonstrated a unique autophagy induction property in our pre-clinical *in vitro* and *in vivo* models through provoking robust cellular vacuolization. Thus, autophagy can serve as biomarker for patient response to ESK981 treatment.

1.4.2 Angiogenesis inhibition

Considering ESK981's anti-VEGFR-A property and our *in vivo* preliminary data that ESK981 strongly attenuated angiogenesis, we believe that angiogenesis can be a biomarker for patient response to ESK981 treatment.

1.4.3 Phosphorylation reduction

According to the kinase inhibition profile data from ESANIK, ESK981 inhibits a group of kinases that have been validated in our preliminary *in vitro* models. Using immunohistochemistry (IHC) to determine phosphorylation levels of these targets can help to assess ESK981 pharmacodynamics in patient.

1.4.4 Tumor cellular proliferation rate

Preliminary data have shown that ESK981 significantly inhibited tumor cell proliferation *in vitro* and *in vivo* xenografts. Therefore, we believe that determining proliferation rate by Ki67 IHC will also be a potential biomarker for patient response to ESK981 treatment.

1.4.5 Other potential markers

Overall patient survival, assessment by radiography, radiographic progression free survival, and PSA progression free survival can be monitored clinically as surrogates for treatment outcomes. Mutation analysis by whole genome sequencing, immunohistochemistry to quantify PD-1 and PD-L1 expression and presence of CD8+ tumor infiltrating lymphocytes can be useful in exploratory evaluation. In addition, select genetic and epigenetic characteristics of the patients' tumors will be assessed as surrogate biomarkers for clinical benefit. These evaluations are expected to identify genomic correlates of response (biochemical recurrence, onset of pain), complications (osteoblastic lesions in bone, cachexia), and survival. The specific biomarker candidates were identified based on their inclusion in either of the following actionable/informative categories: (i) immediate link with the ESK981 mechanism of action, (ii) high prevalence in mCRPC and/or routine clinical testing, (iii) potential less-invasive detection in biofluids. Respectively, the proposed correlative endpoints include expression levels of ESK981 target kinases, ETS-status, and AR amplification/levels in circulating tumor cells. The rationale for each biomarker choice and correlative assessment is listed in section 1.4, while methodological and statistical considerations for the genomic screening are reviewed in section 11.3.

2. OBJECTIVES

2.1 Primary Objective

The primary objectives of this study are:

- To determine the PSA ≥ 50% response rate (PSA50) from baseline using the PCWG3 criteria to ESK981 as a single agent in men with mCRPC who have progressed on enzalutamide (an oral androgen-receptor inhibitor) and/or oral abiraterone acetate (an androgen synthesis inhibitor).
- To assess the safety and tolerability of ESK981 as a single agent. Adverse events of all grades will be captured by the National Cancer Institute - Common Terminology Criteria for Adverse Events, version 4.03 (NCI-CTCAE v4.03) and GCP standards.

2.2 Secondary Objectives

- To determine the time to PSA response to ESK981 in mCRPC patients.
- To determine the duration of PSA response to ESK981 in mCRPC patients.
- To determine PSA progression rates as defined by the PCWG3 criteria.
- To determine PSA progression free survival as defined by the PCWG3 criteria

2.3 Correlative/Exploratory/Tertiary Objectives

- To assess exploratory biomarkers from blood and tumor biopsies. Examples of these may include, but are not limited to: whole exome sequencing, capture whole transcriptome analysis, AR and ARv7 analysis, ki-67, apoptosis, etc.

3. PATIENT SELECTION

A total of 27 patients with mCRPC will be included in this study.

Each subject will be counseled by a study investigator about the planned study procedures, risk and benefits of participating in the study, and alternative therapeutic options. Each subject must sign an Institutional Review Board (IRB)/ Independent Ethics Committee (IEC)-approved consent form and written authorization to use protected health information as outlined in the Health Insurance Portability Act (HIPAA) of 1996, prior to the initiation of any study related procedures.

3.1 Inclusion Criteria

Patients should meet all of the following criteria at the time of registration:

3.1.1 Have signed an informed consent document indicating that the subject understands the purpose of and procedures required for the study and are willing to participate in the study.

3.1.2 Be willing and able to adhere to the prohibitions and restrictions specified in this protocol.

3.1.3 Males 18 years of age and above.

3.1.4 Eastern Cooperative Group (ECOG) performance status ≤1.

3.1.5 Patient must have progressive disease while receiving androgen deprivation therapy (ADT) defined by any one of the following as per the PCWG3 criteria for PSA, measureable or non-measurable (bone) disease and must have a castrate serum testosterone level (i.e. ≤ 50 ng/dL) at screening:

3.1.5.1 PSA: At least two consecutive rises in serum PSA, obtained at a minimum of 1-week intervals, with the final value ≥ 2.0 ng/mL.

3.1.5.2 Measurable disease (by RECIST 1.1): ≥20% increase (with an absolute increase of at least 5mm) in the sum of diameters of all measurable lesions or the development of one or more new lesions. The short axis of a target lymph node must be more than 15mm to be assessed for change in size.

3.1.5.3 Non-measurable (bone) disease: The appearance of two or more new areas of uptake on bone scan consistent with metastatic disease compared to previous imaging during castration therapy. The increased uptake of pre-existing lesions on bone scan will not be taken to constitute progression, and ambiguous results must be confirmed by other imaging modalities (e.g. X-ray, CT or MRI).

3.1.6 Documented histologically confirmed adenocarcinoma of the prostate.

3.1.7 Metastatic prostate cancer (M1) as documented by appropriate medical imaging (i.e. CT-Scan, PET scan or bone scan).

3.1.8 Treatment failure of either abiraterone and/or enzalutamide per PCWG3 criteria for PSA, measurable disease or non-measurable (bone) disease.

3.1.9 Willingness to use contraception by a method that is deemed effective by the Investigator throughout the treatment period and for at least 30 days following the last dose of therapy.

3.1.10 Willingness and ability to comply with study procedures and follow-up examination.

3.1.11 Able to swallow and retain oral medication.

3.1.12 Willingness and ability to undergo mandatory tumor biopsy at baseline and week 8/cycle 3.

3.1.13 Willingness and ability to undergo mandatory whole blood sample collections at baseline, days 8, 15, and 22 in the first cycle, and then monthly.

3.2 <u>Exclusion Criteria</u>

3.2.1 Current systemic therapy (other than a GnRH agonist/antagonist) for CRPC including:

a. CYP-17 inhibitors (e.g. ketoconazole, abiraterone)
b. Antiandrogens (e.g. bicalutamide, nilutamide)
c. Second generation antiandrogens (e.g. enzalutamide, ARN-509, Galeterone)
d. Immunotherapy (e.g. sipuleucel-T, ipilimumab)
e. Chemotherapy (e.g. docetaxel, cabazitaxel)

3.2.2 Prior chemotherapy (e.g. docetaxel, cabazitaxel) for CRPC. Prior docetaxel administered in the castrate-sensitive space is allowed.

3.2.3 Prior radiopharmaceutical therapy (e.g. radium-223, strontium-89, samarium-153, etc.) within the past year.

3.2.4 Have any condition that, in the opinion of the investigator, would compromise the well-being of the subject or the study or prevent the subject from meeting or performing study requirements.

3.2.5 The patient has any of the following hematologic values:

- absolute neutrophil count (ANC) less than 1500/mm^3
- platelet count less than 100000/mm^3
- hemoglobin less than 9 g/dL.

3.2.6 The patient has any of the following hepatic function values:

- bilirubin greater than 1.5 times the upper limit of normal (ULN)
- alanine aminotransferase (ALT) greater than 2.0 times the ULN in the absence of known hepatic metastases
- aspartate aminotransferase (AST) greater than 2.0 times the ULN in the absence of known hepatic metastases,
- ALT or AST greater than 3.0 times the ULN in the presence of known hepatic metastases.

3.2.7 The patient has a serum creatinine value greater than 1.5 mg/dL.

3.2.8 The patient has active brain metastases.

3.2.9 The patient is currently on warfarin or heparin therapy.

3.2.10 The patient has any pre-existing coagulopathy, recent hemoptysis, gross hematuria or gastrointestinal bleeding.

3.2.11 The patient has a history of a clinically significant cardiovascular or cerebrovascular event within 6 months prior to study entry.

3.2.12 The patient has uncontrolled hypertension defined as a blood pressure measurement greater than 150 mm Hg systolic or 90 mm Hg diastolic with medication.

3.2.13 The patient has received any investigational drug within the past 4 weeks.

3.2.14 The patient has previously been enrolled in the study or received ESK981.

3.2.15 The patient has known hypersensitivity to gelatin or lactose monohydrate.

3.2.16 The patient has taken a medication known to be a potent inducer of CYP1A2, CYP2C8, or CYP3A4 within 4 weeks prior to the first dose of study drug.

3.2.17 The patient has taken a medication known to be a potent inhibitor of CYP1A2, CYP2C8, or CYP3A4 within 2 weeks prior to the first dose of study drug.

3.3 Screen Failures

Minimal data for subjects who fail screening will be collected such as demographic information and the reason for screen failure. Such subjects may be re-screened at the discretion of the Principal investigator. The reason for the need to re-screen a subject will be documented in the subject's source documents.

4. ENROLLMENT PLAN AND SUBJECT REGISTRATION

4.1 Registration Procedure

All patients shall be registered with the Cancer Center Clinical Trials Office at (313) 576-9837 (Kim Dobson). At the time of registration, a pre-study form and all information required to verify eligibility shall be necessary on each patient prior to treatment.

4.2 Survival Follow-up

Subject survival information will be collected preferably via office visit or telephone contact every 3 months from the date of end of treatment visit until 5 years post treatment.

- The following information will be collected: The subject's survival status, and if deceased, the date of death.
- The method by which the survival status was assessed and the date it was assessed.
- Any subsequent anti-cancer therapy received after discontinuation from study drug.

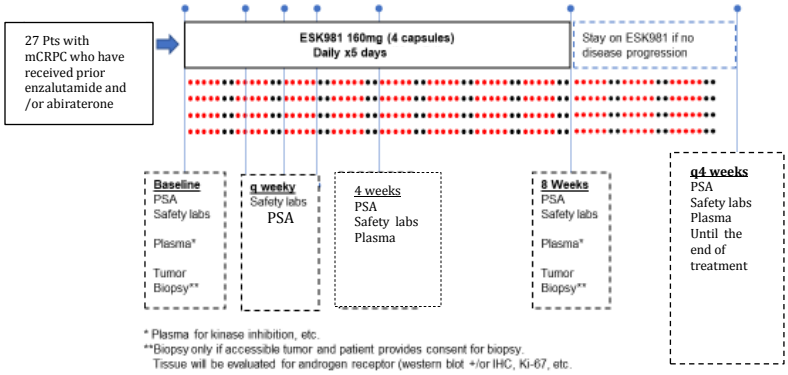
The subject will be determined to be lost to follow-up only after at least 3 attempts to contact him have been made over a 4 month period. At least two of the attempts to contact must be made through certified letters.

5. TREATMENT/INTERVENTION PLAN

This is an open-label, non-randomized single-stage Phase II design of ESK981 in patients with mCRPC who have had prior enzalutamide and/or abiraterone. Patients will receive 8 weeks of ESK981, and then be evaluated for their rate of PSA decline of ≥ 50% from baseline (PSA50), using the PCWG3 criteria.

Assessments and procedures that will occur during the study are detailed in Appendix C.

An overall study schema appears below:



5.1 Removing Subjects from the Protocol

In the absence of treatment delays because of adverse events, treatment will continue for 8 weeks. If at the conclusion of the 8-week period, no disease progression has occurred, the participant can stay on trial until one of the following criteria applies:

5.1.1 Subject decides to withdraw from the study.

5.1.2 Disease progression

- symptomatic disease progression at any time
- objective clinical disease progression

5.1.3 Subject is no longer clinically benefitting (NLCB) from treatment.

5.1.4 Intercurrent illness that prevents further administration of treatment.

5.1.5 Unacceptable adverse event(s) that may or may not be directly related to treatment but that, in the judgment of the treating physician, makes it dangerous for the subject to be retreated.

5.1.6 General or specific changes in the patient's condition that render the patient unacceptable for further treatment, in the judgment of the investigator.

Because an excessive rate of withdrawals can render the study uninterruptable, unnecessary withdrawal of subjects should be avoided. When a subject discontinues treatment early, the investigator should make every effort to contact the subject and to perform a final evaluation. The date and the specific reason(s) for withdrawal should be recorded.

5.2 Definition of compliance with the self-administered oral agent ESK981

Based on pill counts made by the Research Coordinator from the bottles of ESK981 returned by the patient, the % of intended ESK981 pills presumably consumed will be calculated. If that % is ≥ 75% over Cycle 1 (the first 28 days of ESK981 treatment), then the patient will be deemed "compliant" with the intended oral ESK981 treatment regimen.

6. THERAPEUTIC AGENT

6.1 Description of Treatments

Patients will receive 160mg (4 capsules) of ESK981 dosed once daily for five consecutive days followed by a two consecutive day treatment break, for a cycle of 28 days. Drugs can be taken in the fed or fasted state. If at the conclusion of the 8-week period, no disease progression has occurred, refer to section 5.1 to determine if participant can stay on trial.

6.2 ESK981 Drug Supply and Return

Shipment and Receipt

Esanik Therapeutics, Inc has contracted with Sharp Clinical Services Inc. as the vendor for the storage and shipping of ESK981 (CEP11981); therefore, all drug shipments will be received from Sharp and not directly from Esanik. All drug shipments will be sent to the attention of the appropriate pharmacy contact, which will be identified in advance by the Investigator as responsible for the management of investigational product. This includes study drug receipt, accountability, storage/handling, and proper dispensation.

Note: The initial shipment will be at least 12 bottles of ESK981, and subsequent shipments will be at the request of the Investigator based on site enrollment. Sites should have enough ESK981 (CEP11981) in stock for initial 8 week treatment course prior to enrolling a patient into the study. If pharmacy space is limited, please contact your Esanik representative for in-treatment resupply plan.

ESK981 will be shipped via an express courier under refrigeration directly to each participating center from Sharp upon authorization from Esanik. Esanik will be notified of the shipping date when confirmed by Sharp. If the shipment does not arrive as scheduled, please notify your Esanik representative immediately.

Upon receipt of the shipment, the package should be inspected for excessive damage or evidence of opening prior to arrival. If the package appears compromised, sequester the shipment and contact Esanik for further instructions. This supply must not be placed in the active inventory or used in the clinical trial without Esanik's approval.

The Pharmacy should open the package as soon as possible and compare the Drug Shipment Form to the contents of the shipment upon receipt. If there is a discrepancy between the Drug Shipment Form and the shipment contents, if bottles arrive damaged, or a temperature deviation is noted from the monitoring device (see below),

the Pharmacy should note the problems on the packing invoice and contact Esanik immediately for further instruction.

All shipments will arrive in insulated shippers at approximately 2-8°C, and will include a temperature monitoring device to verify temperature during transit. Upon receipt of the shipment, please follow the steps below as expeditiously as possible:

1. Open the package, locate temperature log device.
2. Follow the instructions provided to review the temperature tracking.
3. If a temperature deviation is noted, please contact Esanik.
4. If no temperature deviations are noted, the study drug may be accepted into the active inventory.

Storage

Upon acceptance, ESK981 must be immediately removed from the shipping cooler and stored refrigerated at 2-8°C. The storage temperature should be monitored and recorded daily to ensure temperatures are maintained at 2-8°C. A temperature log is provided in Attachment 1; however, sites may use their own temperature log or the one provided in this manual. Sites unable to record daily temperatures must provide verification that there are no temperature deviations.

ESK981 must be kept in a secure place with limited access under the appropriate storage conditions at all times. All personnel involved in the dispensing of investigational product should be aware of its location. Any deviations in accountability and/or storage should be reported to Esanik.

Resupply of Investigational Product

All investigational product resupply requests should be made by faxing or e-mailing the Site Investigational Drug Shipping Request Form (Attachment 2) your CRA or designee. A minimum of two weeks (10 business days) is required for routine resupply; therefore, pharmacies are encouraged to proactively manage drug inventory to avoid supply problems.

Drug Return

At the conclusion of the study, the pharmacy will receive specific instructions on how to handle any undispensed investigational product. Pharmacists are not to return or destroy any unused investigational product until these instructions are received.

6.3 DRUG ACCOUNTABILITY

General Statement

Investigators are required to establish a record of receipt, use, and disposition of all investigational product for a period of two years following the date that the investigational product is approved by the regulatory authority. These drug accountability records must be readily available upon request, and will be reviewed during the study. To avoid error, the investigator should contact Esanik Therapeutics, Inc. before the destruction of any records pertaining to the study to ensure they no longer need to be retained.

Study related forms located in the Attachment Section of this manual should be completed using permanent black ink only. Any error should be crossed through with a single pen stroke, initialed and dated. Do not attempt to cover up an error by

scratching it out or concealing it with correction fluid. The correct information should be recorded next to the error in an appropriate space.

GC4419 Drug Accountability Record

Pharmacies may use institutional forms and systems for managing inventory and accountability provided they meet regulatory requirements for investigational agents. A log entry is made each time ESK981 is received and dispensed by the site.

Attachments

Attachment 1: Temperature Monitor Log

Attachment 2: Site Investigational Drug Shipping Request Form

Attachment 1:

TEMPERATURE MONITORING LOG

Clinical Site: _____ Site Number: _____ PI Name: _____

Location of Refrigerator: _____

Record daily refrigerator temperatures on the log provided. Temperatures must be maintained between 2-8°C. If refrigerator fluctuates from the specified range, contact your CAP CRA for appropriate action and follow, if necessary.

Month: _____, 20____

	Day of Month																													
	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	29	30	31
Temp																															
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12ºC																															
13ºC																															
>14ºC																															

Attachment 2:

SITE INVESTIGATIONAL DRUG SHIPPING REQUEST FORM
Esanik Therapeutics, Inc.
Protocol GT-001

Please Email to: BRIAN WOOD
EMAIL: Brian@esanik.com

Protocol No./Investigational Agent: **ESK981 (CEP11981)**		**Site No./Investigator:**
Request Date:	**Date Required On Site:**	**Number of Vials Requested:**
Ship To: (include attention personnel, full mailing address, phone and email for receiving party)		

Comments/Special Instructions:

Institutional Requestor:

Name	**Signature**	**Phone Number**

CAP Approval*:

Name	**Signature**	**Date**

The completed, original form must be filed in the Pharmacy Binder for this study.

Pharmacokinetics

Note: CEP-11981 refers to the development name for ESK981.

The pharmacokinetic, ADME, and toxicokinetic characteristics of ESK981 have been extensively studied in Sprague Dawley rats and cynomolgus monkeys after intravenous and oral doses and preliminarily in Balb/c nude mice after oral doses. In order to facilitate comparisons between studies and species, all doses are presented in this summary as milligrams of ESK981 free base per kilogram of body weight, regardless of the form administered, unless specifically noted otherwise.

The intravenous dose in rats was administered in 3% dimethylsulfoxide, 30% Solutol®, and 67% phosphate-buffered saline at a volume of 1 mL/kg. In monkeys, the intravenous vehicle was 47.4% polyethylene glycol (PEG) 400, 31.1% water, 20.7% hydroxy-β-cyclodextrin (HPBCD), and 0.8% Pluronic® F-68 at a volume of 0.5 mL/kg. Pharmacokinetic parameters obtained after single intravenous (bolus) administration of ESK981 are summarized in Table 4.

Table 4. Pharmacokinetics After Single Intravenous Administration of ESK981.

Parameter	Male rat[a] (0.8 mg/kg)	Female rat[a] (0.8 mg/kg)	Monkey[b] (0.5 mg/kg)
Clearance (L/hr/kg)	0.26	0.17	1.1±0.2
Half-life (hr)	1.9	2.5	1.7
Volume of distribution V_{ss} (L/kg)	0.68	0.59	2.6±0.3
Volume of distribution V_z (L/kg)	0.71	0.61	2.8±0.3

[a] Parameters were generated from composite mean plasma concentration-versus-time data.
[b] Values presented are mean±standard deviation, except half-life, which is harmonic mean.
V_{ss}=volume of distribution at steady state; V_z=volume of distribution after a single dose.

In rats, the systemic clearance is low relative to the estimated total hepatic plasma flow; the volume of distribution is approximately that of total body water. There are apparent sex-related differences in the pharmacokinetics of ESK981 in rats, with lower clearance and longer half-life in female rats than in male rats. The volume of distribution is numerically less in female rats, but the significance of this difference is doubtful.

In monkeys, the half-life is similar to that in rats, but the systemic clearance is substantially larger than that in rats, as is the volume of distribution. The difference in clearance is consistent with the higher rate of metabolism of ESK981 in incubations with monkey liver microsomes than with rat liver microsomes. The origin of the larger volume of distribution in monkeys is not known.

Pharmacokinetic parameters obtained after single, low-dose oral administration of ESK981 are summarized in Table 4.

Table 5. Pharmacokinetics After Single Oral Administration of ESK981.

Parameter	Female nude mouse[a] (3.0 mg/kg)	Male rat[a] (5.0 mg/kg)	Female rat[a] (5.0 mg/kg)	Monkey[b] (3.0 mg/kg)
C_{max} (ng/mL)	726	1330	1913	491±172
t_{max} (hr)	0.5	2	3	2 (1.5-2)
Half-life (hr)	3.7	3.0	2.7	2.4
$AUC_{0-\infty}$ (ng·hr/mL)	6102	8559	15635	2482±751
Bioavailability (%)	NC	43.9	51.7	93.0±29.3

[a] Parameters were generated from composite mean plasma concentration-versus-time data.
[b] Values presented are mean±standard deviation, except half-life, which is harmonic mean, and t_{max}, which is median [range].
[c] NC=not calculable.
C_{max}= maximum observed plasma drug concentration; t_{max}=time to maximum observed plasma drug concentration; $AUC_{0-\infty}$= area under the plasma drug concentration by time curve from time zero to infinity.

Dose administration in mice was as a suspension of the free base in 0.6% methylcellulose:Tween 80 (99.5:0.5) at a volume of 25 mL/kg. In rats and monkeys, the dose was administered as a suspension of the tosylate salt in Ora-Plus oral suspending vehicle at volumes of 2 and 5 mL/kg, respectively.

Significant oral bioavailability was obtained in both rats and monkeys. The lack of an intravenous comparator dose in mice precluded calculation of the absolute bioavailability in that species, but the similarity in systemic exposure in mice relative to rats would suggest that oral bioavailability is comparable.

The effect of fed versus fasted state on the pharmacokinetics of ESK981 was assessed in a preliminary study in rats. C_{max} and $AUC_{0-\infty}$ were minimally affected by the fed/fasted state of the animals, but the t_{max} was shifted from 2.7 to 5.3 hours postdose when the animals had been fed before dosing.

At higher oral doses of ESK981, there was evidence of dose-dependent pharmacokinetics in all three species. In mice, single-dose systemic exposure increased in a slightly more than dose-proportional manner between 1 and 10 mg/kg, but did not show consistent further increase at 20 or 30 mg/kg. After 23 daily doses, the dose response and concentrations were similar to those after single doses at 1, 3, or 10 mg/kg, but were approximately two times higher in the 20- and 30-mg/kg groups on day 23 versus day 1 of dosing. Systemic exposure after multiple daily doses of 20 and 30 mg/kg was dose-related, although not dose-proportional, relative to that at 10 mg/kg.

In male rats, C_{max} was approximately dose proportional between 5 and 15 mg/kg, but was less than dose proportional between 15 and 40 mg/kg. The increase in $AUC_{0-\infty}$ was greater than dose-proportional between 5 and 15 mg/kg, but was approximately dose proportional between 15 and 40 mg/kg.

In monkeys, increases in both C_{max} and $AUC_{0-\infty}$ were greater than dose proportional between 3 and 13.5 mg/kg. Similar (i.e., greater-than-dose-proportional) increases in systemic exposure were also noted in a preliminary study that tested single oral doses as high as 100 mg/kg in monkeys.

Due to changes in the form of ESK981 that was obtained from successive synthetic campaigns, a series of single-dose oral studies were conducted in rats and monkeys to which different forms of the compound and/or different dosage regimens were administered. The outcome of those studies indicated that milled ESK981 tosylate, administered as a suspension in aqueous methylcellulose and provides systemic exposures that are in the targeted ranges in both species. It is that form with

which the bulk of the nonclinical toxicity testing was done and which will be the form of the compound used in early clinical testing.

The tissue distribution of [14C]- ESK981 was studied in male albino (Sprague Dawley) and pigmented (Lister hooded) rats after single doses orally at 10 mg/kg and intravenously at 1 mg/kg, respectively, as the tosylate salt. Radioactivity was broadly distributed, with highest concentrations (exclusive of the gastrointestinal tract) in the liver, kidney, and adrenal gland.

Minimal radioactivity was detected in barriered tissues, i.e., brain and testis, but there was apparent association with melanin-containing structures such as uveal tract and pigmented skin in the pigmented animals. The bulk of the radioactivity was removed from the tissues during the 168- and 24-hour test periods in the albino and pigmented animals, respectively.

ESK981 is extensively metabolized *in vitro* by liver microsomes from male Swiss CD1 mice, male Sprague Dawley rats, male New Zealand rabbits, male beagle dogs, male cynomolgus monkeys, and humans. The relative order of loss of parent compound during 20 minutes of incubation was: monkeys > humans > mice ≈ dogs > rats ≈ rabbits.

Incubations with individual recombinant human CYP enzymes suggested that three enzymes, ie, CYP1A2, CYP2C8, and CYP3A4, may play significant roles in the metabolic elimination of ESK981. Other CYP enzymes that were also capable of metabolizing the compound *in vitro* are either minimally expressed in adult human liver (i.e., CYP1A1 and CYP3A7) or are polymorphic, being expressed in a minority of individuals (i.e., CYP3A5). The results of experiments assessing the effect of CYP-specific inhibitors on loss of ESK981 during incubation with human liver microsomes also identified CYP3A enzymes as playing a key role in metabolic elimination of the compound.

After oral administration of [14C]- ESK981, radioactivity was predominantly recovered in the feces in both rats and monkeys, with less than 1% of the radiochemical dose recovered in urine of either species. Hence, metabolic profiling was conducted in rat bile, in which parent [14C]- ESK981 was the most prominent radioactive component, especially in the female rat.

The most prominent metabolite in both sexes, but especially in the male, was a monohydroxylated derivative. Overall, the reactions previously observed *in vitro*, i.e., ring hydroxylation, N-demethylation, and reduction (didehydrogenation) accounted for all of the ESK981-derived compounds detected *in vivo*.

The potential for ESK981 to affect the metabolic elimination of co-administered drugs was tested *in vitro* through examination of its capacity for induction of CYP enzymes activities in primary human hepatocyte cultures and for inhibition of CYP activities in human hepatic microsomal preparations.

The levels of mRNA for CYP1A1, CYP1A2, CYP2C9, and CYP3A4 were measured after incubation of ESK981 with hepatocytes for 30 hours at concentrations of 1 to 30 μM. No indication of induction was obtained, but suppression of CYP2C9 and CYP3A4 message was observed at the highest concentration. However, due to apparent cellular toxicity at that concentration, this result was likely an artifact.

ESK981 did not inhibit the activities of CYP2B6, CYP2C9, CYP2C19, CYP2D6, or CYP4A9/11. Marginal inhibition of CYP1A2 was observed, but with an estimated Ki that is higher than exposures expected clinically. CYP3A4/5 activity was inhibited with a Ki of 2.6 and 2.2 μM for the two reactions examined, *i.e.*, testosterone 6β-hydroxylation and midazolam 1′-hydroxylation, respectively. Much of the inhibition was reversible and competitive in nature, with a metabolism-dependent component, but at least some of the inhibition was irreversible for both reactions tested.

In addition to the pharmacokinetic and ADME studies, bioanalytical and pharmacokinetic support has been provided to the toxicokinetics portions of nonclinical safety studies in rats and monkeys.

The longest nonclinical safety study conducted to date in rats was a 4-week oral toxicity study at daily doses of 0 (vehicle only), 5, 10, 20 (lowered to 15 on day 8), and 40 mg/kg. ESK981 was administered as suspensions of its tosylate salt in Ora-Plus oral suspending vehicle. Key toxicokinetic parameters from the study are tabulated in Table 6.

Table 6. Toxicokinetics Results for the 4-Week Oral Toxicity Study in Rats.

	Day 1/Day 28			
Parameter[a]	**5 mg/kg**	**10 mg/kg**	**20→15 mg/kg**	**40 mg/kg**
Males (day 1/day 28)				
C_{max} (µg/mL)	0.94/1.03	1.70/2.20	3.45/NAV[b]	5.28/NAV
t_{max} (hr)	4/1	2/4	2/NAV	4/NAV
Half-life (hr)	NC[c]/3.6	5.7/3.2	4.0/NAV	4.2/NAV
$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (µg·hr/mL)[d]	5.34 (AUC_{0-t})/9.47	17.7/27.1	42.4/NAV	73.6/NAV
Females (day 1/day 28)				
C_{max} (µg/mL)	1.17/1.73	2.69/2.72	5.19/NAV	8.21/NAV
t_{max} (hr)	4/2	4/2	2/NAV	2/NAV
Half-life (hr)	2.9/3.2	2.6/5.7	3.7/NAV	4.6/NAV
$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (µg·hr/mL) [d]	12.5/15.8	28.5/37.2	70.6/	118.7/NAV

[a] Parameters were generated from composite mean plasma concentration-versus-time data.
[b] NAV=not available (insufficient data to estimate parameter)
[c] NC=not calculable.
[d] $AUC_{0-\infty}$ for day 1 and $AUC_{0-\tau}$ for day 28.
C_{max}=maximum observed plasma drug concentration; t_{max}=time to maximum observed plasma drug concentration; $AUC_{0-\infty}$=area under the plasma drug concentration by time curve from time zero to infinity; AUC_{0-t}=area under the plasma drug concentration versus time curve from time zero to the time of the last measurable plasma drug concentration; $AUC_{0-\tau}$=AUC from time zero through 1 dosing interval.

As noted in the pharmacokinetic studies, the systemic exposures were higher in female rats than in male rats and, in groups having both single-dose and multiple-dose datasets, were higher on day 28 than on day 1. Values of C_{max} and AUC were dose-related over the dose range tested, but increases in AUC were more than dose-proportional over portions of the dose range, suggesting that 1 or more of the pharmacokinetic characteristics of ESK981 are dose-dependent.

Two 4-week oral toxicity studies of ESK981 have been conducted in monkeys. In the first study (study DS-2006-020), daily doses of 0 (vehicle only), 5, 10, and 25 mg/kg were administered as suspensions of the free base in Ora-Plus oral suspending vehicle. Due to a subsequent change in the form of the compound to its tosylate salt, a second 4-week study (study DS-2006-042) was conducted testing daily doses of 0 (vehicle only) and 3 mg/kg. Key toxicokinetic parameters from the study are tabulated in Table 7.

Table 7. Toxicokinetics Results for the 4-Week Oral Toxicity Studies in Monkeys.

	Day 1/Day 28			
Parameter[a]	**5 mg/kg**	**10 mg/kg**	**25 mg/kg**	**3 mg/kg**
Males (day 1/day 28)				
C_{max} (µg/mL)	0.061±0.026/0.093±0.058	0.53±0.33/0.67±0.23	0.70±0.27/0.11[c,e]	0.24±0.12/0.20±0.09
t_{max} (hr)	4 (all 4)/3 (2-6)]	4 (4-6)/2 (2-4)	4 (2-6)/8[c,e]	4 (2-4)/2 (2-4)
Half-life (hr)	1.4 (n=1)/1.9	2.1/2.0	2.3/NC[d]	2.2/2.0
$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (µg·hr/mL)[f]	0.24±0.13 ($AUC_{0-\tau}$)/ 0.86±0.18	3.65±2.31/3.13±1.58	5.23±2.24/1.32[c]	1.30±0.71/1.19±0.39
Females (day 1/day 28)				
C_{max} (µg/mL)	0.12±0.09/0.06±0.04	0.44±0.27/0.43±0.32	0.59±0.40/0.46±0.28[e]	0.20±0.09˙0.14 ± 0.05
t_{max} (hr)	2 (2-4)/3 (2-4)	4 (4-6)/3 (2-4)	5 (4-6)/2 (all 2)[e]	2 (2-4)/2 (2-4)
Half-life (hr)	1.6/1.8	1.6/1.6	1.9/1.6[e]	2.0/2.2
$AUC_{0-\infty}$ or $AUC_{0-\tau}$ (µg·hr/mL)[f]	0.86±0.18/0.27±0.23	3.13±1.58/2.5±1.99	3.87±2.28/2.92±2.73	1.19±0.39/0.83±0.31

[a] Parameters were generated from composite mean plasma concentration-versus-time data.
[b] Values are mean±standard deviation, except t_{max}, which is median (range), and half-life, which is harmonic mean.
[c] n=1 due to mortality in the group.
[d] NC = not calculable.
[e] Sampled on day 14 or 15 of dose administration.
[f] $AUC_{0-\infty}$ for day 1 and $AUC_{0-\tau}$ for day 28.
C_{max}=maximum observed plasma drug concentration; t_{max}=time to maximum observed plasma drug concentration; $AUC_{0-\infty}$=area under the plasma drug concentration by time curve from time zero to infinity; AUC_{0-t}=area under the plasma drug concentration versus time curve from time zero to the time of the last measurable plasma drug concentration; $AUC_{0-\tau}$=AUC from time zero through 1 dosing interval.

The monkeys did not show the sex-related differences in systemic exposure that were evident in the rats, but there was a suggestion, as in the pharmacokinetic studies, that some aspect(s) of the pharmacokinetics of the compound might be dose-dependent, especially at higher doses.

Overall, the pharmacokinetic and ADME properties of ESK981 have been studied *in vitro* using blood and tissue fractions from mice, rats, rabbits, dogs, monkeys, and humans and *in vivo* in mice, rats, and monkeys. The estimated half-life in rats and monkeys after an intravenous dose is approximately 2 to 3 hours. Despite its low aqueous solubility, ESK981 is generally well absorbed in mice, rats, and monkeys at dose levels associated with pharmacologic responses in preclinical disease models. At higher doses, evidence of dose-dependent pharmacokinetics has been obtained in all three species. After an oral or intravenous dose, [14C]-ESK981-derived radioactivity is broadly distributed into tissues, generally at concentrations higher than those in blood. Only in barriered tissues, *e.g.*, brain and testis, are concentrations lower than those in blood. The compound is extensively metabolized, but is also excreted in rats via the bile as the parent drug. Excretion is predominantly in the feces in both rats and monkeys.

6.4 Dosage Selected, Preparation, and Schedule of Administration

Treatment will be administered on an outpatient basis.

6.4.1 Supply and packaging

ESK981 monotosylate is a fully synthetic drug substance containing a core indolocarbazolone ring system. ESK981 monotosylate drug substance is a yellow to orange powder that has a molecular weight of 649.77 g/mol. The drug substance contains 73.5% theoretical weight percent of the active moiety ESK981 (free base).

ESK981 drug product is an off-white to orange opaque, hard gelatin capsule containing 40 mg (free base equivalents) of ESK981 monotosylate. The drug product is packaged in a

high-density polyethylene (HDPE) bottle with a child resistant closure and an induction-sealed inner seal. The formulation also contains lactose monohydrate.

6.4.2 Storage and handling

ESK981 capsules should be stored refrigerated at 2°C to 8°C (36°F to 46°F). The stability of the drug substance and drug product continues to be monitored.

6.5 Dose Modifications

Patients with NCI-CTCAE Grade 3 or Grade 4 adverse events judged to be (at least possibly) related to ESK981 will have their ESK981 held until the adverse event resolves to Grade 2 or lower. Standard management of adverse events should be utilized. Patients may then be restarted on ESK981 at a reduced dose of 120mg (3 capsules) daily for 5 days, 2 days off. Following a dose reduction, patients who have a second NCI-CTCAE Grade 3 or Grade 4 adverse event judged to be at least possibly related to ESK981 will be discontinued from the study. If drug cannot be restarted within 3 weeks of being held, ESK981 should be discontinued. Patients will also be discontinued for any of the following reasons:

1. Worsening symptoms that can be attributed to prostate cancer
2. Unacceptable adverse event(s) including development of a urinary outlet obstruction requiring urinary catheterization and/or surgical intervention,
3. Intercurrent illness that prevents further participation,
4. Patient refuses further treatment through the study and/or withdraws consent to participate,
5. Patient is noncompliant with respect to taking drugs, keeping appointments, or having tests required for the evaluation of drug safety and efficacy, or
6. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in this study in the judgment of the investigator.

Under no circumstance will care of a withdrawn patient be adversely affected by a decision to withdraw or be withdrawn from the study

6.6 Management of Expected Toxicities

Event Name	**Nausea**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: antiemetics.	

Event Name	**Vomiting**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: antiemetics.	

Event Name	**Diarrhea**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Loperamide anti-diarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.	

Event Name	**Neutropenia**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Use of growth factors is not recommended. Management of neutropenia should be by dose reduction. For grade 4 febrile neutropenia, discontinue treatment.	

Event Name	**Anemia**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Treat by using local standards.	

Event Name	**Thrombocytopenia**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Treat by using local standards.	

Event Name	**Hypertension**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5** .
Recommended management: Treat by optimizing or adding antihypertensive medications to achieve well-controlled blood pressure of < 150/90 mmHg.	

Event Name	**Left Ventricular Dysfunction**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Treat by optimizing or adding cardiovascular medications for management of congestive heart failure.	

Event Name	**Cardiac Arrhythmias**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Identify appropriate arrhythmias such as atrial fibrillation, atrial flutter, atrioventricular blocks, and other conduction disorders. Treat appropriate medications or procedures as established by standard of care.	

Event Name	**Fatigue**
Grade of Event	**Management/Next Dose**
Grade 1	No change in dose
Grade 2	No change in dose
Grade 3-4	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated per **Section 6.5**.
Recommended management: Optimize supportive care.	

6.7 Concomitant Medications and Supportive Care

Because of the potential for drug-drug interaction, the concurrent use of all other drugs, over-the-counter medications, and alternative therapies must be documented on the CRF. The principal investigator should be alerted if the patient is taking any agent found in Appendix B (a listing of medications with the potential for drug-drug interaction).

6.7.1 Supportive Care Medications

Patients should receive appropriate supportive care for CRPC and appropriate treatment for comorbidities, subject to the restrictions due to potential drug-drug interactions detailed in Section 6.7.3.

6.7.2 Prohibited before enrollment and during administration of study treatment

Concomitant systemic treatments for CRPC (other than a GnRH agonist/antagonist) are prohibited including: CYP-17 inhibitors (e.g. ketoconazole, abiraterone), antiandrogens (e.g. bicalutamide, nilutamide), second generation antiandrogens (e.g. enzalutamide, ARN-509, Galeterone), immunotherapy (e.g. sipuleucel-T, ipilimumab), and chemotherapy (e.g. docetaxel, cabazitaxel).

6.7.3 Potential for drug-drug interactions

ESK981 is metabolized via CYP1A2, CYP2C8, and CYP3A4. Inhibitors of CYP1A2, CYP2C8, and CYP3A4 have the potential to increase plasma concentration of ESK981. Inducers of

CYP1A2, CYP2C8, and CYP3A4 have the potential to decrease plasma concentrations of ESK981. Concomitant use of a medication known to be a potent inhibitor of CYP1A2, CYP2C8, or CYP3A4 (see Appendix B), or a potent inducer of CYP1A2, CYP2C8, or CYP3A4 (see Appendix B) should be avoided unless deemed to be medically necessary by the investigator. In addition, nonclinical studies have identified the potential for ESK981 to inhibit CYP3A4/5 (Ki 2.2μM). ESK981 has the potential to increase plasma concentration of concomitant mediations that are CYP3A4/5 substrates. Caution should be used, or alternative treatments considered, if concomitant treatment with CYP3A4/5 substrates that have a narrow therapeutic range is needed (see Appendix B).

7. SAFETY EVALUATION

A safety review of the first six patients who have been observed for 8 weeks will occur. Enrollment will pause until all six patients have been assessed for toxicity evaluation.

7.1 Definitions

7.1.1 Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment (ICH E2A). An adverse event for the purposes of this protocol is the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) occurring after signing the informed consent even if the event is not considered to be related to the study drug(s). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

An abnormality identified during a medical test (e.g. laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets one of the following criteria:

- Requires active intervention
- Requires interruption or discontinuation of study medication

The abnormality or investigational value is clinically significant in the opinion of the investigator. An adverse event will be recorded and followed from treatment administration to 30 days post-treatment or resolution.

7.1.2 Expected Adverse Events

Expected adverse events are those that have been previously identified as resulting from administration of the agent. An adverse event can be considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

7.1.3 Unexpected Adverse Events

Adverse reactions are those that the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product) (ICH E2A).

Contact the Principal Investigator or sponsor to confirm unexpected adverse events when necessary.

7.1.4 Adverse Drug Reaction (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions (ICH E2A).

The phrase "response to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, *i.e.*, the relationship cannot be ruled out. ‘‘Reasonable possibility’’ means there is evidence to suggest a causal relationship between the drug and the adverse event.

7.1.5 Serious Adverse Event (SAE)

An SAE/ADR as defined in the Code of Federal Regulations (21 CFR § 312.32) is any event that:

- results in death
- is life-threatening
- results in inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect
- is medically significant in the opinion of the investigator

Events that are **not** considered serious adverse events include:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and did not worsen since signing the informed consent
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

7.1.6 Progression of malignancy

Progression of a patient’s malignancy should not be considered an AE, unless in the investigator’s opinion, study treatment resulted in an exacerbation of the patient’s condition. If disease progression results in death or hospitalization while on study or within 30 days of the last dose, progressive disease will be considered an SAE.

7.1.7 Life-threatening

An adverse event or suspected adverse reaction is considered “life-threatening “if, in the view of either investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death (FDA 21 CFR § 312.32).

7.1.8 Hospitalization (or prolongation of hospitalization)

Hospitalization encompasses any inpatient admission (even for less than 24 hours) resulting from a precipitating, treatment-emergent adverse event. For chronic or long-term patients, inpatient admission also includes transfer within the hospital to an acute or intensive care inpatient unit. Hospitalizations for administrative reasons or a nonworsening preexisting condition should not be considered AEs (e.g., admission for workup of a persistent pretreatment laboratory abnormality, yearly physical exam, protocol-specified admission, elective surgery). Preplanned treatments or surgical procedures should be noted in the baseline documentation. Hospitalization because of an unplanned event will be deemed an SAE.

Prolongation of hospitalization is any extension of an inpatient hospitalization beyond the stay anticipated or required for the original reason for admission.

7.1.9 Persistent or Significant disability/incapacity

Any AE that results in persistent or significant incapacity or substantial disruption of the patient's ability to conduct normal life functions.

7.1.10 Congenital anomaly

If the female partner of a male patient becomes pregnant during the course of the study, the treating physician must be notified immediately. All confirmed pregnancies must be immediately reported to the sponsor-investigator. All pregnancies will be followed until resolution (*i.e.*, voluntary or spontaneous termination or birth) and assessed for congenital anomalies and birth defects.

7.2 Recording and Grading of Adverse Events

7.2.1 Recording

All observed or volunteered adverse events, regardless of treatment group, severity, suspected causal relationship, expectedness, or seriousness will be recorded on the Medical History/Current Medical Conditions Electronic Case Report Form.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

a. The severity grade (CTCAE grade 1-4)

b. Its relationship to each study drug (suspected/not suspected)

c. Its duration (start and end dates or if continuing at final exam)

d. Action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)

Adverse events (but not serious adverse events) occurring before starting study treatment, but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions Electronic Case Report Form. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant or require therapy (e.g., any hematologic abnormality that requires

transfusion or cytokine treatment), and should be recorded on the Adverse Events CRF under the signs, symptoms or diagnosis associated with them. In addition, isolated abnormal laboratory values that are considered clinically significant (*e.g*., cause study discontinuation or constitutes in and of itself a Serious Adverse Event) should be recorded on the Adverse Events CRF. SAEs occurring after the start of treatment are recorded on the Adverse Event CRF.

A clinically significant change in a physical examination finding or an abnormal test result should be recorded as an AE, if it:

- is associated with accompanying symptoms,
- requires additional diagnostic testing or medical or surgical intervention,
- leads to a change in study dosing or discontinuation from the study,
- requires additional concomitant drug treatment or other therapy, or
- is considered clinically significant by the investigator.

An abnormal test result that is subsequently determined to be in error does not require recording as an adverse event, even if it originally met one or more of the above criteria.

7.2.2 Grading severity

Adverse events will be assessed according to the Common Toxicity Criteria for Adverse Events [CTCAE] version 4.03. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, **or** Grades 1 - 4, will be used. CTCAE Grade 5 (death) will not be used in this study; rather, this information will be collected in the End of Treatment or Survival Information CRF page. Adverse event monitoring should be continued for at least 4 weeks following the last dose of study treatment.

7.2.3 Attributing causality

After assigning a grade to an adverse event, the investigator must evaluate all AEs for possible causal relationship to the investigational agent. Causality attribution will be decided using the criteria outlined in Table .

Table 8. Relationship of Adverse Event to Study Drug

Relationship	Description
Unrelated	AE is clearly not related
Unlikely	AE is doubtfully related
Possible	AE may be related
Probable	AE is likely related
Definite	AE is clearly related

7.3 Adverse Events Reporting

7.3.1 Regulatory and reporting requirements

These will be followed per IND requirements (if applicable) as well as sponsor requirements and WSU IRB requirements.

7.3.2 WSU IRB Adverse Event Reporting Guidelines

Unexpected Problem – Risk to participant or others. A problem that is a) unforeseen, b) indicates that participants or others are at increased risk of harm, and c) is related or possibly related to the research. The following are examples of unexpected problems:

1. Adverse Event: Any harm experienced by a participant regardless of whether the event was internal (on-site) or external (off-site) and regardless of whether the event meets the FDA definition of "serious adverse event", which in the opinion of the principal investigator are both **unexpected, related (definitely, probably, or more likely than not), and suggests that participants are at greater risk than was previously known or recognized.**
    a. An adverse event is "unexpected" when its specificity and severity are not accurately reflected in the informed consent document, the protocol or the investigator's brochure.
    b. An adverse event is "related to the research procedures" if in the opinion of the principal investigator it was more likely than not to be caused by the research procedures, or if it is more likely than not that the event affects the rights and welfare of current participants.
2. **Any harm experienced by a participant or others** as a result of involvement in research activities (internal or external **excluding** adverse events).
3. Information that indicates a **change to the risks or potential benefits** of the research. For example:
    a. An interim analysis or safety monitoring report indicates that frequency or magnitude of harm or benefit may be different than initially presented to the IRB.
    b. A paper is published from another study that shows the risks or potential benefits to your research may be different than initially presented to the IRB.
    c. Study put on hold by the PI, FDA, or the Sponsor for reasons that may include safety, toxicity and/or efficacy.
4. **A change in FDA labeling** or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
5. **Change to the protocol** taken without prior IRB review to eliminate an apparent immediate hazard to a research participant.
6. **Research conducted without prior WSU IRB approval.**
7. **Event that requires prompt reporting to the sponsor.**
8. **Unanticipated adverse device effect**: Any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device if that effect, problem, or death was not previously identified in natured, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.
9. **Sponsor-imposed suspension for risk.**
10. **Complaint of a participant** when the complaint indicates unexpected risks or cannot be resolved by the research team.
11. **A breach of confidentiality.**

12. **Protocol violation/deviation** (meaning an accidental or unintentional change to the IRB-approved protocol) that harmed participants or others, that indicates participants or others may have been placed at increased risk of harm, or rights of research participants were violated. For example:
    a. Failure to draw safety labs
    b. Request for continuation submitted late to the IRB three years in a row
    c. Wrong informed consent signed or failure to obtain informed consent
13. **Incarceration of a participant** in a protocol not approved to enroll prisoners.
14. **All deaths that have happened at WSU or one of its affiliates within 30 days of the last study intervention, and not related to progressive disease.**
15. **Any death, if the PI feels that it is significant** no matter when it occurs.

IRB Policy

Principal investigators must report any of the above to the IRB as soon as possible, **but in all cases within 5 working days.**

7.4 Serious Adverse Event Reporting

Within 24 hours of awareness of a serious adverse event, whether or not related to the study drug, the investigator will complete and submit a Medwatch 3500a form, containing all required information (reference CFR § 312.32). The investigator will submit a copy of this Medwatch 3500a form to the sponsor-investigator and Esanik by either e-mail or fax, within the same timeframe.

The SAE documentation, including the Medwatch 3500A form and available source records should be emailed or faxed to:

Email: drugsafety@esanik.com

Fax: 610-408-0321

The following minimum information is required:

- study number
- subject number, sex and age
- the date of report
- a description of the SAE (event, seriousness of the event)
- causal relationship to the study drug. Follow-up information for the event should be sent within 7 days as necessary. Copy of above should also be sent to coordinating site (PI- Dr. Elisabeth Heath).

Sponsor-Investigator:
Elisabeth I. Heath, MD FACP
4100 John R
Detroit, MI 48201

Tel: 313-576-8717
Fax: 313-576-8767
Email: heathe@karmanos.org

7.4.1 Safety Reporting Requirements for IND Holders

In accordance with 21 CFR § 312.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

Expedited IND Safety Reports:

7.4.2 Calendar-Day Telephone or Fax Report:

The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of ESK981. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

15 Calendar-Day Written Report:

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the use of ESK981. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Esanik Drug Safety, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter).

FDA fax number for IND Safety Reports:

1 (800) FDA-0178

IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report. All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Esanik.

7.5 Procedure in Case of Pregnancy

The effect of ESK981 in pregnant and lactating women is not known, and the exposure of a fetus or nursing infant is considered a potential risk. ESK981 can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Subjects receiving ESK981 are advised to use two acceptable methods of birth control (one of which must include a condom as a barrier method of contraception) starting at the time of screening for an ESK981 study and continuing throughout the course of treatment and for at least three months after ESK981 is discontinued.

If, during the conduct of the clinical trial, a male subject impregnates his partner, the subject should report the pregnancy to the investigator. The investigator should report the pregnancy to the sponsor as an SAE within 24 hours of awareness of the event. The expected date of delivery or expected date of the end of the pregnancy, last menstruation,

estimated fertility date, pregnancy result and neonatal data etc., should be included in this information.

The investigator should report the outcome of the pregnancy (independent of outcome, e.g., full term delivery, pre-term delivery, spontaneous abortion, induced abortion, stillbirth, death of newborn, congenital anomaly [including anomaly in a miscarried fetus, etc.]) in accordance with the same reporting procedure as for SAEs. The date of outcome of the pregnancy, gestational age, date of birth and neonatal data etc., should be included in this information.

8. CRITERIA FOR OUTCOME ASSESSMENT/THERAPEUTIC RESPONSE

8.1 Outcome Assessment

All baseline evaluations will be performed as closely as possible to the beginning of treatment (<4 weeks). For subsequent evaluations, the method of assessment and techniques will be the same as those used at baseline.

8.1.1 Measurement of clinical lesions

Only superficial clinical lesions (e.g., skin nodules and palpable lymph nodes) are considered measurable. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

8.1.2 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 to x-ray.

8.1.3 Conventional CT, MRI, positron emission tomography (PET), bone scan

To assess the antitumor effect of a treatment, imaging-based evaluation is preferable to evaluation by clinical examination. CT and MRI should be performed using contiguous slices of 10 mm or less. Spiral CT should be performed using a 5-mm contiguous reconstruction algorithm (interval). If a 5-mm contiguous reconstruction algorithm (interval) is not used, as a general rule, the lesion diameter should be no less than double the reconstruction algorithm (interval) and must be at least 20 mm for a nonspiral CT or at least 10 mm for spiral CT. (If available, spiral CT is preferred in all instances.) A technetium-99m bone scan will be performed at screening and at each tumor assessment during the study to evaluate bone metastasis. For adequate assessment of bone lesions, it is expected that the radiologist will adjust window leveling accordingly. PET scan will be used for bone scan if requested by physician. This applies to tumors of the chest, abdomen, pelvis and bone. Head and neck tumors and those of the extremities typically require specific protocols.

8.1.4 Ultrasound

When the primary endpoint of the study is objective response, ultrasound should not be used to measure tumor lesions. Ultrasound may be appropriate, however, as a possible alternative to clinical measurements of superficial, palpable lymph nodes, subcutaneous lesions, or thyroid nodules, or to confirm the complete disappearance of superficial lesions (usually assessed by clinical examination).

8.1.5 Endoscopy/laparoscopy

Using endoscopy of laparoscopy for objective tumor evaluation has not yet been fully validated and should be restricted. These techniques may be useful, however, to confirm complete pathological response when biopsies are obtained.

8.1.6 Tumor markers

Tumor markers alone cannot be used to assess response. If markers are initially above the ULN, they must normalize for a patient to be considered in complete clinical response.

8.1.7 Cytology/histology

In certain situations, cytology and histology can be used to differentiate response (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). When the measurable tumor has met criteria for response or stable disease, cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment is mandatory to differentiate between response, stable disease, and progressive disease.

8.2 Therapeutic Response

Response and progression will be evaluated in this study using a combination of the revised Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria[15] and the guidelines for prostate cancer endpoints developed by the Prostate Cancer Clinical Trials Working Group (PCWG3).[3]

Trial objectives are defined based on controlling, relieving, or eliminating disease manifestations that are present when treatment is initiated, or preventing or delaying the development of disease manifestations that are expected to occur (or in some cases both). Identify whether control/relieve/eliminated or prevent/delay endpoints. Traditional measures of response reflect when a treatment is working and measures of progression indicate when a drug should be stopped. Because assessing response in bone (the most common site of prostate cancer spread) is uncertain and the clinical significance of PSA changes in response to therapy is not a reliable predictor of response, measures of response have been expanded in consortium trials to include measures of progression.

Patients will need to be reevaluated for response every cycle (or more frequently if indicated), according to the guidelines below.

8.2.1 PSA

In this study, PSA testing is being performed on days 8, 15, and 22 of Cycle 1, Day 1 of Cycle 2 and each subsequent cycle (every 4 weeks) with the threshold PSA level at 2.0 ng/mL. To report PSA-based outcomes, PCWG3 recommends that the percent of change in PSA from baseline to 8 weeks (or earlier for those who discontinue therapy) and the maximum decline in PSA that occurs at any point after treatment be reported for each patient using a waterfall plot.[3] Because the rate of rise has shown prognostic significance, estimate a pretreatment PSA doubling time (PSA-DT) if at least 3 values are available, but do not delay either treatment or enrollment onto a trial simply to estimate PSA-DT. Because declines in serum PSA, if they occur, may not do so for several weeks, PSA measurements obtained during the first 8 weeks should not be used as the sole criterion for clinical decision making.

8.2.2 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT or MRI scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters. NOTE: tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to

include then, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT or MRI scan (CT scan slice thickness recommended t be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease and abdominal masses (not followed by CT or MRI) are considered as non-measurable. Non-measurable also includes lesions that are < 20mm buy chest x-ray. Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable or non-measurable) sine they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 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 (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow- up.

8.2.3 Methods for Evaluation of Disease

Conventional CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up must be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases.

Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

8.2.4 Methods for Evaluation of Bone Disease

Evaluation of Radionuclide Bone Scans: Bone disease will be evaluated using radionuclide bone scan. NOTE: Will follow PCWG3

Interpretation of serial changes in a radionuclide bone scan is well recognized to be highly subjective. Thus, the primary outcome will be whether the bone scan is stable or improved, vs. worse or progression. Changes in intensity will not be used as an outcome measure. The 2+2 rule will be applied for interpretation of bone scans:

Stable or Improved: Include the appearance of at least 2 new skeletal lesions on the first post-treatment scan performed 6 or more weeks later or changes in intensity of a preexisting lesion will be considered stable disease unless associated with other signs of progression.

Progression (Non-Response): Must be documented as the appearance of at least two additional new lesions on the next scan after the first 2 new lesions were noted. If at least two additional new lesions are seen on the next (confirmatory) scan the date of progression is the date of the first post treatment san when the first two new lesions were documented. *An increase in the size or intensity of known skeletal lesions will not be considered progression*.

8.3 Response Criteria

8.3.1. Evaluation of Target Lesions

Complete Response (CR)

Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR)

At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. NOTE: The appearance of one or more new lesions is also considered progression (see "Evaluation of New Lesions" below).

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. (Note: a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease)

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 8 weeks.

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 (e.g., fine needle aspirate or biopsy) before confirming the complete response status.

Changes in nodal and visceral sites should be recorded and reported separately, and lymph nodes in the pelvis must measure at least 2 cm in greatest diameter to be considered target lesions. Complete elimination of disease at a particular site should be recorded separately. Any favorable change should be confirmed using a second follow-up scan.

8.3.2 Evaluation of Non-Target Lesions

Complete Response (CR)

Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Non-CR/Non-PD

Persistence of one or more non-target lesion(s) and/or the maintenance of tumor marker levels above the normal limits.

Progressive Disease (PD)

Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (see "Evaluation of New Lesions" below). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

When the patient also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

8.3.3 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it:

- increases in size to ≥ 15 mm in the short axis, or
- there is new pathological confirmation that it is disease (regardless of size).
- new effusion or ascites that appears during treatment should only be reported as a new lesion (and therefore progressive disease) if it has cytological confirmation of malignancy.

8.3.4 PSA Response

As long as patient safety is the primary concern, in the absence of other indicators of disease progression, therapy should not be discontinued solely on the basis of a rise in PSA after week 8.

PSA progression is defined as the date that a 25% or greater increase and an absolute increase of 2.0 ng/mL or more from the nadir is documented and confirmed by a second value obtained 3 or more weeks later. Where no decline from baseline is documented, PSA progression is defined as a 25% increase from the baseline value along with an increase in absolute value of 2.0 ng/mL or more after 8 weeks.

8.3.5 Evaluating best overall response

The best overall response is the best response recorded from the start of treatment until either disease progression or recurrence. The investigator's determination of best overall response will be based both on response criteria and on confirmation criteria (Table 9). To be assigned a status of partial response or complete response, changes in tumor measurements must be confirmed by repeat assessment performed 4-6 weeks after the criteria for response are first met. To confirm stable disease, follow-up measurements must meet SD criteria at a minimum interval of 6-8 weeks after SD was first documented.

Note: If unconfirmed progression noted at the first time point is not confirmed at a second time point, the next assessment time point after the first that meets the criteria for progression will be treated as time point 1.

Table 9. Assessing Overall Response.

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

Abreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Subjects with global deterioration of health status who require treatment to be discontinued without objective evidence of disease progression should be classified as having symptomatic deterioration. Every effort should be made to document their objective progression, even after discontinuing treatment.

Subjects who do not have tumor response assessment due to rapid progression or toxicity will be considered nonresponders, will be included in the denominator for the response rate, and will be classified into one of the categories listed below:

- death attributed to disease progression
- early discontinuation attributed to disease progression
- death attributed to drug toxicity
- early discontinuation attributed to drug toxicity

Note: If a subject receives subsequent therapy before tumor progression is documented, the reason for changing therapy must be reported. Reasons include clinical progression, drug toxicity, or secondary therapy for maintaining tumor response.

8.4 Confirmatory Measures/Duration of Response (see Table 10 for specific PCWG3 measures)

8.4.1 Confirming time-to-event outcomes

Any post-treatment change in disease status, be it favorable or unfavorable, should be confirmed using a second assessment at a later time 6-8 weeks.

8.4.2 Duration of overall response

Duration of overall response is measured from the time when partial response or complete response is first noted until the date when recurrent or progressive disease is objectively documented. Duration of overall complete response is measured from the time the criteria for complete response are first met until the first date that recurrent disease is objectively documented. Duration of stable disease is measured from the start of treatment until the criteria for progression are met.

8.4.3 Progression-free survival

Progression-free survival (PFS) is a composite endpoint defined as the time from study entry or random assignment to disease progression in bone or soft-tissue, symptoms, or death. Use an interval-censored approach in which all assessments of the composite PFS endpoint (i.e., PSA, bone, CT scans, and symptom assessments) are performed at the same time points. All assessments of disease should be collected at the same time interval (e.g., bone scan, CT scan, and PSA at 12-week intervals). In addition to PSA, confirm post-treatment changes in measurable target lesions, radionuclide bone scans, and symptoms.

Table 10. Prostate Cancer Clinical Trials Working Group 3 (PCWG3) Outcome Measures.

Variable	Control/Relieve/Eliminate Endpoints	Prevent/Delay Endpoints
PSA	Record the percent change from baseline (rise or fall) at 8 weeks and, separately, the maximal change (rise or fall) at any time using a waterfall plot	**Decline from baseline:** record time from start of therapy to first PSA increase that is ~25% and ~2.0 ng/mL above the nadir, and which is confirmed by a second value 3 or more weeks later (i.e., a confirmed rising trend) † Recording the duration of PSA decline of little value **No decline from baseline:** PSA progression ~25% and ~2.0 ng/mL after 8 weeks
Soft-tissue lesions	Use RECIST with caveats: Only report changes in lymph nodes that were ~2 cm in diameter at baseline Record changes in nodal and visceral soft tissue sites separately Record complete elimination of disease at any site separately Confirm favorable change with second scan Record changes using waterfall plot	Use RECIST criteria for progression, with additional requirement that progression at first assessment be confirmed by a second scan 6 or more weeks later Note that for some treatments, a lesion may increase in size before it decreases
Bone	Record outcome as either *new lesions* or *no new lesions* First scheduled reassessment: No new lesions: continue therapy New lesions: perform a confirmatory scan 6 or more weeks later Confirmatory scan: No new lesions: continue therapy Additional new lesions: progression Subsequent scheduled reassessments: No new lesions: continue New lesions: progression	The appearance of ~2 new lesions, and, for the first reassessment only, a confirmatory scan performed 6 or more weeks later that shows at least 2 or more additional new lesions The date of progression is the date of the first scan that shows the change
Symptoms	Consider independently of other outcome measures Document pain and analgesia at entry with a lead-in period and measure repeatedly at 3- to 4-week intervals Perform serial assessments of global changes in HRQOL, urinary or bowel compromise, pain management, additional anticancer therapy Ignore early changes (~12 weeks) in pain or HRQOL in absence of compelling evidence of disease progression Confirm response or progression of pain or HRQOL endpoints ~3 weeks later	

Abbreviations: PSA, prostate-specific antigen; HRQOL, health-related quality of life.
†Particularly important when anticipated effect on PSA is delayed or for biologic therapies.

9. CORRELATIVES

The correlative assessments in this section are proposed as tertiary or exploratory objectives of the study. If exceptional responders/non-responders are observed, the comprehensive sequencing based profiling of tumors is expected to delineate putative molecular biomarkers, and refine the understanding of ESK981's mechanism of action.

9.1 The Association of Somatic and Germline Mutations with Exceptional Response/Resistance to ESK981

In addition to the prevalent TP53 mutations, AR amplifications, and ETS-fusions, mutations in the PTEN-PI3K-AKT pathway as well as germline and somatic events in the DNA repair pathway are frequently observed in mCRPC tumors. We propose integrative genomic sequencing to comprehensively profile the spectrum of genetic aberrations. The specific endpoints will be: activation through a hotspot mutation or amplification (gain-of-function), and inactivation through two-hit missense/nonsense mutation or deletion (loss-of-function) for genes from select pathways recurrently altered in mCRPC. Somatic and germline mutations will be identified through DNA sequencing of tumor and matched patient normal tissue utilizing the Oncoseq1700 platform. NGS-based analyses of copy-number alterations (CNA) and loss-of-heterozygosity (LOH) will be employed to probe for focal amplifications (e.g. AR, CCND1, PIK3CB) and deletions (e.g. PTEN, RB1).

9.2 The Association of ETS / Kinase Gene Fusions with Exceptional Response/Resistance to ESK981

Capture RNA sequencing will be used to detect further genetic events, such as activating and inactivating genes fusions. It is expected that this will identify activating ETS fusions (ERG, ETV1, ET4, ETV5), driver kinase fusions (BRAF, RAF1, FGFR2, FGFR3), as well as additional inactivating events in tumor suppressors (e.g. PTEN, TP53, RB1, BRCA1/2, ATM). To increase statistical power, mutations will be evaluated for association with clinical outcomes, both independently and grouped by molecular pathways: PTEN-PI3K-AKT, homologous recombination (e.g. BRCA1, BRCA2, PALB2), cell-cycle checkpoints (e.g. RB1, CCND1).

9.3 The association of AR signaling with Exceptional Response to ESK981

Furthermore, since modulation of androgen receptor (AR) signaling through tyrosine kinases is observed as a mechanism of resistance to androgen deprivation therapy and androgen-independence, we hypothesize that the loss of dependence on AR signaling will be an additional independent predictor of response and outcomes. The trial is designed to enrich for AR-independent patients in the Enza/Abi-refractory cohort who will allow us to evaluate our hypotheses. Aggregate scores of AR-dependence and AR-independence will be derived from the expression levels of canonical AR targets and trans-differentiation markers.

9.4 Metastatic Kinome Activity Profiles as Predictive Biomarkers for Response to ESK981

Since the mechanism of ESK981 is based on the multiplex inhibition of a set of protein kinases we hypothesize that a tumor's dependence on kinase signaling pathways (kinome activity profile) will correlate with sensitivity to multiplex kinase inhibition by ESK981. Similarly, kinome activity profiles will be quantified through the expression levels of individual tyrosine and serine/threonine kinases targeted by ESK981 and, by proxy of gene expression signatures, the activity of their downstream signaling pathways. The activity of each targeted kinase will be correlated with the clinical endpoints independently, and in conjunction with the tumor's AR status and other genetic alterations.

9.5 Evaluation of Circulating and Disseminated Tumor Cells as Pharmacodynamic Biomarkers of ESK981 response.

The burden of circulating (CTC) and disseminated tumor cells (DTC) has been associated with survival in a number of solid tumors. Further, a decrease in the number of CTC is a reliable indicator of response for a number of oncological therapies. Therefore, we hypothesize that ESK981 treatment will reduce the burden of CTCs in the blood and DTCs obtained from the bone marrow. Blood samples will be collected at intervals of 4 weeks for the total duration of treatment (from screening to end of treatment). Circulating Tumor Cells (CTC) will be enriched from 7.5ml of whole blood using the AdnaTest ProstateCancerSelect assay. The expression of marker genes associated with androgen signaling, cell proliferation, kinome activity, autophagy, will be assessed pre-amplified cDNA (AdnaTest ProstateCancerDetect) using qPCR. In addition, blood samples will be processed using RareCtye platform for further characterizing CTCs in single cell level. Similarly, to detect DTCs bone marrow aspirates will be collected. DTCs will be enriched by negative (lymphocyte markers) and positive (epithelial markers) selection using flow cytometry. The enriched DTCs will be profiled for single-cell gene expression using the 10X genomics sequencing platform (The Chromium System). Specifically, we will evaluate whether clinical response is associated with: (i) the pre-treatment CTCs/DTC burden, (ii) a reduction in CTCs/DTCs during response, (iii) changes CTC/DTC expression profiles. Forty (40) ml of whole blood will be collected at each respective time-point, please see lab manual for collection and processing guidelines.

9.6 Pathological assessment of phenotypic tumor and host responses to ESK981 treatment.

ESK981 treatment results in a striking and conserved phenotype (autophagy) in preclinical *in vitro* and *in vivo* models. However, the drug can in principle target other kinases expressed in normal cells from the tumor microenvironment for example. To differentiate between the direct and indirect effects of ESK981, we will collect tumor biopsies from baseline and 8 weeks of treatment for histopathological evaluation. We will perform immunohistochemistry (IHC) for the following protein markers: Ki67 (cell proliferation); AR (androgen receptor); CD31, NG2 and desmin (angiogenesis); PDGFR1/2, VEGFR1/2, Tie2, c-Kit, FGFR (tyrosine kinase receptor); LC3 (autophagy). We will evaluate whether treatment response is associated with a phenotypic change in the tumor histology (vacuolization), microenvironment (vascularization, immune infiltration), and the tumor's' molecular characteristics (AR status, kinome profile).

10. DATA REPORTING AND REGULATORY REQUIREMENTS

10.1 Data Collection and Management

Data collected during this study will be entered into a secure database.

10.1.1 Electronic Case Report Forms (eCRFs)

Data will be collected and maintained on study specific electronic case report forms in the Oncore Research Enterprise system at Karmanos Cancer Institute. Training and support will be provided for all staff entering data upon local regulatory approval. Data entry must occur within 2 weeks of visits along with submission of applicable source documentation. KCI is responsible for ensuring eCRF's are completed accurately and submitted in a timely manner.

10.1.2 Source documents

Source documentation refers to original records of observations, clinical findings and evaluations that are subsequently recorded as data. Source documentation will be made available to support the subject's research record.

10.1.3 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents and study-related documents. Records are to be retained and securely stored until the later of: (a) two (2) years following the date a New Drug Application is approved for the Study Drug that is the subject of the Clinical Trial; or (b) two (2) years after the Investigational New Drug Application for such Study Drug is terminated or withdrawn, or such longer period of time as may be required by Participant policies, applicable laws, rules or regulations.

10.1.4 Data Submission Timelines

All data should be transmitted within 14 days of visit except for SAE submission (see section 7.4).

10.1.5 Data Review and Queries

Karmanos will review data and source documentation as it is submitted. Data will be monitored against source documentation as necessary and discrepancies will be sent as queries. In addition, the Karmanos monitor will review data for logic, consistency, and obvious anomalies. Queries will be sent by the Karmanos monitor.

10.1.6 Protocol Amendments or Changes in Study Conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed and approved. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB. A copy of the written approval of the IRB must be provided. Examples of amendments requiring such approval are:

1. increases in drug dose or duration of exposure of subjects,
2. significant changes in the study design (e.g. addition or deletion of a control group),
3. increases in the number of invasive procedures,
4. addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons PI must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include:

1. changes in the staff used to monitor trials
2. minor changes in the packaging or labeling of study drug

10.2 Data Safety and Monitoring Board

10.2.1 Scheduled meetings will be held monthly or more frequently depending on the activity of the protocol. These meetings will include the protocol investigators and data managers involved with the conduct of the protocol.

10.2.2 During these meetings the investigators will discuss matters related to:

- Safety of protocol participants (Adverse Event reporting)
- Validity and integrity of the data
- Enrollment rate relative to expectation of target accrual, characteristics of participants
- Retention of participants, adherence to the protocol (potential or real protocol violations)
- Data completeness on case report forms and complete source documentation

10.2.3 Completed Data and Safety Monitoring Reports of these regular investigator meetings will be kept on file in the office of the Clinical Trials Core (see form in Appendix F). The data manager assigned to the clinical trial will be responsible for completing the report form. The completed reports will be reviewed and signed off by the Principal Investigator (PI) or by one of the Co-PI's in the absence of the PI. The signed off forms will then be forwarded to the Director, Clinical Trials Core for review of completeness and processing with the Data and Safety Monitoring Committee.

10.2.4 The Barbara Ann Karmanos Cancer Institute, Data and Safety Monitoring Committee will meet on a monthly basis to review the prior months Serious Adverse Event forms and Data and Safety Monitoring study specific reports that have been filed.

11. STATISTICAL CONSIDERATIONS

11.1 Objectives

The **primary objectives** of this study are:

1) To determine the PSA ≥ 50% response rate (PSA50) from baseline using the PCWG3 criteria to ESK981 as a single agent in men with mCRPC who have progressed on enzalutamide (an oral androgen-receptor inhibitor) and/or abiraterone acetate (an androgen synthesis inhibitor).

2) To assess the safety and tolerability of ESK981 as a single agent.

The **secondary** objectives of this study are:

1) To determine the time to PSA response (TTPR) to ESK981 in patients with mCRPC

2) To determine the duration of PSA response (PRD) to ESK981 in patients with mCRPC

3) To determine PSA progression rates as defined by the PCWG3 criteria.

4) To determine PSA progression free survival (PPFS) as defined by the PCWG3 criteria.

The **correlative/exploratory/tertiary objectives** of this study are: to assess exploratory biomarkers from blood and tumor biopsies. Examples of these may include, but are not limited to: whole exome sequencing, capture whole transcriptome analysis, AR and ARv7 analysis, ki-67, apoptosis, etc.

11.2 Endpoints

11.2.1 The first primary endpoint (PSA50) is a PSA decline of ≥ 50% from baseline (PSA50), using the PCWG3 criteria. Refer to Section 8.2.1.

The second primary endpoint(s) (toxicities) is (are) defined in Section 7. Adverse events of all grades will be captured by the National Cancer Institute - Common Terminology Criteria for Adverse Events, version 4.03 (NCI-CTCAE v4.03) and GCP standards.

11.2.2 The secondary endpoints are defined as follows:

Time to PSA response (TTPR) is defined as the time from treatment start until the first documented occurrence of PSA50. PSA50 is defined in Section 1.3.

Duration of PSA response (PRD) is defined as the time from start of PSA50 until PSA progression (defined in Section 8.3.2).

PSA progression free survival (PPFS) is defined in Section 8.3.2 except that it will be based on PSA assessments only. PSA progression rates will be estimated from the PSA-only PFS distribution.

11.2.3 The correlative/exploratory/tertiary endpoints (and the methods of their determination) are described in Section 9.

11.3 Design

11.3.1 We will utilize a single group single-stage Phase II design for the study. Patients will receive 160mg (4 capsules) of ESK981 dosed once daily for five consecutive days followed by a two consecutive day treatment break, for a cycle of 28 days. After 8 weeks (2 cycles) of ESK981, patients will be evaluated for their rate of PSA decline of ≥ 50% from baseline (PSA50), using the PCWG3 criteria. Extrapolating from the data summarized on PSA50 response at any time by Cheng et al,[16] we hypothesize that the 8-week PSA50 rate in patients similar to patients eligible for this protocol is approximately 20%. We also hypothesize that the 8-week PSA50 rate for ESK981 treated patients will be approximately 30%.

11.3.2 We seek preliminary evidence that the true 8-week PSA50 rate for patients on ESK981 exceeds the hypothesized reference value of 20%. Having a directional hypothesis, the PSA50 rate will be estimated with a 1-sided Wilson type 90% lower confidence interval (CI). With precision defined as a CI half-width (distance from the PSA50 estimate to the lower 90% confidence limit (CL)) of 0.100, a sample size of N=27 patients and a sample estimate of PSA50 = 30% would have a lower CL = 0.201. This would indicate that the true 8-week PSA50 rate was > 20%, with 90% confidence.

11.3.3 The required sample size of N=27 was determined from the "Confidence Intervals for One Proportion" program within the PASS 15 software.[17]

11.3.4 Sample size adjustment for non-compliance will be made as follows. A major goal of this study is to evaluate the efficacy (in addition to the effectiveness) of ESK981 upon the PSA50 rate, safety, and tolerability. For each individual patient, we will allow for an ESK981 treatment non-compliance of up to 25% (i.e., up to 25% *of pills* were not taken).

Our initial analytic focus will be on the patients who are eligible (per Section 3), and evaluable (i.e., were on treatment for at least Cycle 1 [28 days]), and compliant (took at least 75% of the intended ESK981 pills during Cycle 1).

The patients meeting all 3 such requirements will be considered the eligible, evaluable, and compliant ("EEC") subset. We anticipate that up to 15% *of the enrolled patients* will not become EEC for one reason or another.

To obtain the requisite 27 ESK981 compliant patients (i.e., *completely* EEC) allowing for an overall attrition rate ("a") of *up to* 15% of patients, we will need to enroll *up to* N' = [N / (1 – a)] = [27 / (1 – 0.15)] = 27 / 0.85 = 32 patients.

The consequence of non-compliance (i.e, not being completely EEC) is that such patients would still be utilized in analyses of the effectiveness of ESK981. For example, that would focus on the patients who were "EE" but not compliant. Another effectiveness analysis would focus on the patients who were eligible, but neither evaluable or compliant. All of these analyses of non-EEC patients would be viewed as *sensitivity analyses of* the efficacy analysis of only the EEC patients. These supplemental analyses would provide a more complete assessment of the feasibility and efficacy *combined* of the oral ESK981 treatment in this study population.

11.4 Analysis

11.4.1 For the Primary Objectives, all analyses point and (1-sided Wilson type 90% lower) CI estimates of the primary endpoint (PSA50) will be calculated.

Adverse events will be tabulated by incidence and severity graded according to the National Cancer Institute – Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Other statistics will include point and (2-sided) CI estimates of overall toxicity and of specific types of toxicity.

11.4.2 For Secondary Objective 1, descriptive statistics of TTPR will be used to summarize the time to PSA response. These descriptives will include N, median, mean, standard deviation (SD), interquartile range (IQR), minimum, and maximum.

11.4.3 For Secondary Objectives 2 and 3, the censored distributions of duration of response (RD) and PSA progression free survival (PFS) will be summarized with the Kaplan-Meier (K-M) survivorship estimate. A graph of the K-M curve for PRD and for PPFS will be generated along with the Hall-Wellner 90% confidence band, and a display of the number of patients at risk at several time points, below the X-axis. Summary statistics (6-month rate, 12-month rate, median, etc.) will be calculated from the K-M life table, each one with its respective 80% confidence interval (CI).

11.4.4 For the Correlative (Exploratory) Objectives, frequency distributions of all categorical variables (grade of specific types of toxicity, and categorical biomarkers or other correlatives) will be generated. Serial PSA levels will be displayed using spaghetti plots, and changes in PSA using waterfall plots.

Descriptive statistics will be used to summarize all continuous correlatives (e.g., PSA, continuous biomarkers and other correlatives), and changes in them pre/post ESK981 treatment. Gene expression levels will be $\log_2$ transformed prior to descriptive analysis. Multiple boxplots and scatterplots will be used to display summary statistics of biomarkers/correlatives and interrelationships between continuous correlatives, respectively.

Details of the planned determination of bioinformatics correlatives are given in Section 9. Response (whether PSA50 or overall), a categorical clinical outcome, will be statistically modeled as a function of each correlative using exact logistic regression. TTPR, a continuous

uncensored clinical outcome (limited to only the PSA50 responders), will be statistically modeled as a function of each correlative using linear regression.

Time to event clinical outcomes (PRD and PPFS) will be statistically modeled as a function of each correlative using Cox proportional hazards regression. Examples of categorical correlatives include: gene mutation status (Sec. 9.1 and 9.2); AR dependence (Sec. 9.3); and vacuolization status or vascularization status (Sec. 9.6). Examples of continuous correlatives include: kinase expression levels (Sec. 9.4); CTC and DTC counts (Sec. 9.5); and levels of Ki67, AR, and LC3 (Sec. 9.6).

11.5 Expected Accrual Rate, Accrual Duration, and Total Study Duration

11.5.1 This will be a clinical trial involving Karmanos Cancer Institute (KCI) only. We expect to accrue 10-12 patients/year.

11.5.2 At that rate, we can accrue 32 patients (to obtain 27 EEC patients) in 32-38 months. Allowing an additional 4 months to finish determining all biomarkers and correlatives in all patients yields an estimated total study duration of 36-42 months.

12. REGULATORY AND PROTECTION OF HUMAN SUBJECTS

12.1 Roles and Responsibilities

12.1.1 Sponsor Investigator

The Sponsor Investigator is responsible for performing the following tasks:

- Responsibility for the overall conduct of the study and for monitoring the progress of the study
- Reviewing and ensuring reporting of Serious Adverse Events (SAEs)
- Reviewing data

12.2 Ethical Considerations

This study will be conducted in compliance with the protocol, GCP guidelines established by the International Conference on Harmonisation, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: www.laakariliitto.fi/e/ethics/helsinki.html).

12.3 Written Informed Consent

Before obtaining consent, members of the study team will review the rationale for the treatment program with the patient. The discussion will review the alternatives available (including hormonal therapy, chemotherapy, or supportive care as appropriate), the potential benefits of this program, the risks and the probability of their occurrence, and the procedures to minimize these risks. Should an adverse event occur, the provisions available to ensure medical intervention will also be reviewed. Why the risks are reasonable in relation to the anticipated benefits, incentives, or costs that will or may be incurred as a result of participating in the study, as well as the efforts to maintain confidentiality, will also be discussed with the patient.

Patients will be required to sign and date an informed consent form that meets the requirements of the Code of Federal Regulations (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the IRB. The medical record will include a statement that written informed consent was obtained (and document the date that it was obtained) before the patient is enrolled in the study. The original signed document will become part of the patient's medical record, a copy will be forwarded to the lead site/sponsor pursuant to sponsor registration and to the PCCTC and a copy will be sent home with each patient.

The consent form will include the following:

- the nature and objectives, potential toxicities, and benefits of the intended study
- the length of therapy and likely follow-up required
- alternatives to the proposed therapy (including available standard and investigational therapies)
- the name of the investigator(s) responsible for the protocol
- the right of the patient to accept or refuse treatment and to withdraw from participation in this study
- Text regarding the consortium and the coordinating center should be added to all institutional informed consent documents and sections in the research authorization/HIPAA forms (e.g., "Prostate Cancer Clinical Trial Consortium")

12.4 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. After this discussion, they will be asked to sign a Notice of Privacy Practice research authorization/HIPAA form. The original signed documents will become part of the patient's medical records, and each patient will receive a copy of the signed documents. The use and disclosure of protected health information will be limited to the individuals described in the research authorization form. The research authorization form must be completed by the principal investigator and approved by the IRB.

12.5 Terminating or Modifying the Study

Adverse event and laboratory data from this trial will be assessed by the lead site or the sponsor's medical monitor on an ongoing basis. SAEs will be reviewed as they are reported to the lead site/sponsor, and the medical monitor will make an assessment regarding the safety of continuing or modifying the study. This assessment will be shared with the investigators either in writing or as part of a teleconference. Should the assessment of either the lead site/sponsor or the principal investigator be that the study should be terminated; the study will be closed to further accrual. Patients who are receiving an investigational agent will be assessed individually by the investigator to see if it is in the patients' best interest to continue, which might be the case for a patient that is responding to the intervention. Follow-up safety assessments will be performed for all patients who are terminated from the study prematurely.

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APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Description**	**%**	**Description**
0	Normal activity. Fully active, able to continue all predisease 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 (eg, 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 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 > 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: MEDICATIONS WITH THE POTENTIAL FOR DRUG-DRUG INTERACTIONS

Please refer to Tables 3-2 and 3-3 of the FDA drug interactions labeling website for examples of clinical inhibitors and clinical inducers of P450, respectively.[18]

Potent Inhibitors of CYP1A2, CYP2C8, or CYP3A4
Potent inhibitors of the CYP1A2, 2C8, or 3A4 include, but are not limited to, the following:
CYP1A2
- Fluvoxamine
- Ciprofloxacin
- Mexiletine
- Propafenone
- Zileuton

CYP2C8
- Gemfibrozil

CYP3A4
- Ketoconazole
- Indinavir
- Ritonavir
- Itraconazole
- Clarithromycin
- Telithromycin
- Aprepitant
- Diltiazem
- Erythromycin
- Fluconazole
- Grapefruit juice
- Verapami

This list is not comprehensive as new information is continually being identified.

Potent Inducers of CYP1A2, CYP2C8, or CYP3A4

Potent inducers of the CYP1A2, 2C8, or 3A4 include, but are not limited to, the following:
CYP1A2
- Smoking

CYP2C8
- Rifampicin

CYP3A4
- Rifampicin
- Carbamazepine
- Griseofulvin
- This list is not comprehensive as new information is continually being identified.

Substrates of CYP3A4/5 With a Narrow Therapeutic Range

Substrates of CYP3A4/5 with a narrow therapeutic range include, but are not limited to:

- Alfentanil
- Astemizole
- Cisapride
- Cyclosporine
- Diergotamine
- Ergotamine
- Fentanyl
- Pimozide
- Quindine
- Sirolimus
- Tacrolimus
- Terfenadine

This list is not comprehensive as new information is continually being identified.

APPENDIX C: STUDY CALENDAR

	Screening	**Treatment/Intervention Period**					**End of Tx (30 Days from last dose)**	**Follow-up Q 3 mos**
		Cycle 1				**Cycle 2 + beyond**		
	Day -28 to Day -1	Day 1 (±3 d)	Day 8 (±3 d)	Day 15 (±3 d)	Day 22 (±3 d)	Day 1 (±3 d)	(±3 d)	
Informed consent and research authorization/ HIPAA form	X[a]							
Demographics, medical history, histologic and radiologic confirmation of disease	X							
Physical examination, vitals, weight, blood pressure	X	X	X	X	X	X	X	
EKG	X							
Height	X							
Performance status	X	X	X	X	X	X	X	
Concomitant meds	X	X	X	X	X	X	X	
Toxicity assessment	X	X	X	X	X	X	X	
Tumor measurements[b]	X	Tumor measurements will be repeated every 8 weeks (+/- 10 d)						
Laboratory tests[c]	X		X	X	X	X	X	
Correlative samples	X					X[d]	X	
Tumor Biopsies	X					X[e]		
Follow-up								X[f]

a. May be obtained up to 60 days prior to initiation of study treatment.
b. Radiographic evaluations will include a CT or MRI abd/pel and a bone scan or PET. Chest x-ray should be done if necessary for tumor assessment. Radiologic documentation will be provided for patients removed from study for progressive disease.
c. Complete blood count, comprehensive chemistry panel including renal function (BUN, creatinine) and liver function tests (AST, ALT, total bilirubin), PSA, testosterone (at baseline).
d. Whole blood samples will be collected every 4 weeks from screening to end of treatment (see lab manual).
e. Tumor biopsies performed at the Cycle 3 visit (week 8, +/- 10 d) (see lab manual).
f. Patients will be followed every three months for 5 years from end of treatment for survival and subsequent treatment.

APPENDIX D: GLOSSARY OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
CFR	Code of Federal Regulations
CRF	case report form
CRPC	castration resistant prostate cancer
CT	computerized tomography
CTC	circulating tumor cell
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FGFR1	fibroblast growth factor receptor 1
HIPAA	Health Insurance Portability and Accountability Act
IC50	50% inhibition of activity
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	intent-to-treat population
LD	longest diameter
mCRPC	metastatic castration-resistant prostate cancer
MTD	maximum tolerated dose
MTKI	multi-tyrosine kinase inhibitor
NCI	National Cancer Institute
NLCB	no longer clinically benefitting
NOD	non-obese diabetic
PCCTC	Prostate Cancer Clinical Trials Consortium
PCWG3	Prostate Cancer Working Group 3
PD	progressive disease
PFS	progression-free survival
PI	principal investigator
PR	partial response
PSA	prostate-specific antigen
PSA50	PSA ≥ 50% response rate

RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SCID	severe combined immunodeficiency
SD	stable disease
TIE1, TIE2	tyrosine kinase with immunoglobulin-like and EGF-like domains (1, 2)
TMZ	temozolomide
ULN	upper limit of normal
VEGF	vascular endothelial growth factor

APPENDIX E.

2017-065 Pill Diary

An Open-Label, Parallel, Phase II Study of Single-Agent Oral ESK981 in Men with Castrate-Resistant Prostate Cancer (CRPC)

Subject #_____ Subject Initials:_____ Cycle Number:

ESK981 ***Total Daily Dose Prescribed: _____ mg / Total Number of Pills to Take Daily: _____***

Day	Date	Time of Dose	# of Pills Taken	Initials	Comments
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					

This Section to be completed by clinic staff
Number of Pills Returned: _____ Received by: _____ Date: _____

APPENDIX F. DSM REPORT FORM

PROTOCOL#:		**REPORT DATE:**	

PROTOCOL TITLE			
PROTOCOL ACTIVITY SINCE LAST REPORT			
Accrual Goal:	Eligible:		Total number of AE's to date:
Accrual to Date:	Ineligible: (provide reason):		
Accrual Since Last Monthly Report:			

SPECIFICALLY FOR PHASE I TRIAL &/OR DOSE ESCALATING TRIALS:	
DOSE LEVEL	ACCRUAL

RECORD ALL GRADE 3, 4, AND 5 ADVERSE EVENTS (AE). GROUP BY CATEGORY OF AE. RECORD THE DATE OF THE OCCURRENCE, ATTRIBUTION AND IF REPORTABLE TO THE IRB. SHADE THE ROWS OF THE AE'S THAT HAVE OCCURRED FOR THIS REPORT. ATTACH THE HIC UP REPORT FORM FOR THESE REPORTABLE EVENTS THAT OCCURRED ON THIS REPORT.

Pt. ID#	Category and type of adverse reaction	Date of Occurrence	Grade[1]	Attribution[2]	Reportable to IRB (Y/N) Yes with date

1. Grade: 1-Mild, 2-Moderate, 3- Severe, 4-Life-threatening, or 5- Death.

2. Attribution: 1-unrelated, 2 - unlikely, 3 - possibly, 4 - probably, or 5 - definitely

OFF TREATMENT □ Provide reason [progression, death, toxicity, completed therapy, etc].			
PROTOCOL VIOLATIONS □ Deviations from protocol treatment, monitoring, or study calendar.			
PROTOCOL AMENDMENTS □ Include date submitted to regulatory bodies and date approved.			
OTHER COMMENTS			
Investigator Signature:		**Data Manager Signature:**	