KCI Protocol # 2011-185

Pilot Trial of oral cabozantinib/ XL184 in metastatic castrate resistant prostate cancer to explore the changes in bone and tumor imaging related pathways

Version date: Nov 17 2011, Dec 29, 2011, Mar 19, 2012, June 28, 2012, Sept 6, 2012, Aug 16 2013, June 25, 2014, Aug 14, 2014

Sponsor- NOMIC grant Dept of Defense.

Cabozantinib/XL184 and grant support will be provided by Exelixis Inc.

IND#: 114758

Principal Investigator: Ulka Vaishampayan M.D.
Karmanos Cancer Institute
4100 John R, HW04HO
Detroit, MI 48201

Tel # 313-576-8715

Fax # 313-576-8487.

E-mail vaishamu@karmanos.org

Co-Investigators:

Elisabeth Heath M.D.

Karmanos Cancer Institute
4100 John R, HW04HO
Detroit, MI 48201

Tel# 313-576-8715

Fax # 313-576-8767

E mail heathe@karmanos.org

Anthony Shields, MD, PhD
Associate Cancer Center Director for Clinical Sciences
Professor of Oncology
Karmanos Cancer Institute
4100 John R, HW04HO
Detroit, MI 48201

Office: 313 576 8735
Fax: 313 576 8767
Email: shieldsa@karmanos.org

Joseph Fontana M.D.
VA Medical Center
Oncology 11M-HO
Room C3687
4646 John R
Detroit MI 48201.
Tel# 313-576-3659
E mail Joseph.Fontana@med.va.gov

Lance Heilbrun Ph.D.
Biostatistics Unit
Karmanos Cancer Institute
4100 John R, MM05BI
Detroit, MI 48201

Detroit, MI 48201.
Tel# 313-576-8652
E mail heilbrun@karmanos.org

Izabela Podgorski, PhD
Assistant Professor
Department of Pharmacology
Karmanos Cancer Institute, Wayne State University
Lab: 313 577 0941
Office: 313 577 0514
E Mail ipodgors@med.wayne.edu

Susan Land PhD
Applied Genomics Technology Center
CS Mott Center Bldg.
275 East Hancock St.
Detroit MI 48201
313-577-9605 (office phone)
313-577-0024 (lab phone)
E mail Sland@med.Wayne.edu

Aliccia Bollig-Fischer, Ph.D.
Applied Genomics Technology Center
Karmanos Cancer Institute
Wayne State University
275 E. Hancock
Detroit, MI 48201
Tel 313-577-9605
E mail bollig@karmanos.org

Study Coordinator	Brenda Dickow, RN Clinical Trials Office 4100 John R, MM03CT Detroit, MI 48201 Tel # 313-576-9372 E mail: dickowb@karmanos.org
Grants and Contracts:	Mary Jo O'Loughlin Karmanos Cancer Institute 4100 John R, MM00RA Detroit, MI 48201 Tel # 313-578-4405 Fax # 313-578-4419 e-mail: oloughli@karmanos.org

Pilot Trial of oral cabozantinib/XL184 in metastatic castrate resistant prostate cancer to explore the changes in bone and tumor imaging related pathways

SYNOPSIS

Pilot trial of oral cabozantinib/ XL184 in metastatic castrate resistant prostate cancer to explore the changes in bone and tumor imaging related pathways

- Primary Objective:

  To conduct a pilot clinical trial to evaluate the timing, physiology, and magnitude of changes in tumor imaging, and pharmacodynamic markers with XL184 treatment in metastatic castrate resistant prostate cancer.

  .

- Secondary Objectives:

  1) To estimate the progression-free survival (PFS) achieved with XL184 in metastatic CRPC patients.
  2) To evaluate the safety and tolerability of the therapy, and the toxicities associated.
  3) To evaluate OS in chemotherapy naïve metastatic CRPC patients

Study Treatment

XL184 at a starting dose of 60 mg orally daily taken after fasting 2 hours prior and one hour after administration.

Dose modifications for treatment related severe or intolerable adverse events.
-1, and -2 Dose levels will be 40 mg and 20 mg respectively.

Study Assessments

Pretherapy CT, standard methylene diphosphonate (^{99m}Tc-MDP) bone scan, sodium ^{18}F-fluoride (^{18}F-fluoride) PET bone scan, and FMAU {[2'-^{18}F]-1-(2'-deoxy-2'-fluoro-beta-

D-arabinofuranosyl)thymine} PET scan. Pretherapy bone biopsy and correlative pharmacodynamic sample collection (Blood and urine). The FMAU and ^{18}F-fluoride PET scans will be done under separately approved imaging protocols.

Start XL184, monitor for toxicity every 2 weeks for the first 3 months, then once every 4 weeks.

At 2 -3 weeks post therapy repeat ^{18}F-flouride PET / FMAU PET scans, bone scans and tumor biopsy. The timing of the scan maybe further changed depending on the optimal time to detect changes as determined from the first 5 patients enrolled. At 6 weeks, 20 weeks and then every 12 weeks thereafter until patient continues on cabozantinib, CT and 99Tc-MDP bone scan will be done to evaluate tumor status.
The week 4 timepoint scan will be discontinued for all present and future patients.
We will also review each patients baseline scan to see if there is disease detectable. If no readily visible cancer is found then the 2nd scan with FMAU PET will be eliminated for that patient. So far of the 6 patients imaging, only patient 1 had no easily visible tumor on the baseline scan. Longer follow up of the patients has revealed that it remains a challenge to determine if the patients are continuing to derive clinical benefit. The PSA level is frequently discordant with response. Hence we will obtain an additional ^{18}F-flouride PET scan and a FMAU scan (if the patient had <3 FMAU scans prior) at week 32 (+/- 10 weeks) for the patients continuing on study therapy at that timepoint. For the patients who discontinue therapy, prior to the 32 weeks timepoint,, the scan/s will be done at the time of treatment discontinuation (+/- 2 weeks). This timepoint was chosen to capture the maximum number of patients currently on the study for this scan, and since conventional CT scan and bone scan is also conducted at the same timepoint for comparison.

Tissue, Blood and Urine collection:

Tumor tissue biopsy will be obtained within 2 weeks prior to and within 2-3 weeks post XL184 therapy. Part of each tissue sample will be fresh-frozen for genomic analysis and the rest will be decalcified, fixed and embedded in paraffin for histological and immuhistochemical analyses. Blood and urine samples will be collected from each patient for correlative studies. For the first 5 patients, samples will be taken at pretherapy, 1, 2 and 4 weeks after treatment begins. Timeline for additional 15 patients will be adjusted based on results obtained in first 5 patients and will be correlated with the imaging schedule. For instance if no changes are seen at a particular time point in all

5 patients, then that timepoint maybe excluded. Blood sample collection will be performed using Vacutainer CPT tubes. This method will generate serum samples for the analysis of biochemical markers, and mononuclear cells for future analyses of DNA and RNA. All isolated samples will be stored at -80° C until use.

Clinical Monitoring and testing

Patients will be monitored for response (measurable disease, PSA levels, clinical response and bone scans) progression, survival and toxicity.

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the plasma drug concentration time curve
C_{max}	maximum plasma concentration
cPR	confirmed partial response
CR	complete response
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
DVT	deep vein thrombosis
EC	ethics committee
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ED_{50}	dose required for 50% inhibition
ESC	Exelixis Safety Committee
FDA	Food and Drug Administration
FMAU	[2'-^{18}F]-1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)thymine
FLT3	FMS-like tyrosine kinase 3
FSH	follicle-stimulating hormone
GB	glioblastoma
GCP	Good Clinical Practice
GI	gastrointestinal
GEJ	gastroesophageal junction
GnRH	gonadotropin-releasing hormone

HCC	hepatocellular carcinoma
HGF	hepatocyte growth factor
IC_{50}	concentration required for 50% inhibition
ICH	International Conference on Harmonisation
IME	important medical event
INR	International Normalized Ratio
IRB	Institutional Review Board
LHRH	luteinizing hormone-releasing hormone
MDP	methylene diphosphonate (^{99m}Tc-MDP) bone scan
MedDRA	Medical Dictionary for Regulatory Activities
MTC	medullary thyroid cancer
MTD	maximum tolerated dose
NCI	National Cancer Institute
PET	Positron Emission Tomography

LIST OF ABBREVIATIONS (continued)

NSCLC	non-small-cell lung cancer
PD	progressive disease
PE	pulmonary embolism
PFS	progression-free survival
PFS6	progression-free survival at 6 months
PI	principal investigator
PIB	powder-in-bottle
PK	pharmacokinetic
PO	oral
PPE	palmar-plantar erythrodysesthesia
PR	partial response
PT	prothrombin time
PTT	partial thromboplastin time
qd	once daily
QTc	corrected QT
RPLS	reversible posterior leukoencephalopathy syndrome
RTK	receptor tyrosine kinase
SAE	serious adverse event
SD	stable disease
SCLC	small-cell lung cancer
$t_{1/2, z}$	terminal-phase half-life
ULN	upper limit of normal
VEGF(R)	vascular endothelial growth factor (receptor)

1 BACKGROUND AND RATIONALE

1.1 Hypothesis:

XL-184 is a cmet inhibitor that results in abrupt clinical changes in bone metabolism represented as an abrogation of 99mTc-MDP uptake on bone scan. This is likely due to inhibition of osteoclast function and decrease in osteoblast activity. We hypothesize that XL-184 uniquely targets the cross talk between cMet and VEGFR axis, downstream cathepsin K driven pathways and novel recetor tyrosine kinases (such as DDR-1 and DDR-2) in bone tumor microenvironment. Hence we propose a pilot trial designed to study the pathophysiology and genomic changes in bone metastases and correlate these with response and clinical outcome data in metastatic CRPC patients treated with XL-184 therapy.

1.2 Study Objectives

The objectives of this study are as follows:

- Primary Objective:

  To conduct a pilot clinical trial to evaluate the timing, pathophysiology, and magnitude of changes in tumor imaging and pharmacodynamic markers with XL184 treatment in metastatic castrate resistant prostate cancer.

  .

- Secondary Objectives:

  1) To estimate the progression-free survival (PFS) achieved with XL184 in metastatic CRPC patients.
  2) To evaluate the feasibility of the therapy, and the toxicities associated.
  3) To evaluate OS in metastatic CRPC patients post ADT treated with XL-184.

1.3 Background: Prostate Cancer and Targeted Therapy

Cancer is a worldwide clinical and economic problem. The American Cancer Society estimates that there will be nearly 1.5 million cancer diagnoses and

566,000 cancer-related deaths in the United States in 2008 (American Cancer Society 2008). The estimated overall cost of cancer in 2006 (reflecting, for example, clinical treatment, lost work time and productivity for patients and families, and mortality) was $206.3 billion. Conventional approaches to treating cancer include surgery, radiotherapy, and cytotoxic chemotherapy as single modalities or as combined therapies. Recently, targeted therapies including antibodies and small molecule inhibitors have also demonstrated clinical benefit. Further studies into the pathways and mechanisms of action of these novel agents will help guide patient selection for future therapies and determine resistance mechanisms.

Bone metastases in prostate cancer are primarily osteoblastic with evidence of deposition of new bone. The increased osteoblastic activity results in incorporation of Tc99-labeled methylene-diphosphonate allowing visualization of the lesions on bone scan. Met and its ligand, hepatocyte growth factor (HGF) have demonstrated increased expression after androgen ablation as well as with disease progression to lymph nodes and soft tissue. XL184 is a VEGF and c-met inhibitor that has demonstrated promising clinical efficacy in metastatic CRPC. Not only did the agent produce 89% aggregate response and stable disease rate in measurable disease, but marked improvement of bone scans was noted (Smith DC et al. GU ASCO symposium 2011). This is an unusual phenomenon, not seen even with any of the other agents that produce high levels of anti-tumor efficacy such as ADT. The physiologic changes occurring with the rapid normalization of bone scans (within the first 6 weeks) are unknown to date. We propose a pilot trial to study the changes occurring in bone metastases and in tumor tissue and to correlate with clinical outcome, with XL184 therapy. We hypothesize that XL184 uniquely targets the crosstalk between cMet and VEGFR axis, downstream cathepsin K-driven pathways and novel RTKs (e.g., DDR-1, DDR-2) in the bone tumor microenvironment. XL184 action leads to abrupt changes in bone metabolism and abrogation of ^{99m}Tc-MDP uptake due to the inhibition of osteoclast activity and subsequent decrease in osteoblast function.

1.3.1 Signal Pathways

The MET receptor tyrosine kinase (RTK) (receptor for hepatocyte growth factor [HGF]) has been implicated as a mediator in many important aspects of tumor pathobiology,

including tumor survival, growth, angiogenesis, invasion, and dissemination (Sattler et al. 2004; Jiang et al. 2005), and several MET RTKs have been reported to show activity in cell lines and animal models (Sattler et al. 2004). Recently, inhibitors of MET including XL880 and ARQ 197 have shown signs of antitumor activity in Phase 1 studies (Eder et al. 2007; Garcia et al. 2007; Ross et al. 2007). The vascular endothelial growth factor receptor 2 (VEGFR2 [KDR]) is a central mediator of tumor angiogenesis, and several small molecule and protein therapeutics targeting this receptor are currently in clinical development. Recently, bevacizumab (Avastin®), a monoclonal antibody directed against VEGF, has been shown to improve overall survival (OS) when combined with chemotherapy in patients with metastatic colorectal cancer (Hurwitz et al. 2004) and in lung cancer (Sandler et al. 2005). In addition to their individual roles in tumor pathobiology, nonclinical data suggest that Met and VEGFR2 play synergistic roles in promoting tumor angiogenesis and subsequent dissemination (Bottaro and Liotta 2003).

Compounds that simultaneously inhibit VEGF and MET RTKs may be more effective anticancer agents than agents that target each of these receptors individually (Pennacchietti et al. 2003). The investigational drug in this study, XL184, is a potent RTK inhibitor that targets primarily MET and VEGFR2 RTKs. XL184 has activity against other RTKs that have been implicated in tumor pathobiology, including KIT, FMS-like tyrosine kinase 3 (FLT3), and Tie-2. In addition, XL184 is known to inhibit RET, a RTK known to be causative for malignancy in the setting of hereditary medullary thyroid cancer (MTC).

1.4 XL184 Background

A summary of XL184 clinical and nonclinical experience is contained in the Investigator's Brochure supplied by Exelixis (or designee). The Investigator's Brochure should be reviewed in conjunction with this study protocol.

1.4.1 Spectrum of XL184 Activity

XL184 is a new chemical entity that inhibits multiple RTKs with growth-promoting and angiogenic properties. The primary biochemical targets of XL184 are MET, VEGFR2, RET, and KIT (Table 1-1).

Table 1-1: XL184 IC_{50} Values in Biochemical, Enzymatic Assays

Kinase	IC_{50} (biochemical) [nM]
MET	1.8
VEGFR2	0.035
RET	3.8
KIT	4.6

IC_{50}, concentration required for 50% target inhibition; VEGFR2, vascular endothelial growth factor receptor 2.

XL184 is orally bioavailable as demonstrated by pharmacokinetic (PK) experiments in rodent and nonrodent models. Target modulation studies show that the administration of XL184 in mouse models causes dose-dependent inhibition of MET, VEGFR2, a mutationally activated form of RET, and multiple mutationally activated forms of KIT. Immunohistochemical studies demonstrate rapid effects on the tumor endothelium, resulting in breakdown of the vasculature and tumor cell death within 8 hours after administration of XL184. These effects translate into significant tumor growth inhibition after XL184 treatment in multiple tumor models (Table 1-2). In addition, in all of the models examined (human breast cancer, human lung carcinoma, human MTC, and rat glioblastoma [GB]), marked tumor regression and excellent tolerability were observed .

Table 1-2: XL184 ED_{50} Values in Tumor Models

Tumor Line	Species	Tumor Type	ED_{50} (mg/kg/day)	Treatment Duration
C6	Rat	Glioblastoma	< 1	qd × 12
MDA-MB-231	Human	Breast	2	qd × 14
H441	Human	NSCLC	3	qd × 14
TT	Human	Medullary thyroid carcinoma	11	qd × 21
U87	Human	Glioblastoma	≤ 30	qd × 14

ED_{50}, dose associated with 50% tumor growth inhibition; NSCLC, non-small-cell lung cancer; qd, once daily.

Data from pharmacodynamic experiments show that in vivo XL184 inhibits key RTKs that promote tumor cell proliferation and/or angiogenesis (MET, VEGFR2, and RET) (Table 1-3). XL184 inhibited RTKs MET in the liver, and xenograft tumors and VEGFR2 in lung tissue, with ED_{50} (dose required for 50% inhibition of receptor phosphorylation) values of 5, 9, and 26 mg/kg, respectively. Durations of action were sustained with > 50% inhibition 10-24 hours post-dose at a dose level of 100 mg/kg. Immunohistochemical evaluations indicated that XL184 inhibited angiogenesis and cell proliferation and stimulated necrosis in tumor tissue in vivo.

Table 1-3: Summary of XL184 In Vivo Target Modulation Studies in Mice

		Dose Response			Duration of Action		
Target	Model	Maximum Inhibition (%)	Estimated ED_{50} (mg/kg)	Estimated IC_{50} (μM)	Dose (mg/kg)	Maximum Inhibition (%)	Sustained Inhibition > 50% (hours)
MET	Liver (+ HGF)	97	5	2	100	99	10
	H441	96	9	7	100	92	10
VEGFR2	Lung (+ VEGF)	98	26	2	100	99	10
RET	TT	89	11.1	7.4	100	84	4[a]

ED_{50}, dose associated with 50% inhibition of receptor phosphorylation; HGF, hepatocyte growth factor; IC_{50}, plasma concentration associated with 50% inhibition of receptor phosphorylation; VEGF, vascular endothelial growth factor.

[a] 44% inhibition after 24h, no intermediate timepoint between 4h and 24h was tested.

Nonclinical experiments in nude mice demonstrated that XL184 crosses the blood-brain-barrier in healthy animals. Four hours after drug administration at 10 mg/kg, XL184 brain exposure was approximately 16% (1.3 μM) of the XL184 concentration in plasma (8.2 μM).

Overall, the data generated in vivo demonstrate that the target profile of XL184 translates to potent anti-angiogenic activity and potent antitumor efficacy. The anti-VEGFR2 activity of XL184, combined with additional growth-inhibitory properties mediated by its activity against MET, may result in enhanced antitumor activity in GB. Phase 1 and 2 clinical data suggest that XL184 has robust clinical activity in several tumor types, including MTC and GB.

More extensive summaries of XL184 pharmacology are contained in the Investigator's Brochure supplied by Exelixis (or designee). This document should be reviewed in conjunction with this study protocol.

1.4.2 XL184 Nonclinical Toxicology

In nonclinical toxicity studies in rodents and nonrodents, histopathological changes associated with XL184 administration were observed in gastrointestinal (GI) tract, bone marrow, lymphoid tissues, kidney, adrenal and reproductive tract tissues. Histopathological changes present in bone and pancreas were considered secondary to XL184 administration. XL184 was negative in in vitro bacterial, in vitro mammalian cell, and in vivo mammalian genotoxicity bioassays. In reproductive toxicity studies, XL184 was embryotoxic in rats, produced fetal soft tissue changes in rabbits, and decreased fertility in male and female rats.

Safety pharmacology studies of XL184 administration did not demonstrate adverse effects on neurobehavioral or respiratory-system function in rats; furthermore, no significant changes in electrocardiographic parameters (including corrected QT [QTc] interval) were observed in telemeterized dogs.

XL184 was not an inhibitor of cytochrome P450 (CYP) 3A4 in vitro and is not predicted to have significant effects on CYP3A4 induction. XL184 was shown to be an inhibitor of CYP2C8, CYP2C9*3, and CYP2C19 isozymes in vitro and was also a substrate of CYP3A4-mediated metabolism. The mean plasma protein binding by XL184 in vitro was greater than 98%.

Additional toxicology information may be found in the Investigator's Brochure.

1.4.3 Clinical Experience (Detailed and current clinical information is available in current version of the Investigators brochure)

1.4.3.1 Clinical Summary

There are nine ongoing studies for XL184, including four Phase 1 studies, one Phase 1b/2 study, three Phase 2 studies, and one Phase 3 study. As of 01 June 2010, there have been 747 subjects enrolled across all nine studies. The single-agent maximum tolerated dose

(MTD) on the once daily (qd) oral (PO) dosing schedule has been determined to be 175 mg. Details of all studies may be found in the Investigator's Brochure.

1.4.3.2 Clinical Safety Profile

The adverse event (AE) and serious adverse event (SAE) data summarized in the following section include those reported and entered in the clinical database and safety database as of 01 June 2010. The clinical studies with XL184 are ongoing, thus the AE data from the clinical database do not yet include all SAEs. Data from double-blinded studies are not presented. The severity of AEs was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

1.4.3.2.1 Adverse Events

As of 01 June 2010, more than 561 subjects have been studied in open-label clinical trials with XL184, including treatment with XL184 as a single agent, as well as XL184 in combination with temozolomide and radiation therapy, and XL184 in combination with erlotinib. The available pooled data in this database include 483 subjects who received open-label XL184 in Studies XL184-001, XL184-002, XL184-201, XL184-202, and XL184-203 (lead-in stage only). The most frequently observed AEs (> 15%), regardless of the relationship to XL184, were fatigue, diarrhea, decreased appetite, nausea, constipation, vomiting, dysphonia, hypertension, rash, aspartate aminotransferase (AST) increased, alanine aminotransferase (ALT) increased, palmar-plantar erythrodysesthesia (PPE) syndrome, headache, dyspnea, cough, and weight decrease. Some of these events, including fatigue, diarrhea, nausea, dysphonia, hypertension, rash, AST increased, ALT increased, and PPE syndrome, resulted in permanent study drug discontinuation,. Effects that may be related to inhibition of VEGF, including hypertension, thromboembolic events, GI perforation and hemorrhage, wound dehiscence, and proteinuria, have been observed in clinical studies with XL184.

1.4.3.2.2 Serious Adverse Events

Of the 561 subjects enrolled in open-label clinical trials with XL184, 223 subjects (40%) experienced one or more SAEs recorded in the Argus safety database, and 95 subjects experienced one or more SAEs that were assessed to be related to treatment with XL184.

The most commonly reported SAEs assessed as drug related were pulmonary embolism (PE) (in 14 subjects), diarrhea (in nine subjects), deep vein thrombosis (DVT) (in seven subjects), nausea (in seven subjects), hypertension (in five subjects), thrombocytopenia (in five subjects), dehydration (in four subjects), vomiting (in four subjects), abdominal pain (in three subjects), and perirectal abscess (in three subjects). In addition, one late-breaking case of reversible posterior leukoencephalopathy syndrome (RPLS) was reported after the data cut-off in the double-blind placebo-controlled Study XL184-301.

Fifty-two deaths were reported within 30 days of the last dose of study drug; the majority was due to disease progression, and five deaths were assessed to be related to XL184. They were the result of GI hemorrhage (in one subject), PE (in two subjects), respiratory failure (in one subject), and hemoptysis (in one subject).

Detailed information regarding the safety profile of XL184 from all studies may be found in the Investigator's Brochure.

1.4.3.3 Clinical Pharmacokinetics

Pharmacokinetic analysis showed dose proportional increases in maximum plasma concentration (C_{max}) and area under the plasma concentrationtime curve (AUC) both for the powder-in-bottle (PIB) formulation (dose range: 0.08 to 11.52 mg/kg) and the capsule formulation (dose range: 125 to 175 mg). Terminal-phase half-life ($t_{1/2, z}$) values were 59.1 to 136 hours. More detailed information regarding XL184 PK from all studies and product metabolism in humans may be found in the Investigator's Brochure.

1.4.3.4 Clinical Activity

Preliminary clinical activity data are available for Studies XL184-001, XL184-201, XL184-202, and XL184-203.

Eighty-five subjects with advanced solid tumors were enrolled in the XL184-001 study. Of the 85 subjects, 18 subjects experienced a tumor shrinkage of ≥ 30%, including 17 (49%) of 35 subjects with MTC with measurable disease. In the response evaluable subset of subjects with MTC, 10 (29%) of 35 subjects had confirmed partial responses (cPRs). In addition, 15 subjects with MTC had stable disease (SD) for at least 6 months.

In Study XL184-201, 196 subjects with relapsed GB have been enrolled. At the dose of 125 mg qd, cPRs were observed in 11 of 37 (30%) subjects who had not received prior anti-angiogenic therapy, with a median duration of response of 5.1 months (range, 0.9+ to 6.7+). In subjects who had not received prior anti-angiogenic therapy, the 6-month PFS rate (PFS6) assessed by Kaplan-Meier estimate was 25%, with a 30% rate of censoring at the time of analysis. The median PFS was 16.0 and 7.9 weeks for anti-angiogenic naïve and anti-angiogenic pretreated subjects, respectively. At the dose of 175 mg qd, cPRs were observed in 7 of 34 (21%) subjects without prior anti-angiogenic therapy, with a median duration of response of 2.9 months (range,1.9 to12.8). In subjects who had not received prior anti-angiogenic therapy, the Kaplan-Meier estimate of PFS6 was 10%. The median PFS was 15.9 and 14.3 weeks for anti-angiogenic naïve and anti-angiogenic pretreated subjects, respectively.

In Study XL184-202, 65 subjects with non-small-cell lung cancer (NSCLC) have been enrolled. As of 01 June 2010, the MTD of the combination of XL184 and erlotinib in the Phase 1 portion has not been determined. To date, 8 of 53 evaluable subjects treated in Phase 1 have experienced a $\geq$ 30% decrease in the sum of tumor measurements as compared to baseline measurements. Confirmed partial response was achieved in 4 of 53 (8%) evaluable subjects.

In Study XL184-203, 198 subjects with advanced solid tumors have been enrolled. Six subjects achieved a cPR during the 12-week open-label lead-in stage, two with hepatocellular carcinoma [HCC], two with NSCLC, one with melanoma, one with prostate cancer). Of the 105 evaluable subjects (with minimum 12 weeks of follow-up), 43 achieved SD (11 with melanoma, eight with NSCLC, five with pancreatic cancer, five with prostate cancer, five with HCC, four with gastric/ gastroesophageal junction [GEJ] adenocarcinoma, four with ovarian cancer, and one with small-cell lung cancer [SCLC]). The overall disease control rates (partial response [PR] + SD) at Week 12 were 88% in the HCC cohort, 86% in the ovarian cancer cohort, 67% in the prostate cancer cohort, 50% in the melanoma cohort, and 50% in the NSCLC cohort.

Genotyping data in the XL184-001 study suggest the absence of a correlation between RET mutations and either clinical response or time on study. In an ongoing analysis in the XL184-201 study, genotyping analysis of several genomic alterations frequently found in GB revealed that none appear to confer resistance to XL184.

Data from subjects enrolled in ongoing clinical studies demonstrated statistically significant changes in plasma biomarkers such as sMET and sKIT. Analysis of serial hair and/or skin samples revealed substantial inhibition of the phosphorylation of XL184 targets such as MET, RET, and KIT, as well as of downstream signaling molecules AKT and ERK, following administration of XL184.

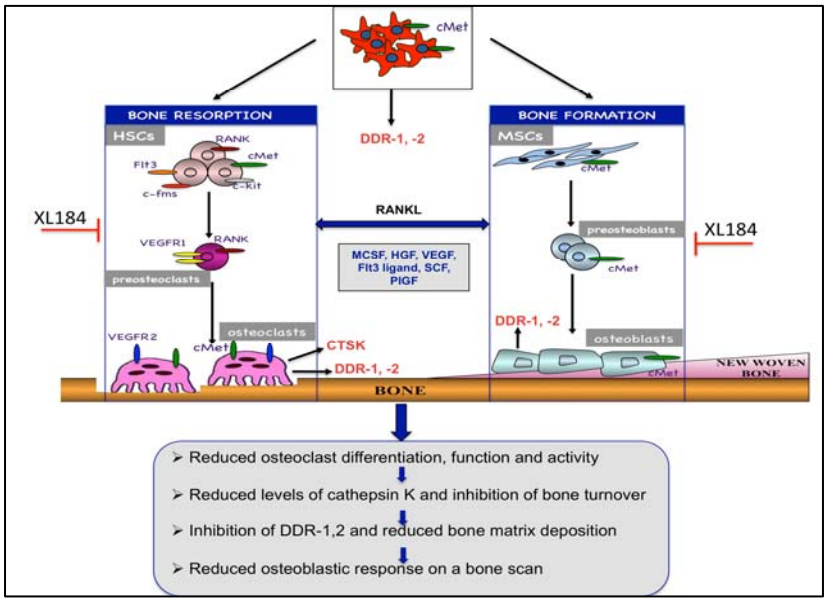
1.5 RATIONALE

Presence of disease progression by PSA, clinical symptoms, or on scans, despite androgen deprivation therapy (ADT), signals the onset of castration resistant prostate cancer (CRPC). At this stage, the expected median survival is 18-20 months, despite treatment with docetaxel based chemotherapy (Petrylak et al. N Engl J Med 2004 and Tannock et al. N Engl J Med 2004). Although novel therapies are showing promising efficacy, this disease remains incurable, and development of novel therapies that are likely to induce complete remissions is important. Also avoiding the side effects of chemotherapy is a worthy goal in the patient population with prostate cancer, that is frequently elderly or with significant comorbidities (Oh W et al. Urology 2006 and Berthold DR et al. J Clin Oncol 2005). Given the limited life expectancy and the significant morbidity of metastatic prostate cancer (MPC), the development of novel therapies to improve outcomes is an “unmet need”. We propose to conduct a pilot trial in the metastatic CRPC patient population to explore the bone effects and tumor tissue effects of XL184. As this is a pilot study with exploratory endpoints, and prior treatment with standard of therapy is allowed, a single arm study design is reasonable.

Bone metastases in prostate cancer are primarily osteoblastic with evidence of deposition of new bone. The increased osteoblastic activity results in incorporation of Tc99-labeled methylene-diphosphonate allowing visualization of the lesions on bone scan. Met and its ligand, hepatocyte growth factor (HGF) have demonstrated increased expression after

androgen ablation as well as with disease progression to lymph nodes and soft tissue. XL184 is a VEGF and c-met inhibitor that has demonstrated promising clinical efficacy in metastatic CRPC. Not only does the agent produce promising response rates in measurable disease, but a marked change in bone scans is noted (Smith D. et al. ASCO GU Symposium 2011 Abstract #127). This is an unusual phenomenon, not seen even with any of the other agents that produce high levels of anti-tumor efficacy such as ADT. The physiologic changes occurring with the rapid normalization of bone scans (within the first 6 weeks) are unknown to date. We propose a pilot trial to study the changes occurring in bone metastases and in tumor tissue and to correlate with clinical outcome, with XL184 therapy. We hypothesize that XL184 uniquely targets the crosstalk between cMet/VEGFR2 axis, downstream cathepsin K-driven pathways and novel RTKs (e.g., DDR-1, DDR-2) in the bone tumor microenvironment. XL184 action leads to abrupt changes in bone metabolism and abrogation of ^{99m}Tc-MDP uptake due to the inhibition of osteoclast activity and subsequent decrease in osteoblast function.

Proposed Model for XL 184 Mechanism of Action in Prostate Cancer



Proposed Mechanism of Action of XL 184 in Prostate Cancer.
XL184 predominantly targets RTKs involved in tumor-induced bone resorption. Inhibition of osteoclast activity by XL184 results in reduced levels of the key osteoclast collagenase CTSK and overall inhibition of bone turnover. Inhibition of DDR-1, and -2 directly by XL184 and indirectly by reduced availability of resorbed collagen abrogates collagen-induced osteoblast differentiation and woven bone deposition. Abbreviations: RANKL ((Receptor Activator of NF-κB ligand), VEGF (Vascular Endothelial Growth Factor), VEGFR1-, 2- (VEGF Receptors), HGF (Hepatocyte Growth Factor), cMet (HGF Receptor), MCSF (Macrophage Colony Stimulating Factor), c-fms (MCSF Receptor), SCF (Stem Cell Factor), c-kit (SCF Receptor), FLt3 (FMS-like Tyrosine Kinase 3), DDR-1,2 (Discoidin Domain Receptor), PlGF (Placental Growth Factor), CTSK (Cathepsin K), TRAcP (Tartrate-Resistant Acid Phosphatase), NT_x (N-Telopeptide), CT_x (C-Telopeptide), ET-1 (Endothelin-1).

Imaging Studies

^{99m}Tc-MDP bone scan is a most widely used, standard of care method for evaluating skeletal metastases of prostate cancer. The ^{99m}Tc-MDP accumulates in new (woven) bone and is an indicator of changes in bone metabolism associated with prostate cancer-induced osteoblastic response. However, ^{99m}Tc-MDP scan findings are not specific in determining the cause of increased or decreased uptake and are indirect markers of response to treatment [Schoder H, et al. Semin Nucl Med 2004]. ^{18}F-flouride PET scans have improved anatomic detail over ^{99m}Tc-MDP scans, a higher accuracy in detecting both osteolytic and osteoblastic metastases and allow quantification of the extent of metastatic lesion [Shreve PD et al. Radiology 1996]. This imaging modality may be superior to 18F-FDG PET for prostate cancer, since the bone metastases in prostate cancer are primary osteoblastic. Osteoblastic metastases tend to exhibit a high rate of fluoride incorporation (Schoder H, et al. Semin Nuc Med 2004) and may have low FDG uptake (Shreve PD et al. radiology 1996). Additionally, ^{18}F-flouride PET has improved sensitivity and specificity when compared to standard ^{99m}Tc-MDP bone scintigraphy, and importantly, PET offers the ability for rigorous quantification.(Heicappell R et al. Eur Urol 1999, Yeh SD, et al. Nuc Med Biol 1996 and Schirrmeister H, et al. J Clin Oncol 1999). It is currently being evaluated in association with other therapeutic trials in prostate cancer (American College of Radiology Network, ACRIN Trial # 6687) to detect pharmacodynamic effects of novel agents. We will utilize the ^{18}F-flouride PET in this study to trace the absorption of fluoride ion by bone tissue and quantify bone response.

PET obtained with the thymidine analog ^{18}F-FMAU scan then be used to image tumor metabolism and it is based on incorporation of the tracer by mitochondrial thymidine kinase 2 (TK2) [Shields EF, et al. J Nucl Med 1998 and Tehtani OS, et al. Eur J Nucl Med Mol Imaging 2008]. The three modalities complement each other in distinguishing bone changes vs. tumor response to XL184. Distribution of ^{18}F-FMAU is useful in measuring cellular metabolism. Measurements of metabolism made during or after therapy would be far more sensitive measures of anti tumor effects than the more

conventional approach of observing the tumor for changes in size or just detecting location of the bone turnover as seen with ^{99m}Tc-MDP conventional bone scans. A decrease in mean SUV by 20% is considered a PET response and will be used as the threshold to detect changes. We have previously reported on the use of FMAU scans for detection of prostate cancer bone metastases from a study conducted at our institution [Sun H et al. Eur J Nucl Med Mol Imaging 2005]. We will utilize the^{18}F-FMAU PET to detect and quantify changes in tumor metabolism due to incorporation by mitochondrial thymidine kinase.

This study will utilize standard of care ^{99m}Tc-MDP bone scan, high resolution ^{18}F-flouride PET to measure effects of XL184 treatment on bone tissue, and ^{18}F-FMAU PET to measure changes in tumor metabolism in response to therapy. Imaging will be performed using a gamma camera and PET/CT scanners. ^{99m}Tc-MDP bone scans, ^{18}F-flouride PET and ^{18}F-FMAU PET is already approved for NOMIC-funded study (C-2335, Shields, AF; PI). We propose evaluating patients with ^{18}F-flouride imaging pre and post XL184 therapy to determine the optimal timing when bone scan normalization occurs. So approximately 5 patients will be evaluated with ^{18}F-flouride imaging pre and within 2-3 weeks post XL184. Then we will determine the optimal timepoint to perform imaging with FMAU PET scans to evaluate for anti tumor effect, as well as the possible osteoclast and osteoblast inhibition resulting from the medication, and the mechanisms underlying it.

Serum, Urine and tumor Tissue Correlates

XL184 uniquely targets osteoclast-driven events in the bone tumor microenvironment that ultimately affect osteoblast differentiation and new bone formation. Gene expression profiling analyses will validate novel molecular signatures with therapeutic potential identified by gene expression analysis. We propose to perform aCGH (array comparative genomic hybridization), expression profiling of human bone biopsy samples to determine molecular signatures prior to and in response to XL184 treatment.

This study will generate precious bone biopsy samples from bone lesions identified by imaging. These samples offer unique opportunity for analysis of changes in gene expression profiles in response to treatment. Particularly, imaging-guided assessment of early bone/tumor responses can potentially lead to identification of novel targets that distinguish responsive lesions from ones that progress.

We will also examine the effects of XL184 treatment on bone turnover and tumor growth and survival via histological, immunohistochemical and serum bone marker analyses in human bone biopsy samples. We will utilize established protocols and procedures for aCGH, expression profiling, as well as histological and serum marker analyses. Performing aCGH will allow us to identify genetic translocation such as TMPRSS2-ERG. In addition, archival tissue will be collected and c-met and VEGF expression will be evaluated pre and post therapy. Pre and post therapy CTC counts and phenotype will be tested. Post therapy biopsies will be obtained for checking for VEGF and c-met expression, and pre and post therapy serum and urine bone markers to assess bone turnover will be examined. Baseline tumor tissue will be evaluated for oncogene mutations and correlated with response and clinical outcome. Although there are no common point mutations in oncogenes, we have the capability (such as the pyrosequencer and sequenom) to perform mutational assays on any gene discovered in the future.

The clinical response and outcome will be correlated with changes noted in tumor imaging and tissue changes to explore the pathways influencing antitumor efficacy of this novel agent.

Based on prior clinical trial enrollment, about 40% of the patient population is expected to be of African American ancestry. This will enable evaluation of XL184 effects in a population where it has not been systematically studied. Given the likely differences in prostate cancer disease course and biology, and associated comorbidities such as hypertension, this experience would broaden the safety and efficacy data regarding XL184.

1.3.1 Rationale for Cabozantinib Dose Selection

A cabozantinib starting dose of 100 mg daily has been studied in 171 CRPC subjects enrolled to the Phase 2 XL184-203 RDT. Despite relatively high rates of cabozantinib dose reductions to the next lowest dose of 60 mg daily within the first 12 weeks of therapy (51%), this starting dose resulted in high rates of pain relief, bone scan improvement, and overall disease control.

Preliminary data from a separate and ongoing dose-ranging study looking at lower doses of cabozantinib in CRPC coupled with results from a retrospective review of the Phase 2 XL184-203 RDT indicate that lower doses below 100 mg daily are likely to retain efficacy while improving upon tolerability:

Preliminary results from an ongoing dose-ranging study: To date, 9 subjects with metastatic CRPC enrolled to the first cohort (starting dose of 40 mg qd) are evaluable for bone scan response. All 9 subjects exhibit evidence of response on bone scan including two complete responses. Although most subjects did not have pain at baseline, one subject reported pain at baseline which resolved by Week 6. No dose reductions or interruptions have been reported to date, although one subject discontinued study treatment for fatigue that was present at baseline and another subject discontinued because of a pathologic fracture. This provides preliminary evidence that lower doses are pharmacologically active in a patient population with advanced CRPC.

Retrospective review of Phase 2 XL184-203 RDT: While the overall rate of dose reduction from 100 mg to 60 mg was 51%, only 14% required an additional reduction in dose from 60 mg to the next lowest dose of 40 mg, which is consistent with an overall improvement in tolerability profile at the 60-mg dose level. The majority (69%) of subjects with pain at baseline who experienced early dose reduction (before Week 6) to 60 mg went on to report pain improvement at Week 6. Moreover, 80% of these subjects remained progression-free and continued to report pain relief at the Week 12 time point. Thus the dose of 60 mg qd appears to offer improved tolerability while maintaining efficacy in a patient population with advanced CRPC and cancer-related pain at baseline.

Further analysis of the timing of AEs that led to dose reductions or interruptions was conducted. The median time to first AE triggering a dose reduction or interruption at 100 mg qd was 29 days, with very few subjects experiencing significant toxicity in the first 2 weeks of study treatment.

As such this study will adopt a starting cabozantinib dose of 60 mg qd. The goal of this regimen is to improve the overall tolerability of cabozantinib while maintaining efficacy in this patient population.

2 STUDY DESIGN

2.1 STUDY DESIGN

2.1.1 Overview of Study Design

The study consists of open label daily, oral administration of XL184 or cabozantinib at a starting dose of 60mg to eligible patients.

Administration

Subjects will be provided with a sufficient supply of study treatment and instructions for taking the study treatment on days without scheduled clinic visits. After fasting (with exception of water) for 2 hours, subjects will take study treatment daily with a full glass of water (minimum of 8 oz/ 240 mL) and continue to fast for 1 hour after each dose of study treatment. Subjects should record dosing time and doses taken in a study drug dosing diary while on study treatment. If doses are withheld, the original schedule of assessments should be maintained when XL184 is restarted. The subject should be instructed to not make up the missed doses and to maintain the planned dosing schedule. Subjects must be instructed to not make up missed doses that are vomited.

This study consists of the following: (1) a Study Treatment Period consisting of continued treatment with XL184, and (2) a Post-Treatment Period.

2.1.2 Study Treatment Period

The Study Treatment Period will consist of continued treatment during which time subjects will receive XL184 until either disease progression or the occurrence of unacceptable drug-related toxicity. Subjects will be assessed for safety at least every 2 weeks for the first 3 months and then at least every 4 weeks thereafter. Adverse events will be collected throughout the study and recorded in the subject's clinical records

Subjects' tumors will be objectively assessed by PET scans and CT scans and bone scans. The purpose of these assessments is to allow the investigator to determine if the subject is benefiting from XL184 and to explore the mechanisms of changes occurring in bone and

tumors. The response will be assessed by PSA working group criteria and by RECIST 1.1 criteria for measurable disease. Karmanos Cancer institute purchases clinical services (i.e. CT scans, PET/CT scans, pulmonary function test, etc.) from the Detroit Medical Center (DMC), a clinical services provider on the medical campus. The consent form language informs the subjects that they may receive services in a DMC facility.

2.3 STUDY CALENDAR:

Prestudy labs should be done within 21 days of day 1. Day 1 labs can be done within 3 days of day 1. A time period of +/- 3 days is permitted for each of the days listed in the calendar

Testing	Prestudy	Day 1	Wk 1	wk 2	wk 4	wk 6	Wks 8, 10 12	Q 4 wks post wk 12	Q12 wks post wk 8[10]
H and P	X	X	X	X	X	X	X	X	
VS, Wt	X	X	X	X	X	X	X	X	
PS	X	X		X	X	X	X	X	
Tox	X	X	X	X	X	X	X	X	
CBCdiff,plt	X	X	X	X	X	X	X	X	
Lytes,glu[1]	X	X	X	X	X	X	X	X	
Labs/LFTs[2]	X	X		X	X	X	X	X	
PSA	X	X	X	X	X	X	X	X	
Testosterone, PT/PTT/INR	X								
TSH, free T4	X					X			X
UA/UPC	X		X		X	X	X	X	
ECG[3]	X				X		X[3]	X[3]	X[3]
Biopsy	X			X					
CT/CXR[4,5]	X					X			X
MDP-Bone scan[5]	X			X		X			X
^{18}F-flouride-PET[6]	X			X					X
XL184		X	X	X	X	X	X	X	X
FMAU PET[7]	X			X					
CTC[8]	X			X					
PD/Bone markers[9]	X		X	X	X				

1. Lytes include lytes, BUN and creatinine assessment. Fasting glucose should be measured.

2. Labs include: Amylase, lipase, Albumin, alkaline phosphatase, total bilirubin, calcium, chloride, magnesium, γ-GT, LDH, inorganic phosphorus, total protein, SGOT, SGPT. If the total bilirubin concentration is increased above 1.5 times the upper normal limit (UNL), total bilirubin should be differentiated into the direct and indirect reacting bilirubin.

3. ECG assessments prestudy, once every 4 weeks through week 12 and then every 8 weeks beyond week 12.

4. CXR is required pretherapy unless pulmonary metastases are noted. If pulmonary metastases, then chest CT is needed at baseline and for subsequent tumor assessment. If CT chest is done pretherapy, then CXR is not needed

5. All tumor evaluation/measurement scans must be within 42 days prior to treatment. CT abdomen /pelvis is required for pretreatment and subsequent tumor assessments.

6. FMAU scans are done under a separate protocol and patients will have to sign consent for those studies separately. Repeat bone scans and PET scans will be done during week 2 on first 5 patients and the schedule will be re–evaluated based on findings. ^{18}F-flouride PET scans should preferably be done before the bone scan in week 2. At week 32+/- 10 weeks or at the time of discontinuing study therapyif this occurs prior to week 32, a ^{18}F-flouride PET scan will be done. A FMAU scan will also be done at the same time period if patient has had <3 FMAU scans in his lifetime.

7. The schedule for FMAU PET will also be determined based on the timeline of changes observed in bone scans and PET scans in the first 5 patients.

8. CTC samples will be drawn at pretherapy, week 2 (post therapy) and at progression.

9. PD samples and bone markers ; blood samples and urine will be collected per schedule; pretherapy day 8 (week 1), day 15 (week 2) and day 29 (week 4). For bone markers, 4cc of blood should be collected in a tiger top tube and the urine NTx requires a 24 hour urine collection.

10. End of treatment and follow up within 4 weeks of discontinuing therapy: End of treatment visit- EKG, urine analysis and CBC, multiphasic and PSA. Repeat tumor

assessment if not performed within the last 6 weeks. At week 32+/- 10 weeks or at the time of discontinuing study therapy if this occurs prior to week 32, a ^{18}F-flouride PET scan and /or FMAU scan (only if patient has had <3 FMAU scans in his lifetime) will be done.

All study bottles and diaries will be collected and submitted. Patients will be followed for a maximum of 5 years after end of treatment. Follow up will be for survival, progression and next line of therapy.

2.1.3 Post-Treatment Period

Subjects will return to the study site to complete end-of-study assessments 4 weeks (± 2 week) after their last dose of XL184. Patients will be followed for progression free survival and overall survival.

2.4 Treatment Assignment

It is the responsibility of the investigator to assign a subject number prior to treating each subject with XL184.

2.5 Study Sites

This study will be conducted at Karmanos Cancer Center, Detroit MI.

2.6 Withdrawals

Subjects may discontinue study treatment or withdraw their consent to participate in the study at any time without prejudice. The investigator may withdraw a subject from study treatment or from the study if, in his or her clinical judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol.

In addition, any of the following conditions require withdrawal of the subject from study treatment:

- An AE or intercurrent illness that in the opinion of the investigator warrants the subject's withdrawal from treatment

- Necessity for treatment with other investigational drug or other anticancer medications prohibited by protocol
- Participation in another clinical study using an investigational agent
- Request by regulatory agencies for termination of treatment of an individual subject or all subjects under this protocol
- Sexually active subjects who refuse to use medically accepted barrier methods of contraception (eg, male condom, or diaphragm with spermicidal gel) during the course of the study and for 3 months following discontinuation of study treatment.
- Inability to tolerate XL184
- XL184 treatment delays > 3 weeks
- Progressive disease (PD) as determined by the investigator.

The reason for study treatment discontinuation will be documented. For subjects who discontinue or are withdrawn from study treatment, every effort must be made to undertake protocol-specified follow-up procedures and end-of-treatment assessments, if possible, unless consent to participate in the study is also withdrawn.

If a subject fails to return for the protocol-defined visits, an effort must be made to determine the reason. If the subject cannot be reached by telephone, at the minimum a registered letter should be sent to the subject (or the subject's legal guardian) requesting contact with the clinic.

If a subject is discontinued from study treatment because of an AE considered to be related to study treatment and the event is ongoing 30 days after the last dose of study treatment, the event must be followed until resolution or determination by the investigator that the event has become stable or irreversible.

If a subject withdraws consent to participate in the study, the reason for withdrawal will be documented, no study procedures or assessments will be performed, and no further study data will be collected for this subject, other than the determination of survival status from public records such as government vital statistics or obituaries.

2.7 TREATMENT PLAN AND DOSE MODIFICATIONS

Composition, Formulation, and Storage

At study site, all study medication will be stored as described in the pharmacy manual and inventoried in accordance with applicable state and federal regulations.

2.7.1 Investigational Treatment

XL184 Tablets

Investigational Treatment: Cabozantinib tablets

Chemical Name: N-{4-[(6,7-dimethoxyquinolin-4-yl)oxy]phenyl}-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, (2S)-hydroxybutanedioate

Exelixis internal number: XL184

Exelexis will provide each investigator with adequate supplies of cabozantinib, which will be supplied as 60-mg, and 20-mg yellow film-coated tablets. The 60-mg tablets are oval, and the 20-mg tablets are round. The components of the tablets are listed below.

Cabozantinib Tablet Components and Composition

Ingredient	Function	% w/w
Cabozantinib Drug Substance (25% drug load as free base)	Active Ingredient	31.7
Microcrystalline Cellulose (Avicel PH-102)	Filler	38.9
Lactose Anhydrous (60M)	Filler	19.4
Hydroxypropyl Cellulose (EXF)	Binder	3.0
Croscarmellose Sodium (Ac-Di-Sol)	Disenegrant	6.0
Colloidal Silicon Dioxide,	Glidant	0.3
Magnesium Stearate	Lubricant	0.75
Opadry Yellow Film Coating which includes: - HPMC 2910 / Hypromellose 6 cp - Titanium dioxide - Triacetin - Iron Oxide Yellow	Film Coating	4.00

Dose, Schedule and Route

Subjects will receive XL184 orally at a (starting) dose of 60 mg once daily (3 Tabs of 20 mg each or 1 Tab of 60 mg each). The study consists of open label daily, oral administration of XL184 or cabozantinib at a starting dose of 60 mg (dose level 1) to eligible patients. Dose adjustment levels are given in table below:

Dose Level	Agent	Dose	Route	Frequency
Starting	XL 184	60 mg	PO	Daily
-1	XL 184	40 mg	PO	Daily
-2	XL 184	20 mg	PO	Daily

Administration

Subjects will be provided with a sufficient supply of study treatment and instructions for taking the study treatment on days without scheduled clinic visits. After fasting (with

exception of water) for 2 hours, subjects will take study treatment daily with a full glass of water (minimum of 8 oz/ 240 mL) and continue to fast for 1 hour after each dose of study treatment. Subjects should record dosing time and doses taken in a study drug dosing diary while on study treatment. If doses are withheld, the original schedule of assessments should be maintained when XL184 is restarted. The subject should be instructed to not make up the missed doses and to maintain the planned dosing schedule. Subjects must be instructed to not make up missed doses that are vomited.

2.7.2General Guidelines for Treatment Delay, Dose Reduction, or Study Drug Discontinuation for Toxicity

Subjects will be monitored continuously for AEs throughout the study and for 30 days after the last dose of study treatment, and for any serious adverse event (SAE) assessed as related to study treatment or study procedures, even if the SAE occurs more than 30 days after the last dose of study treatment.

Subjects will be instructed to notify their physician immediately of any and all AEs. Subjects experiencing one or more AEs due to the study treatment may require a dosing delay or reduction(s) in their dose in order to continue with study treatment.

Re-escalating study treatment after a dose reduction:

- Subjects who required a dose reduction for Grade 3 or 4 non-hematologic toxicity should not be re-escalated
- For other related AEs, subjects may be re-escalated to the previous dose at the discretion of the investigator but not sooner than 2 weeks beyond the resolution to Grade ≤ 1 or to the baseline value of AEs.
- If a subject has been dose-reduced more than once, dose re-escalation can only occur to the next higher dose level. Further dose escalation to higher well-tolerated dose levels is allowed only if clinically indicated per investigator's judgment and dose escalation criteria are met with each escalation (e.g. a minimum 2 week interval between escalations)
- If the AEs that previously led to dose reduction(s) recur upon re-escalation, the dose should be reduced again and no further dose escalation will be permitted.
- Dose re-escalation is not allowed for dose reduction triggered by neutropenia or thrombocytopenia.

Discontinuation from study treatment:

- If the subject does not recover from his or her toxicities to tolerable Grade ≤ 2 within 3 weeks, the subject will have study treatment discontinued unless there is

unequivocal evidence that the subject is benefitting. In this situation, a subject may be able to restart therapy with a dose reduction upon resolution of the toxicity.

.

- The minimum dose of study treatment will be 20 mg daily. Subjects who cannot tolerate 20 mg daily dose, will have study treatment discontinued.

Additional information for dose delays or dose reductions:

- Dose delays for reason(s) other than AEs related to cabozantinib, such as surgical procedures with no anticancer therapy intent, may be allowed with investigator approval. The acceptable length of interruption will be determined by the investigator.

2.8 Warnings, Precautions, and Management of Anticipated Adverse Events

The general adverse event profile of cabozantinib includes GI symptoms (such as nausea, vomiting, and diarrhea), fatigue, anorexia, palmar-plantar erythrodysesthesia (PPE) syndrome, skin rash, elevated ALT and AST, increased pancreatic enzymes with rare cases of pancreatitis, as well as side effects associated with inhibition of VEGF signaling such as thrombotic events (eg, pulmonary embolism [PE] and deep vein thrombosis [DVT]), hypertension, proteinuria, hemorrhagic events, and rare cases of gastrointestinal [GI] perforation and rectal/perirectal abscess. Arterial thromboembolism (transient ischemic attack [TIA], myocardial infarction [MI]) have been reported rarely.

2.9 Management of Adverse Events

In the absence of an unacceptable cabozantinib-related toxicity and/or clinical signs of disease progression, subjects may continue treatment at the discretion of the investigator. Subjects must be instructed to notify their physician immediately for any and all toxicities.

Guidelines for the management of AEs (ie, dose interruptions and dose reductions) are presented in the next sections. Each dose reduction of cabozantinib should be to one dose level lower that the current dose. Dose reductions of more than one dose level are acceptable if agreed to by the Investigator. If study treatment of cabozantinib is restarted after being withheld or interrupted, the subject should be instructed not to make up the missed doses of cabozantinib.

The reason for treatment delay and reduced dose must be recorded on the case report form (CRF).

Dosing may need to be interrupted for AEs considered not related to cabozantinib if this is clinically indicated or if causality is initially uncertain. Study treatment may be resumed at the same dose (or a lower dose per investigator judgment) if the AE is determined not to be related to cabozantinib once the investigator determines that retreatment is clinically appropriate and the subject meets the protocol re-treatment criteria.

2.10 General Guidelines for Non-Hematologic and Hematologic Adverse Events

General guidelines for the management of non-hematologic and hematologic toxicities are provided in Table 3-2 and Table 3-3, respectively. As a general approach, it is suggested that all AEs be managed with supportive care when possible at the earliest signs of toxicity. For more specific guidelines on gastrointestinal AEs (diarrhea, nausea/vomiting, stomatitis/mucositis), hepatobiliary disorders, pancreatic disorders including lipase and amylase elevations, skin disorders (PPE), embolism and thrombus, hypertension, proteinurea, hemorrhage, rectal and perirectal abscess, gastrointestinal perforation and gastrointestinal fistula, wound healing and surgery, and endocrine disorders, refer to Section 3.4.1.2 – Section 3.4.1.13 below. Guidance for the management of fatigue, anorexia, weight loss, non-gastrointestinal fistula, osteonecrosis of the jaw, eye disorders, musculoskeletal and connective tissue disorders, nervous system disorders, respiratory/thoracic/mediastinal disorders and congenital, familial and genetic disorders can be found in the Investigators Brochure.

2.11 Dose Modification Criteria and Management of Adverse Events

Dose Modification levels:

Table 1

Dose Level	Agent	Dose	Route	Frequency
Starting	XL 184	60 mg	PO	Daily
-1	XL 184	40 mg	PO	Daily

-2	XL 184	20 mg	PO	Daily

General Approach to the Management of Study Treatment-Related Non-Hematologic Toxicities

CTCAE Version 4 Grade	Guidelines/Intervention
Grade 1:	Add supportive care as indicated. Continue study treatment at the current dose levels.
Grade 2:	
Grade 2 AEs considered related to study treatment that are subjectively tolerable or easily managed	Add supportive care as indicated. Continue study treatment at the current dose levels.
Grade 2 AEs considered related to study treatment that are intolerable to the subject or deemed unacceptable in the investigator's judgment; or are not easily managed or corrected	Dose reduce • If the AE dose not resolve to Grade ≤ 1 or baseline in 7 to 10 days or worsens at any time, cabozantinib dosing should then be interrupted. Then upon resolution to baseline or Grade ≤ 1, the reduced dose should be restarted. • If the AE does resolves to resolves to Grade ≤ 1 or baseline without a dose interruption, continue the reduced dose.
Grade 3:	
Grade 3 AEs considered related to study treatment which occurred without optimal prophylaxis or which is easily managed by medical intervention or resolved quickly	• Interrupt study treatment and add supportive care as indicated • For AEs that are easily managed (e.g., correction of electrolytes) with resolution to baseline or Grade ≤ 1 within 24 hours, treatment may be resumed at either the same dose or with a dose reduction at the discretion of the investigator unless this is a recurring event at which time the dose should be reduced • For AEs that require supportive care, the dose should be held while supportive care is initiated and optimized. Then upon resolution of the AE to baseline or Grade ≤ 1, treatment should be restarted with a dose reduction. Note: if the investigator believes the likelihood of a reoccurrence of the same Grade 3 AE is small due to continued prophylaxis or other effective intervention, treatment may be resumed without a dose reduction and with very careful monitoring of the subject.
Grade 3 AEs considered related to study treatment that occurred despite optimal prophylaxis or is not easily managed by medical intervention	Interrupt study treatment until recovery to ≤ Grade 1 or baseline, and resume treatment with a dose reduction
Grade 4:	
Grade 4 AEs considered related to study treatment	Permanently discontinue study treatment unless determined that the subject is unequivocally deriving clinical benefit. In this case, upon recovery to Grade ≤ 1 or baseline, the subject may be re-treated at a reduced dose that is to be determined by the investigator.

Dose reductions or delays may occur in the setting of lower grade toxicity than defined above if the investigator believes that it is in the interest of the subject's safety.

Table 0-1 General Approach to the Management of Hematologic Toxicities

CTCAE Version 4 Grade	Intervention
Neutropenia	
Grade 3 neutropenia with documented infection Grade 3 neutropenia ≥ 5 days Grade 4 neutropenia	Interrupt cabozantinib treatment until resolution to Grade ≤ 1, and resume cabozantinib treatment at a reduced dose.
Thrombocytopenia	
Grade 3 thrombocytopenia with clinically significant bleeding or Grade 4 thrombocytopenia	Interrupt cabozantinib treatment until resolution to ≤ Grade 1, and resume cabozantinib treatment at a reduced dose
Febrile Neutropenia	
Grade 3 febrile neutropenia	Interrupt cabozantinib treatment until recovery of ANC to Grade ≤ 1 and temperature to ≤ 38.0°C and resume cabozantinib treatment at a reduced dose.
Grade 4 febrile neutropenia	Permanently discontinue study treatment unless determined that the subject is unequivocally deriving clinical benefit. In this case, upon recovery to Grade ≤ 1 or baseline, the subject may be re-treated at a reduced dose that is to be determined by the investigator.

ANC, absolute neutrophil count; LLN, lower limit of normal

Neutropenia: Grade 1 (LLN ≤ ANC < 1.5 × 10^9/L; Grade 2 (1 × 10^9/L ≤ ANC <1.5 x 10^9/L), Grade 3 (0.5 × 10^9/L ≤ ANC < 1 × 10^9/L), Grade 4 (ANC < 0.5 × 10^9/L).

Febrile Neutropenia: Grade 3 (present); Grade 4 (Life-threatening consequences; urgent intervention indicated).

Thrombocytopenia: Grade 1 (<LLN – 75 x 10^9/L); Grade 2 (<75.0 – 50.0 x 10^9/L); Grade 3 (Platelet count ≤ 50 - 25 × 10^9/L); Grade 4 (Platelet count < 25 × 10^9/L).

2.11.1Diarrhea, Nausea, Vomiting, Stomatitis, and Mucositis

Diarrhea

Subjects should be instructed to notify their physician immediately at the first signs of poorly formed or loose stool or an increased frequency of bowel movements. Administration of antidiarrheal agents is recommended at the first sign of diarrhea as initial management. Loperamide is recommended as standard first line therapy.

Alternatively, diphenoxylate/atropine can be used. Additional agents to consider in subjects with diarrhea that is refractory to the above include deodorized tincture of opium and octreotide (Benson et al. 2004). Some subjects may require concomitant therapy with loperamide, diphenoxylate/atropine, and deodorized tincture of opium to control diarrhea. When combination therapy with antidiarrheal agents does not control the diarrhea to tolerable levels, a dose reduction and/or dose interruption of cabozantinib should be implemented as described. In addition, general supportive measures should be implemented including continuous oral hydration, correction of fluid and electrolyte abnormalities, small frequent meals, and stopping lactose-containing products, high fat meals and alcohol.

Nausea and Vomiting

Anti-emetic agents along with supportive care are recommended as clinically appropriate at the first sign of nausea and vomiting. A dose reductions and/or dose interruption of cabozantinib may be required as described in Table if antiemetic treatment and/or prophylaxis alone is not adequate.

Agents classified as having a high therapeutic index (such as 5-HT3 receptor antagonists, or NK-1 receptor antagonists) per ASCO or MASCC/ESMO guidelines for anti-emetics in oncology or dexamethasone are recommended (Hesketh et al. 2008, ASCO 2006; Roila et al, Annals of Oncology, 2010). Caution is recommended with the use of aprepitant or fosaprepitant and nabilone as cabozantinib exposure may be affected by concomitant administration because aprepitant and fosaprepitant are both inhibitors and inducers of CYP3A4, and nabilone is a weak inhibitor of CYP3A4.

Stomatitis and Mucositis

Preventive measures may include a comprehensive dental examination to identify any potential complications before study treatment is initiated. Appropriate correction of local factors should be instituted as indicated, such as modification of ill-fitting dentures and appropriate care of gingivitis. During treatment with cabozantinib, good oral hygiene and standard local treatments such as non-traumatic cleansing, and oral rinses (eg, with a weak solution of salt and baking soda) should be maintained. The oral cavity should be rinsed and wiped after meals, and dentures should be cleaned and brushed often to remove plaque. Local treatment should be instituted at the earliest onset of symptoms.

When stomatitis interferes with adequate nutrition and local therapy is not adequately effective, dose reduction or temporary withholding of cabozantinib should be considered.

2.11.2 Hepatobiliary Disorders

Elevations of transaminases have also been observed during treatment with cabozantinib. In general, it is recommended that subjects with elevation of ALT, AST, and/or bilirubin have more frequent laboratory monitoring of these parameters. If possible, hepatotoxic concomitant medications and alcohol should be discontinued in subjects who develop elevated transaminases.

Since subjects may enter the study with elevations of AST/ALT at baseline, the following guideline should be used for dose modifications:

Transaminase elevation CTCAE v4.0	Intervention
Subjects with AST and ALT less than or equal to the ULN at baseline	
Grade 1	Continue study treatment with weekly monitoring of liver function tests (LFTs) for at least 4 weeks.. Then resume the standard protocol-defined monitoring of LFTs.
Grade 2	Continue study treatment with at least twice weekly monitoring of LFTs for 2 weeks. Then weekly for 4 weeks. If LFTs continue to rise within Grade 2, interrupt study treatment. Then continue with at least weekly LFTs until resolution to Grade ≤ 1. Study treatment may then be resumed at a one-dose-level reduction of cabozantinib
Grade 3	Interrupt study treatment and monitor with at least twice weekly LFTs until Grade ≤ 2. Then continue with at least weekly LFTs until resolution to Grade ≤ 1. Study treatment may then be resumed at a one-dose-level reduction of cabozantinib.
Grade 4	Discontinue study treatment permanently. LFTs should be monitored as clinically indicated, at least 2-3 times per week, until resolution to Grade ≤ 1. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume treatment at a lower dose as determined by the investigator
Subjects with AST or ALT above the ULN but ≤ 3.0 x ULN (i.e., Grade 1) at baseline	
≥ 1.5 fold transaminases increase (at least one of AST or ALT) and still Grade 1 or Grade 2	Continue study treatment with at least twice weekly monitoring of LFTs for 4 weeks. If LFTs continue to rise, interrupt study treatment. Then continue with at least weekly LFTs until resolution to Grade ≤ 1. Study treatment may then be resumed at a one-dose-level reduction of cabozantinib
≥ 1.5 fold transaminases increase (at least one of AST or ALT) and Grade 3	Interrupt study treatment and monitor with at least twice weekly LFTs until Grade ≤ 2. Then continue with at least weekly LFTs until resolution to Grade ≤ 1. Study treatment may then be resumed at a one-dose-level reduction of cabozantinib.
Grade 4	Discontinue study treatment permanently. LFTs should be monitored as clinically indicated, at least 2-3 times per week, until resolution to Grade ≤ 1. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume treatment at a lower dose as determined by the investigator.
Subjects AST or ALT > 3.0 but ≤ 5.0 x ULN at baseline	
≥ 1.5 fold transaminases increase (at least one of AST or ALT) and still Grade 2 or Grade 3	Interrupt study treatment and monitor with at least twice weekly LFTs until LFTs resolve to baseline and Grade ≤ 2. Study treatment may then be resumed at a one-dose-level reduction of cabozantinib.
Grade 4	Discontinue study treatment permanently. LFTs should be monitored as clinically indicated, at least 2-3 times per week, until resolution to Grade ≤ 1. If the subject was unequivocally deriving clinical benefit, the subject may be able to resume treatment at a lower dose as determined by the investigator.

Cabozantinib treatment should also be interrupted when transaminase increases are accompanied by progressive elevations of total bilirubin, and/or elevations of coagulation tests (eg, International Normalized Ratio [INR]). Monitoring of transaminases should be intensified (2–3 times per week) and cabozantinib should be held until the etiology of the abnormalities is determined and these abnormalities are corrected or stabilize at clinically acceptable levels (INR < 1.5 × ULN, total bilirubin < 1.5 × ULN, aminotransferases < 2.5 × ULN or baseline).

Subjects must have cabozantinib permanently discontinued if transaminase elevations are accompanied by evidence of impaired hepatic function (bilirubin elevation >2xULN), in the absence of evidence of biliary obstruction (i.e., significant elevation of alkaline phosphatase [ALP]) or some other explanation of the injury (e.g., viral hepatitis, alcohol hepatitis), as the combined finding (i.e., Hy's Law cases) represents a signal of a potential for the drug to cause severe liver injury.

All subjects who develop isolated bilirubin elevations of Grade 3 should have study treatment held until recovered to Grade ≤ 1 or baseline (or lower). If this occurs within 6 weeks of the dosing delay, study treatment may continue at a reduced dose. In subjects without biliary obstruction and Grade 4 bilirubin elevation, or with recurrence of Grade 3 bilirubin elevation after a dose reduction, study treatment must be discontinued.

2.11.3 Pancreatic Conditions

Amylase and lipase elevations have been observed in clinical studies with cabozantinib. The clinical significance of asymptomatic elevations of enzymes is not know but in general have not been associated with clinically apparent sequelae. It is recommended that subjects with lipase elevation and/or symptoms of pancreatitis have more frequent laboratory monitoring of lipase and/or amylase (2-3 times per week). Subjects with symptomatic pancreatitis should be treated with standard supportive measures.

Asymptomatic Lipase or Amylase Elevations

Asymptomatic Lipase or Amylase Elevations	
Grade 1 or Grade 2	Continue at current dose level. More frequent monitoring is recommended
Grade 3	• Interrupt treatment • Monitor lipase and amylase twice weekly • Upon resolution to Grade ≤ 1 or baseline, cabozantinib may be restarted at the same dose or at a reduced dose provided that this occurs within 6 weeks. • If retreatment following Grade 3 lipase or amylase elevation is at the same dose and Grade 3 elevations recur, then treatment must be interrupted again and till lipase and amylase levels have resolved to Grade ≤ 1 or baseline and retreatment must be at a reduced dose.
Grade 4	• Interrupt treatment • Monitor lipase and amylase twice weekly • Upon resolution to Grade ≤ 1 or baseline and if resolution occurred within 4 days, cabozantinib may be restarted at the same dose or a reduced dose. If resolution took more than 4 days, the dose must be reduced upon retreatment provided that resolution occurred within 6 weeks. • If retreatment following Grade 4 lipase or amylase elevation is at the same dose and Grade 3 or 4 elevations recur, then treatment must be interrupted again until lipase and amylase have resolved to Grade ≤ 1 or baseline and retreatment must be at a reduced dose.

Symptomatic Pancreatitis

Pancreatitis	
Grade 1	Continue at current dose level. More frequent monitoring of lipase and amylase and radiographically is recommended
Grade 2	• Interrupt treatment • Monitor lipase and amylase twice weekly • Upon resolution to Grade ≤ 1 or baseline , cabozantinib may be restarted at the same dose or at a reduced dose provided that this occurs within 6 weeks. • If retreatment following Grade 2 pancreatitis is at the same dose and Grade 2 pancreatitis recurs, then treatment must be interrupted again and till resolution to Grade ≤ 1 or baseline and retreatment must be at a reduced dose.
Grade 3	• Interrupt treatment • Monitor lipase and amylase twice weekly • Upon resolution to Grade ≤ 1 or baseline , cabozantinib may be restarted at a reduced dose if resolution occurred within 6 weeks
Grade 4	Permanently discontinue treatment. However, if the subject was unequivocally deriving benefit from cabozantinib therapy, treatment may resume at a reduced dose per investigator judgment.

2.11.4 Skin Disorders

Palmar-plantar erythrodysesthesia syndrome (PPE; also known as hand-foot syndrome), skin rash (including blister, erythematous rash, macular rash, skin exfoliation, dermatitis acneiform, and papular rash), pruritus, dry skin, erythema, pigmentary changes, and alopecia have been reported with cabozantinib. All subjects on study should be advised on prophylactic measures including the use of emollients, removal of calluses, avoidance of exposure of hands and feet to hot water leading to vasodilatation, protection of pressure-sensitive areas of hands and feet, and use of cotton gloves and socks to prevent injury and keep the palms and soles dry.

The onset of PPE is variable with paraesthesia (tingling, numbness) being the characteristic initial manifestation, which can be accompanied by slight redness or mild hyperkeratosis. PPE advances with symmetrical painful erythema and swollen areas (edema) on the palms and soles. The lateral sides of the fingers or periungual zones may also be affected. Adequate interventions are required to prevent worsening of skin symptoms such as blisters, desquamations, ulcerations, or necrosis of affected areas.

Aggressive management of symptoms is recommended, including early dermatology referral. Subjects with skin disorders should be carefully monitored for signs of infection (eg, abscess, cellulitis, or impetigo).

In the case of study treatment-related skin changes (eg, rash, hand-foot syndrome), the investigator may request that additional assessments be conducted with the subject's consent. These assessments may include digital photographs of the skin changes and/or a biopsy of the affected skin and may be repeated until the skin changes resolve.

Hand-Foot Skin Reaction and Hand Foot Syndrome (PPE)	
No apparent toxicity	Prophylaxis with Ammonium lactate 12% cream (Amlactin®) twice daily OR heavy moisturizer (e.g. Vaseline) twice daily
Grade 1	Continue treatment at current dose if tolerable or reduce to the next lower dose if intolerable. Start urea 20% cream twice daily AND clobetasol 0.05% cream once daily. Assess subject at least weekly for changes in severity. Subjects should be instructed to notify investigator immediately if severity worsens. If severity worsens at any time or if there is no improvement after 2 weeks, proceed to the management guidelines for Grade 2 PPE
Grade 2	Reduce study treatment to next lower level and/or interrupt dosing. Start/continue urea 20% cream twice daily AND clobetasol 0.05% cream once daily. Pain control with NSAIDs/GABA agonists/narcotics. Assess subject at least weekly for changes in severity. Subjects should be instructed to notify investigator immediately if severity worsens. If severity worsens at any time (eg, peeling, blisters, bleeding, edema, or hyperkeratosis or affects self-care) or if there is no improvement after 2 weeks, proceed to the management guidelines for Grade 3 PPE. If the dose was reduced, then upon resolution to Grade 0 or Grade 1, treatment may continue at the reduced dose. If the dose was only interrupted but not reduced, then treatment may be restarted at one dose level lower.
Grade 3	Interrupt study treatment until severity decreases to Grade 1 or 0. Start/continue urea 20% cream twice daily AND clobetasol 0.05% cream once daily. Pain control with NSAIDs/GABA agonists/narcotics. Treatment may restart at one dose level lower when reaction decreases to Grade 1 or 0. Permanently discontinue subject from study if reactions worsen or do not improve within 6 weeks.

GABA, γ-aminobutyric acid; NSAID, nonsteroidal anti-inflammatory drugs; PPE, palmar-plantar erythrodysesthesia

2.11.5 Embolism and Thrombosis

In clinical studies with cabozantinib, venous thrombotic events (DVT and PE) have been observed in less than 10% of subjects, and arterial thromboembolic events (MI and TIA) have been reported rarely. In addition, subjects with cancer have a significantly increased likelihood of developing thromboembolic complications (Agnelli et al, 2009).

Subjects who develop a PE and/or DVT should have study treatment interrupted until full anticoagulation is established with low molecular weight heparin (LMWH) (Full anticoagulation with warfarin is not permitted). Venous filters (e.g. vena cava filters) are not recommended due to the high incidence of complications associated with their use. Once a subject is fully anticoagulated, treatment can be restarted per investigator judgment at one dose lower. Subjects should permanently discontinue after a second thrombotic event. Although routine prophylactic anticoagulation is not necessary for all

subjects, prophylactic anticoagulation is allowed for individual subjects at the discretion of the investigator.

Cabozantinib should be discontinued in subjects who develop an acute MI or any other clinically significant arterial thromboembolic complication.

2.11.6 Hypertension

Hypertension is a relatively common complication of other VEGF-pathway inhibitors and has been observed in cabozantinib clinical studies.

Decisions to decrease or hold the dose of study treatment must be based on BP readings taken by a medical professional and must be confirmed with a second measurement at least 5 minutes following the first measurement. Other than for hypertension requiring immediate therapy, the presence of new or worsened hypertension should be confirmed at a second visit before taking new therapeutic action. Blood pressure should be monitored in a constant position visit to visit, either sitting or supine. Cabozantinib dosing should be interrupted in subjects with severe hypertension (180 mm Hg systolic or 120 mm Hg diastolic; or sustained ≥ 160 mm Hg systolic or ≥ 110 diastolic) who cannot be controlled with medical interventions and discontinued in subjects with hypertensive crises or hypertensive encephalopathy.

Management of Hypertension Related to Cabozantinib

Criteria for Dose Modifications	Treatment/cabozantinib Dose Modification
Subjects not receiving optimized anti-hypertensive therapy	
> 140 mm Hg (systolic) and < 160 mm Hg OR > 90 mm Hg (diastolic) and < 110 mm Hg	• Increase antihypertension therapy (i.e., increase dose of existing medications and/or add new antihypertensive medications) • Maintain dose of cabozantinib • If optimal antihypertensive therapy (usually to include 3 agents) does not result in blood pressure < 140 systolic or < 90 diastolic, dose of cabozantinib should be reduced.
≥ 160 mm Hg (systolic) and < 180 mm Hg OR ≥ 110 mm Hg (diastolic) and < 120 mm Hg	• Reduce cabozantinib by one dose level. • Increase antihypertension therapy (i.e., increase dose of existing medications and/or add new antihypertensive medications) • Monitor subject closely for hypotension. • If optimal antihypertensive therapy (usually to include 3 agents) does not result in blood pressure < 140 systolic or < 90 diastolic, dose of cabozantinib should be reduced further.
≥ 180 mm Hg (systolic) OR ≥ 120 mm Hg (diastolic)	• Interrupt treatment with cabozantinib Add new or additional anti-hypertensive medications and/or increase dose of existing medications. • Monitor subject closely for hypotension. • When SBP < 140 and DBP < 90, restart cabozantinib treatment at one dose level lower • If optimal antihypertensive therapy (usually to include 3 agents) does not result in blood pressure < 140 systolic or < 90 diastolic, dose of cabozantinib should be reduced further.

BP, blood pressure, SBP systolic blood pressure, DBP diastolic blood pressure

NOTE: If SBP and DBP meet different criteria in table, manage per higher dose-modification criteria

2.11.7 Proteinuria

Proteinuria has been reported with approved drugs that inhibit VEGF pathways as well as with cabozantinib. Development and worsening of proteinuria should be monitored by serial urinalysis (qualitative/semiquantitative assessment by dipstick; quantitative assessment by urine protein/urine creatinine ratio [UPCR], or 24-hour urine protein excretion). When a UPCR exceeds 1 (mg/dL protein / mg/dL creatinine), a repeat UPCR or a 24-hour urine protein and creatinine should be performed to confirm the result. Cabozantinib should be discontinued in subjects who develop nephrotic syndrome

(proteinuria > 3.5 grams per day in combination with low blood protein levels, high cholesterol levels, high triglyceride levels, and edema) or any other relevant renal disease. Also, given the nephrotoxic potential of bisphosphonates, these agents should be used with caution in patients receiving treatment with cabozantinib. Details of management are described in Table below:

Management of Treatment Emergent Proteinuria

Urine Protein/Creatinine Ratio	Action To Be Taken
≤ 1	• No change in treatment or monitoring
> 1 and < 3.5	• Confirm with a 24 hour urine protein excretion within 7 days • If proteinuria of > 1 g/24 hours is confirmed, hold cabozantinib and continue with UPCR monitoring. When UPCR returns to < 1, restart cabozantinib at a reduced dose. Continue monitoring UPCR once every week until two consecutive readings are < 1, then revert to UPCR monitoring frequency specified in the protocol.
≥ 3.5	• Hold cabozantinib immediately and confirm with 24 hour urine protein excretion. • Evaluate for nephrotic syndrome. If present, discontinue cabozantinib treatment permanently, and monitor subject for resolution of nephrotic syndrome. • If proteinuria of ≥ 3.5 g/24 hours is confirmed without diagnosis of nephrotic syndrome, continue to hold cabozantinib and monitor UPCR weekly. If UPCR decreases to < 1, restart cabozantinib at a reduced dose. Continue monitoring UPCR once every week until two consecutive readings are < 1, then revert to UPCR monitoring frequency specified in the protocol.

UPCR, urine protein/urine creatinine ratio

2.11.8 Hemorrhage

Hemorrhagic events have been reported with approved drugs that inhibit VEGF pathways as well as with cabozantinib. As preventive measures, subjects should be evaluated for potential bleeding risk factors prior to initiating cabozantinib treatment and monitored for bleeding events with serial complete blood counts and physical examination while on study. Risk factors for hemorrhagic events may include (but may not be limited to) the following:

- Tumor lesions of the lung with cavitations or tumor lesions which invade, encase, or abut any major blood vessels; non-small cell lung cancer (NSCLC) with squamous cell differentiation is known for significant lung cavitations and centrally located

tumors that may invade major blood vessels. The anatomic location and characteristics of tumor as well as the medical history should be carefully reviewed in the selection of subjects for treatment with cabozantinib.

- Recent or concurrent radiation to the thoracic cavity
- Active peptic ulcer disease, ulcerative colitis, and other inflammatory GI diseases
- Underlying medical conditions which affect normal hemostasis (eg, deficiencies in clotting factors and/or platelet function, or thrombocytopenia)
- Concomitant medication with anticoagulants or other drugs which affect normal hemostasis

Based on the described predisposing risk factors for hemoptysis, many studies with antiangiogenic drugs exclude subjects with NSCLC and squamous cell differentiation. Although enrollment of subjects with NSCLC with squamous cell differentiation has been allowed on cabozantinib studies, cabozantinib studies exclude NSCLC subjects with any of the following: tumors abutting, encasing, or invading a major blood vessel; cavitating lesions; history of clinically significant hemoptysis; or recent (within 3 months) radiation therapy to the thoracic cavity including brachytherapy unless radiation therapy targets bone metastasis.

Cabozantinib should be discontinued in subjects with serious and life-threatening bleeding events or recent hemoptysis (≥ ½ tablespoon of red blood). Treatment with cabozantinib should be interrupted if less severe forms of clinically significant hemorrhage occur and may be restarted after the cause of hemorrhage has been identified and the risk of bleeding has subsided. Therapy of bleeding events should include supportive care and standard medical interventions.

Furthermore, subjects who develop tumors abutting, encasing, or invading a major blood vessel or who develop cavitation of their pulmonary tumors while on study treatment must be discontinued from cabozantinib treatment.

2.11.9 Rectal and Perirectal Abscess

Rectal and perirectal abscesses have been reported, sometimes in subjects with concurrent diarrhea. These should be treated with appropriate local care and antibiotic therapy. Cabozantinib should be held until adequate healing has taken place.

2.11.10Gastrointestinal perforation and GI fistula

Gastrointestinal perforation and GI fistula have been reported with approved drugs that inhibit VEGF pathways as well as with cabozantinib. To allow for early diagnosis, subjects should be monitored for episodes of abdominal pain, especially if known risk factors for developing GI perforation or fistula (Turnage et al. 2008) are present. Such risk factors include (but may not be limited to) the following:

- Intra-abdominal tumor/metastases invading GI mucosa
- Active peptic ulcer disease, inflammatory bowel disease, ulcerative colitis, diverticulitis, cholecystitis or symptomatic cholangitis, or appendicitis
- History of abdominal fistula, GI perforation, bowel obstruction, or intra-abdominal abscess
- Prior GI surgery (particularly when associated with delayed or incomplete healing)

Complete healing following abdominal surgery or resolution of intra-abdominal abscess must be confirmed prior to initiating treatment with cabozantinib.

Additional risk factors include concurrent use of steroid treatment or non-steroidal anti-inflammatory drugs (Rodriguez et al. 2001, Straube et al. 2009). Constipation, consistent with symptoms of bowel obstruction, should be monitored and effectively managed. Discontinue cabozantinib and initiate appropriate management in subjects who have been diagnosed with GI perforation or fistula.

2.11.12 Wound healing and Surgery

VEGF inhibitors can cause wound healing complications and wound dehiscence which may occur even long after a wound has been considered healed. Therefore, surgical and traumatic wounds must have completely healed prior to starting cabozantinib treatment and be monitored for wound dehiscence or wound infection while the subject is being treated with cabozantinib.

Treatment with cabozantinib must be interrupted for any wound healing complication which needs medical intervention. Treatment with cabozantinib can be resumed once wound healing has occurred unless otherwise prohibited in specific protocols. Cabozantinib should be discontinued in subjects with serious or chronic wound healing complications.

The appropriate dose hold interval prior to elective surgery to reduce the risk for wound healing complications has not been determined. In general, cabozantinib should be stopped at least 3 weeks (5 half lives) prior to elective surgery.

2.11.13 Endocrine Disorders

Prospective studies of markers of thyroid functions are currently ongoing in two single-agent studies to characterize the effects of cabozantinib on thyroid function. Preliminary data indicate that cabozantinib affects thyroid function tests (TFTs) in a high number of subjects. In Study XL184-203, 17 of 34 (50%) euthyroid subjects with castration-resistant prostate cancer (CRPC) developed abnormal thyroid-stimulating hormone (TSH) levels 6 weeks after initiation of cabozantinib treatment (6% showed TSH levels between 5 and 7 mU/L, 44% had TSH > 7 mU/L). The median TSH level at week 6 was 5.2 mU/L (range, 0.02-29.7 mU/L). In a Phase 1 combination study of rosiglitazone and cabozantinib (XL184-008) to determine the potential for a clinically significant drug-drug interaction of cabozantinib on the CYP isozyme CYP2C8, subjects with advanced solid tumors (particularly renal cell carcinoma [RCC] and differentiated thyroid cancer [DTC]) are enrolled. Among 11 evaluable subjects, the AE of hypothyroidism was reported in 55% of subjects. Currently available data are insufficient to determine the cause of TFT alterations and its clinical relevance. Routine monitoring of thyroid function and assessments for signs and symptoms associated with thyroid dysfunction is recommended for subjects treated with cabozantinib. Management of thyroid dysfunction (eg, symptomatic hypothyroidism) should follow accepted clinical practice guidelines.

Other endocrine disorders such as hypocalcemia and hyperglycemia, and associated laboratory changes, have been observed in less than 10% of subjects. Monitoring with standard laboratory tests for endocrine disorders and clinical examination prior to initiation and during treatment with cabozantinib is recommended. Cabozantinib should be discontinued in subjects with severe or life-threatening endocrine dysfunction.

2.11.14 Guidelines for Management of Treatment-Emergent Corrected QT (QTc) Prolongation

In preclinical studies, high doses of cabozantinib (up to 1000 mg/kg) in dogs had no effect on electrocardiographic parameters, including QT and QTc intervals. However, the current clinical safety profile of cabozantinib indicates a mild to moderate prolongation effect upon QT and QTc intervals at clinically relevant exposures. Other factors which may contribute to QTc prolongation include

- Treatment with other drugs associated with QTc prolongation (see http://www.qtdrugs.org)
- Treatment with CyP3A4 inhibitors (which may increase cabozantinib drug levels)
- Electrolyte changes (hypokalemia, hypocalcemia, hypomagnesemia)
- Medical conditions which can alter electrolyte status e.g., severe or prolonged diarrhea

Subjects having any of these additional risk factors while on cabozantinib should have ECGs performed approximately one week after the onset of these factors.

A very recent analysis of ECG data from the XL184-301 study, An International, Randomized, Double-Blinded, Phase 3 Efficacy Study of XL184 versus Placebo in Subjects with Unresectable, Locally Advanced, or Metastatic Medullary Thyroid Cancer ,in which 214 subjects were randomized to cabozantinib and 109 subjects randomized to placebo, demonstrates a mild to moderate prolongation of the QTcF in cabozantinib-treated subjects vs placebo-treated subjects. Although subjects were treated with a higher dose in that study, we believe it is appropriate to monitor patients for QTc prolongation. No subject had a QTcF greater that 500 msec.

If at any time on study there is an increase in QTc interval to an absolute value **> 500 msec,** two additional ECGs should be performed within 30 minutes after the initial ECG with intervals not less than 3 minutes apart. If the average QTcF from the three ECGs is > 500 msec, study treatment must be withheld and the following actions should be taken:

- Check electrolytes, especially potassium, magnesium and calcium. Correct abnormalities as clinically indicated
- If possible, discontinue any QTc-prolonging concomitant medications
- Repeat ECG triplets hourly until the average QTcF is ≤ 500 msec or otherwise determined by consultation with a cardiologist

Subjects with QTc prolongation and symptoms must be monitored closely until the QTc elevation has resolved. Cardiology consultation is recommended for evaluation and subject management. Symptomatic subjects must be treated according to standard clinical practice. No additional study treatment is to be given to the subject until after the event has resolved and the subject has been thoroughly evaluated. If any additional study treatment is given (eg, after correction of electrolyte abnormalities and normalization of QTcF), it will be at a reduced dose per protocol dose levels.

2.12 Concomitant Medications and Therapies

2.12.1 Anticancer Therapy

If a subject requires additional systemic anticancer treatment, including steroid treatment for prostate cancer therapy, study treatment must be discontinued. Local intervention is

discouraged unless medically unavoidable. Subjects receiving local intervention (eg, palliative radiation) are allowed to continue to receive study treatment at the investigator's discretion. Subjects with prostate cancer currently receiving luteinizing hormone-releasing hormone (LHRH) or gonadotropin-releasing hormone (GnRH) agonists may be maintained on these agents.

2.12.2 Other Medications

Subjects must be instructed to inform the investigators of the current or planned use or all other medications during the study (including prescription medications, vitamins, herbal and nutritional supplements, and over-the-counter medications). It is the responsibility of the investigator to ensure that details regarding all medications are documented.

Anti-emetics and anti-diarrheal medications should not be administered prophylactically prior to the first dose of cabozantinib. After the first dose of study treatment, at the discretion of the investigator or after the onset of symptoms, treatment (or prophylaxis) with anti-emetic and anti-diarrheal medications may be undertaken per standard clinical practice. Bisphosphonates are allowed if started prior to screening activities or may be initiated during the course of the study to control bone pain.

Colony stimulating factors (eg, erythropoietin and granulocyte colony-stimulating factors) and pain medications administered as dictated by standard practice are acceptable while the subject is enrolled in the study. However, colony stimulating factors should not be administered prophylactically prior to the first dose of study treatment.

No concurrent investigational agents are permitted.

2.12.3 Potential Drug Interactions

Cytochrome P450: Preliminary data from a clinical drug interaction study (Study XL184-008) show that clinically relevant steady-state concentrations of cabozantinib appear to have no marked effect on the AUC of co-administered rosiglitazone, a CYP2C8 substrate. Therefore, cabozantinib is not anticipated to markedly inhibit CYP2C8 in the clinic, and by inference, is not anticipated to markedly inhibit other CYP450 isozymes that have lower [I]/Ki values compared to CYP2C8 (ie, CYP2C9, CYP2C19, CYP2D6, CYP1A2, and CYP3A4). In vitro data indicate that cabozantinib is unlikely to induce cytochrome P450 enzymes, except for possible induction of CYP1A1 at high cabozantinib concentrations (30 μM).

Cabozantinib is a CYP3A4 substrate (but not a CYP2C9 or CYP2D6 substrate), based on data from in vitro studies using CYP-isozyme specific neutralizing antibodies. Preliminary results from a clinical pharmacology study, XL184-006, showed that concurrent administration of cabozantinib with the strong CYP3A4 inducer, rifampin, resulted in an approximately 80% reduction in cabozantinib exposure (AUC values) after a single dose of cabozantinib in healthy volunteers. Co-administration of cabozantinib with strong inducers of the CYP3A4 family (eg, dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbital, and St. John's Wort) may significantly decrease cabozantinib concentrations. The chronic use of strong CYP3A4 inducers should be avoided. Other drugs that induce CYP3A4 should be used with caution because these drugs have the potential to decrease exposure (AUC) to cabozantinib. Selection of alternate concomitant medications with no or minimal CYP3A4 enzyme induction potential is recommended. In addition, caution must be used when discontinuing treatment with a strong CYP3A4 inducer in a subject who has been concurrently receiving a stable dose of cabozantinib, as this could significantly increase the exposure to cabozantinib.

Preliminary results from a clinical pharmacology study, XL184-007, showed that concurrent administration of cabozantinib with the strong CYP3A4 inhibitor, ketoconazole, resulted in a 33-39% increase in the cabozantinib exposure (AUC values) after a single dose of cabozantinib in healthy volunteers. Co-administration of cabozantinib with strong inhibitors of the CYP3A4 family (eg, ketoconazole, itraconazole, clarithromycin, indinavir, nefazodone, nelfinavir, and ritonavir) may increase cabozantinib concentrations. Grapefruit / grapefruit juice and Seville oranges may also increase plasma concentrations of cabozantinib. Strong CYP3A4 inhibitors and other drugs that inhibit CYP3A4 should be used with caution because these drugs have the potential to increase exposure (AUC) to cabozantinib. Selection of alternate concomitant medications with no or minimal CYP3A4 enzyme inhibition potential is recommended.

Because in vitro studies only assessed the metabolizing capacity of the CYP3A4, CYP2C9, and CYP2D6 pathways, the potential for drugs that inhibit/induce other CYP450 pathways (eg, CYP2C8, CYP2C19, CYP2B6, CYP1A2) to alter cabozantinib exposure is not known. Therefore, these drugs should be used with caution when given with cabozantinib.

Please refer to the Flockhart drug interaction tables for lists of substrates, inducers, and inhibitors of selected CYP450 isozyme pathways (Flockhart 2007; *http://medicine.iupui.edu/clinpharm/ddis/table.aspx*).

Protein Binding: Cabozantinib is highly protein bound (approximately 99.9%) to human plasma proteins. Therefore, highly protein bound drugs should be used with caution with cabozantinib because there is a potential displacement interaction that could increase free concentrations of cabozantinib and/or a co-administered highly protein-bound drug (and a corresponding increase in pharmacologic effect). Factors that influence plasma protein binding may affect individual tolerance to cabozantinib. Therefore, concomitant medications that are highly protein bound (eg, diazepam, furosemide, dicloxacillin, and propranolol) should be used with caution. Because warfarin is a highly protein bound drug with a low therapeutic index, administration of warfarin at therapeutic doses should be avoided in subjects receiving cabozantinib due to the potential for a protein binding displacement interaction.

Other Interactions: A relative bioavailability study in dogs suggests that cabozantinib is unlikely to be affected by drugs that alter gastric pH. In vitro data suggest that cabozantinib is unlikely to be a substrate for P glycoprotein (P-gp), but it does appear to have the potential to inhibit the P-gp transport activity.

Additional details related to these overall conclusions are provided in the Investigators Brochure

2.13 Compliance

Drug accountability and subject compliance will be assessed with drug dispensing and return records.

2.14 Study Drug Accountability

The investigator will maintain accurate records of receipt of all cabozantinib, including dates of receipt. In addition, accurate records will be kept regarding when and how much study treatment is dispensed and used by each subject in the study. Reasons for deviation from the expected dispensing regimen must also be recorded. At completion of the study, to satisfy regulatory requirements regarding drug accountability, all unused cabozantinib will be reconciled and destroyed in accordance with applicable state and federal regulations.

3 STUDY POPULATION

3.1 Target Population

Inclusion Criteria:

A subject must meet the following criteria to be eligible for the study:

1. The subject has histologically confirmed prostate adenocarcinoma with radiologic evidence of metastases.
2. If patient are on anti-androgens, these should be discontinued, at least 4 weeks prior for flutamide and at least 6 weeks for bicalutmide or nilutamide.
3. At least 14 days should have elapsed from prior radiation therapy to bone metastases from prostate cancer (see details in exclusion criterion # 3 for other sites of RT).
4. The subject is ≥ 18 years old.
5. The patient has received a maximum of one prior chemotherapy regimen for metastatic prostate cancer.
6. Patients must demonstrate disease progression on or after most recent systemic therapy, either by PSA (minimum PSA has to be ≥ 2.0 ng/ml if no measurable disease present and for PSA only progression, an increase in PSA levels done at least one week apart is required), new bone metastases or by measurable disease criteria per RECIST 1.1 guidelines.
7. Patients should have received either orchiectomy, or be on LHRH analogue or LHRH antagonist for metastatic prostate cancer and have a testosterone level ≤50 ng/dL.
8. The patient has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
9. Bisphosphonate therapy can be continued if started prior to protocol enrollment.

10. Patients must have BP readings ≤150/90 prior to enrollment.

11. The subject has organ and marrow function as follows:

    a. Absolute neutrophil count (ANC) ≥ 1500/mm^3without colony stimulating factor support
    b. Platelets ≥ 100,000/mm^3
    c. Hemoglobin ≥ 9 g/dL
    d. Bilirubin ≤ 1.5 × the upper limit of normal (ULN). For subjects with known Gilbert's disease, bilirubin ≤ 3.0 mg/dL
    e. Serum albumin ≥ 2.8 g/dl
    f. Serum creatinine ≤ 1.5 × ULN or creatinine clearance (CrCl) ≥ 50 mL/min. For creatinine clearance estimation, the Cockcroft and Gault equation should be used:

        Male: CrCl (mL/min) = (140 - age) × wt (kg) / (serum creatinine × 72)

        Female: Multiply above result by 0.85
    g. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN if no liver involvement, or ≤ 5 × ULN with liver involvement
    h. Lipase < 1.5 x the upper limit of normal (except for subjects with adenocarcinoma of the pancreas)
    i. Urine protein/creatinine ratio (UPCR) ≤ 1
    j. Serum phosphorus ≥ LLN

12. The subject is capable of understanding and complying with the protocol requirements and has signed the informed consent document.

13. Patients participating in this trial must also be eligible and willing to sign consent for participation in FMAU scan done under separate protocol.

14. Sexually active subjects (men) must agree to use medically accepted barrier methods of contraception (eg, male condom, or diaphragm with spermicidal gel) during the course of the study and for 4 months after the last dose of study drug(s), even if oral contraceptives are also used by the female partner. All subjects of reproductive potential must agree to use both a barrier method and a second method of birth control.

15. Projected life expectancy of at least 6 months.

16. No prior history of other malignancies in the last 3 years, except for squamous and basal cell skin cancer.

3.2 Exclusion Criteria

A subject who meets any of the following criteria is ineligible for the study:

1. The subject has received cytotoxic chemotherapy (including investigational cytotoxic chemotherapy) or biologic agents (eg, cytokines or antibodies) within 3 weeks, or nitrosoureas/ mitomycin C within 6 weeks before the first dose of study treatment.
2. Prior treatment with cabozantinib
3. The subject has received radiation therapy:
    a. to the thoracic cavity or gastrointestinal tract within 3 months of the first dose of study treatment.
    b. to bone or brain metastasis within 14 days of the first dose of study treatment
    c. to any other site(s) within 28 days of the first dose of study treatment
4. The subject has received radionuclide treatment within 6 weeks of the first dose of study treatment
5. The subject has received prior treatment with a small molecule kinase inhibitor or a hormonal therapy (including investigational kinase inhibitors or hormones) within 14 days before the first dose of study treatment. Patients receiving LHRH or GnRH agonists to maintain castrate levels of testosterone or patients on bisphosphonate/denosumab, may be maintained on these agents.
6. The subject has received any other type of investigational agent within 28 days before the first dose of study treatment.
7. The subject has not recovered to baseline or CTCAE $\leq$ Grade 1 from toxicity due to all prior therapies except alopecia and other non-clinically significant AEs.
8. The subject has a primary brain tumor.
9. The subject has active brain metastases or epidural disease (Note: Subjects with brain metastases previously treated with whole brain radiation or radiosurgery or subjects with epidural disease previously treated with radiation or surgery who are asymptomatic and do not require steroid treatment for at least 2 weeks before starting study treatment are eligible. Neurosurgical resection of brain metastases or brain biopsy is permitted if

completed at least 3 months before starting study treatment. Baseline brain scans are not required to confirm eligibility.)

10. The subject has prothrombin time (PT)/ International Normalized Ratio (INR) or partial thromboplastin time (PTT) test results at screening ≥ 1.3 × the laboratory ULN.
11. The subject requires concomitant treatment, in therapeutic doses, with anticoagulants such as warfarin or warfarin-related agents, heparin, thrombin or Factor Xa inhibitors, or antiplatelet agents (eg, clopidogrel). Low dose aspirin (≤ 81 mg/day), low-dose warfarin (≤ 1 mg/day), and prophylactic low molecular weight heparin (LMWH) are permitted.
12. The subject has experienced any of the following within 3 months before the first dose of study treatment:
    a. clinically-significant hematemesis or gastrointestinal bleeding
    b. hemoptysis of ≥ 0.5 teaspoon (2.5ml) of red blood
    c. any other signs indicative of pulmonary hemorrhage
13. The subject has radiographic evidence of cavitating pulmonary lesion(s)
14. The subject has tumor in contact with, invading or encasing major blood vessels
15. The subject has uncontrolled, significant intercurrent or recent illness including, but not limited to, the following conditions:
    a. Cardiovascular disorders including
        i. Congestive heart failure (CHF): New York Heart Association (NYHA) Class III (moderate) or Class IV (severe) at the time of screening
        ii. Concurrent uncontrolled hypertension defined as sustained BP >_150 mm Hg systolic, or > 90 mm Hg diastolic despite optimal antihypertensive treatment (BP must be controlled at screening)
        iii. Any of the following within 6 months before the first dose of study treatment:
            - unstable angina pectoris
            - clinically-significant cardiac arrhythmias
            - stroke (including TIA, or other ischemic event)
            - myocardial infarction
            - thromboembolic event requiring therapeutic anticoagulation (Note: subjects with a venous filter (e.g. vena cava filter) are not eligible for this study)
    b. Gastrointestinal disorders particularly those associated with a high risk of perforation or fistula formation including:
        i. Any of the following at the time of screening

- intra-abdominal tumor/metastases invading GI mucosa
- active peptic ulcer disease,
- active inflammatory bowel disease (including ulcerative colitis and Crohn's disease), diverticulitis, cholecystitis, symptomatic cholangitis or appendicitis

ii. Any of the following within 6 months before the first dose of study treatment:

(1) history of abdominal fistula

(2) gastrointestinal perforation

(3) bowel obstruction or gastric outlet obstruction

(4) intra-abdominal abscess. Note: Complete resolution of an intra-abdominal abscess must be confirmed prior to initiating treatment with cabozantinib even if the abscess occurred more that 6 months ago.

iii. GI surgery (particularly when associated with delayed or incomplete healing) within 28 days. Note: Complete healing following abdominal surgery must be confirmed prior to initiating treatment with cabozantinib even if surgery occurred more that 28 days ago.

c. Other disorders associated with a high risk of fistula formation including PEG tube placement within 3 months before the first dose of study therapy or concurrent evidence of intraluminal tumor involving the trachea and esophagus.

d. Other clinically significant disorders such as:

i. active infection requiring systemic treatment

ii. serious non-healing wound/ulcer/bone fracture

iii. history of organ transplant

iv. concurrent uncompensated hypothyroidism or thyroid dysfunction

v. history of major surgery within 4 weeks or minor surgical procedures within 1 week before randomization

16. The subject is unable to swallow tablets
17. The subject has a corrected QT interval calculated by the Fridericia formula (QTcF) >470 ms within 21 days of treatment.
18. The subject has a previously identified allergy or hypersensitivity to components of the study treatment formulation.
19. The subject is unable or unwilling to abide by the study protocol or cooperate fully with the investigator or designee.

20. The subject has had evidence within 2 years of the start of study treatment of another malignancy, which required systemic treatment

4 STUDY ASSESSMENTS AND PROCEDURES

4.1 Pre-Treatment Period

During the Pre-Treatment Period, subjects are consented and qualified (screened) for the study. Informed consent must be obtained before initiation of any clinical screening procedure that is performed solely for the purpose of determining eligibility for this study. Evaluations performed as part of routine care before informed consent can be considered as screening evaluations if done within the defined screening period, and if permitted by the site's institutional review board (IRB)/ ethics committee (EC) policies. Informed consent may be obtained within 42 days prior to first dose of study treatment. At informed consent, subjects will be assigned a subject identifier. Subject identifiers are not to be re-assigned if a subject is determined to be ineligible, and subjects are to maintain their original identifier if rescreening is required.

For each subject, the Pre-Treatment Period ends upon receipt of the first dose of study treatment or final determination that the subject is ineligible for the study.

4.2 Study Treatment Period

Subjects are defined as enrolled upon receipt of the first dose of study treatment.

If the subject is unable to have a study assessment taken within the defined time window due to an event outside of his or her control (eg, clinic closure, personal emergency, inclement weather, vacation), the assessment should be performed as close as possible to the required schedule.

Subjects should be instructed to inform the PI of any AEs. Subjects experiencing dizziness, sleepiness, or other symptoms that could influence alertness or coordination should be advised not to drive or operate other heavy machinery.

Subjects may receive study treatment until the earlier of PD or unacceptable toxicity. Regular tumor assessments should be performed according to the guidelines in Section 4.5 to determine if PD is present.

The Treatment Period ends when a subject receives his or her last dose of study treatment; the subject then enters the Post-Treatment Period.
The week 4 timepoint scan will be discontinued for all present and future patients. We will also review each patients baseline scan to see if there is disease detectable. If no readily visible cancer is found then the 2nd scan with FMAU PET will be eliminated for that patient. So far of the 6 patients imaging, only patient 1 had no easily visible tumor on the baseline scan. Longer follow up of the patients has revealed that it remains a challenge to determine if the patients are continuing to derive clinical benefit. The PSA level is frequently discordant with response. Hence we will obtain an additional ^{18}F-flouride PET scan and a FMAU PET scan (if <3 lifetime FMAU scans done on the patient) at week 32 (+/- 10 weeks) For the patients who discontinue therapy. prior to the 32 weeks timepoint, the scan will be done at the time of treatment discontinuation (+/- 2 weeks). This timepoint was chosen to capture the maximum number of patients currently on the study for this scan, and since conventional CT scan and bone scan is also conducted at the same timepoint for comparison.

4.3 Post-Treatment Period

Subjects will return to the study site 4 weeks (± 2 weeks) after the last dose of XL184 for an end-of-treatment assessment. Laboratory and physical examinations will be performed. Remaining study treatment will be returned by the subject, and treatment compliance will be documented. Additional follow-up will occur for subjects with AEs related to study treatment that are ongoing at the time of this visit, and for subjects with SAEs related to study treatment that occur after the time of this visit.

4.4 Laboratory Assessments

Local laboratories will perform all laboratory tests. Blood and urine samples for standard hematology, serum chemistry, and urinalysis panels will be prepared using standard procedures.

Abnormalities in clinical laboratory tests that lead to a change in subject management (eg, dose delayed, withheld, or reduced; additional medication or monitoring required) or that are considered to be clinically significant by the investigator are to be recorded on the AE case report form (CRF). If laboratory values constitute part of an event that meets

criteria for an SAE, the event (and associated laboratory values) must be reported as an SAE (Section 5.1.2).

4.5 Tumor Assessment

4.5.1 Routine Tumor Assessment

Tumor response should be assessed per the study schedule and until the medication is continuing to show benefit, (complete response [CR], PR, or SD) may continue on study. Subjects with disease progression should have their treatment discontinued, and they should enter the post treatment phase of the study. The same method for tumor assessment should be employed at every assessment.

4.6 Response criteria:

For measurable disease response RECIST criteria 1.1 will be used [Eisenhauer E, et al. Eur J Cancer 2009]. The Prostate Cancer Clinical Trials Working Group (PCWG2) criteria will be used to determine a response [Scher H, et al. J Clin Oncol 2008]. PCWG2 recommends a two-objective paradigm in metastatic CRPC: (1) controlling, relieving, or eliminating disease manifestations that are present when treatment is initiated and (2) preventing or delaying disease manifestations expected to occur. PSA decline and changes in imaging will also be reported. The progression for measurable disease will be per RECIST criteria, and for bone metastases will be defined as given below:

4.6.1 THE RECIST 1.1 CRITERIA WITH UNIDIMENSIONAL MEASUREMENT ARE TO BE USED FOR MEASURABLE DISEASE RESPONSE EVALUATION

Methods of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI is also acceptable in certain situations (e.g., for body scans but not for lung).Lesions on a chest X-ray may be considered measurable lesions if they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Clinical lesions will only be considered measurable when they are superficial and ≥10 mm in diameter as assessed using calipers. For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the

lesion, is recommended.Ultrasound (US) should not be used to measure tumor lesions.

Baseline Disease Assessment

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 42 days before the beginning of the treatment.

Measurable lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; when CT scans have slice thickness >5 mm, the minimum size should be twice the slice thickness).

• 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as nonmeasurable).

• 20 mm by chest X-ray.

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

• Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components

that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable if the soft tissue component meets the definition of measurability described above.

• 'Cystic lesions' thought to represent cystic metastases can be considered measurable 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.

Non-measurable lesions

Non-measurable lesions are all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis), as well as truly non-measurable lesions.

• Blastic bone lesions are non-measurable.

• Lesions with prior local treatment, such as those situated in a previously irradiated area or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

Target Lesions

• All measurable lesions up to a maximum of two lesions per organ and five 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) and be representative of all involved organs, as well as their suitability for reproducible repeated measurements.

• All measurements should be recorded in metric notation using calipers if clinically assessed. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions

Non-target Lesions

All lesions (or sites of disease) not identified as target lesions, including pathological lymph nodes and all non-measurable lesions, should be identified as non-target lesions and be recorded at baseline. Measurements of these lesions are not required and they should be followed as 'present', 'absent' or in rare cases, 'unequivocal progression'.

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 diameters of target lesions, taking as reference the baseline sum of diameters.
Progressive Disease (PD):
At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this may include the baseline sum). The sum must also demonstrate an absolute increase of at least 5 mm.
Stable Disease (SD):
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials.
However, in all other circumstances, (i.e., in randomized phase II or III trials or studies where stable disease or progression are the primary endpoints), confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.
In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the
study protocol.

4.6.2 Progression in patients with bone metastases

Majority of the patients with prostate cancer have bone metastases which are not considered measurable sites of disease. Progression in patients with bone metastases will

be defined as either the appearance of a minimum of 2 new lesions on bone scan which are related to metastatic disease per the judgement of the treating physician, or the occurrence of a new skeletal related event. Skeletal-related event is defined as occurrence of new pathologic bone fractures (vertebral or nonvertebral), spinal cord compression, requirement of surgery or radiation therapy to bone metastases (including the use of radioisotopes), or a requirement to change antineoplastic therapy to treat bone pain or other related symptoms.

4.6.3 Response Evaluable Patients

All patients registered on the protocol and completing a minimum of 7 days of therapy followed by clinical, or radiologic or PSA assessment of disease status.

4.6.4 Toxicity- Evaluable patients

All patients registered on the protocol and starting therapy with protocol medication, will be considered toxicity evaluable.

4.6.5 Progression-free survival (PFS)

PFS will be measured from date of registration to date of first documented disease progression, or death from any cause, whichever occurs first. After treatment is discontinued for any reason patients will be followed every 3 months for progression and survival.

4.6.6 Overall survival (OS)

OS will be measured from date of registration to death or last follow up. After treatment is discontinued for any reason patients will be followed every 3 months for progression and survival.

5 ADVERSE EVENT REPORTING AND SAFETY

5.1 Adverse Events and Laboratory Abnormalities

5.1.1 Adverse Events

Toxicity will be graded per CTCAE version 4.0. An AE is any untoward medical occurrence in a patient or clinical investigation subject who has been enrolled in a clinical study and who may have been given an investigational product, regardless of whether or not the event is assessed as related to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, regardless of whether or not the event is assessed as related to the investigational product. Pre-existing medical conditions that worsen during a study should be recorded as AEs. Abnormal laboratory values, electrocardiogram (ECG) findings, or vital signs are to be recorded as AEs if they meet the criteria described in Section 5.2.1.

All untoward events that occur after start of therapy through 30 days after the last dose of study treatment are to be recorded by the investigational site. This requirement includes AEs from unscheduled as well as scheduled visits.

Assessment of the relationship of the AE to the study treatment by the investigator is based on the following two definitions:

- Not Related: A not-related AE is defined as an AE that is not associated with the study treatment and is attributable to another cause.
- Related: A related AE is defined as an AE where a causal relationship between the event and the study treatment is a reasonable possibility. A reasonable causal relationship is meant to convey that there are facts (eg, evidence such as dechallenge/ rechallenge) or other clinical arguments to suggest a causal relationship between the AE and study treatment.

5.1.2 Serious Adverse Events

The SAE definition and reporting requirements are in accordance with the International Conference of Harmonisation (ICH) Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Topic E2A.

An SAE is defined as any untoward medical occurrence that at any dose:

1. Results in death.
2. Is immediately life-threatening (ie, in the opinion of the investigator, the AE places the subject at immediate risk of death; it does not include a reaction that, had it occurred in a more severe form, might have caused death).
3. Requires inpatient hospitalization or results in prolongation of an existing hospitalization.
4. Results in persistent or significant disability or incapacity.

   Note: The term "disability" refers to events that result in a substantial disruption of a subject's ability to conduct normal life function.
5. Is a congenital anomaly or birth defect.
6. Is an important medical event (IME). Note: The term "important medical event" refers to an event that, based upon appropriate medical judgment, may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or require intervention to prevent one of the other serious outcomes listed under the definition of Serious Adverse Event. Examples of IMEs include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias, or convulsions that do not result in hospitalization; or development of product dependency or product abuse.

5.1.3 Serious Adverse Event Reporting

As soon as an investigator becomes aware of an AE that meets the definition of 'serious,' this should be documented to the extent that information is available.

- This report must be submitted by the study site to Exelixis or designee within 24 hours, even if it is not felt to be drug related.
- Email: drugsafety@exelixis.com; Fax 650-837-7392
- The investigator agrees to provide supplementary information requested by the Exelixis Drug Safety personnel or designee.
- Pregnancy, although not itself an SAE, should also be reported on an SAE form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

5.1.4 Regulatory Reporting

All serious unexpected adverse drug reactions (unexpected related SAEs) must be reported to the Food and Drug Administration (FDA) by the investigator as required by 21 CFR 312.32.

- These reports are to be filed utilizing the Form FDA 3500A (MedWatch Form).

The final MedWatch Form must be submitted by the study site to Exelixis at the time of submission to the FDA to allow Exelixis time to cross-report to Exelixis' IND. Email: drugsafety@exelixis.com; Fax 650-837-7392

5.2 Other Safety Considerations

5.2.1 Laboratory Data

All laboratory data required by this protocol and any other clinical investigations should be reviewed. Any abnormal value that leads to a change in subject management (eg, dose reduction or delay or requirement for additional medication or monitoring) or that is

considered to be of clinical significance by the investigator should be reported as an AE or SAE as appropriate.

5.2.2 Pregnancy

Forms for reporting pregnancies will be provided to the study sites upon request. The outcome of a pregnancy (for the partner of a subject) and the medical condition of any resultant offspring must be reported to Exelixis or designee. Any birth defect or congenital anomaly must be reported as an SAE, and any other untoward events occurring during the pregnancy must be reported as AEs or SAEs, as appropriate.

5.2.3 Medication Errors/ Overdose

Any study drug administration error or overdose that results in an AE, even if it does not meet the definition of serious, requires reporting within 24 hours to Exelixis or designee.

5.2.4 Follow-Up of Adverse Events

Any related SAEs or any AEs assessed as related that led to treatment discontinuation, including clinically significant abnormal laboratory values that meet these criteria, ongoing 30 days after the last dose of study treatment must be followed until either resolution of the event or determination by the investigator that the event has become stable or irreversible. This follow-up guidance also applies to related SAEs that occur > 30 days after the last dose of study treatment. The status of all other continuing AEs will be documented as of 30 days after the last dose of study treatment.

6.0 CORRELATIVE STUDIES/PHARMACODYNAMIC STUDIES:

Serum and urine markers

Serum bone markers will be assessed pre and post XL184 therapy. These will include serum bone specific alkaline phosphatase (BSAP) and N-terminal telopeptide of collagen type I (NTx). High levels of these markers ($\geq$146u/Lfor BSAP and $\geq$ 100nmol/mmol for NTx) were noted to be significantly predictive of higher incidence of skeletal complications (relative risk of 3.32, $p< 0.001$), prostate cancer progression (RR 2.02, $p<0.001$) and death (RR of 4.59, $p<0.001$) [Lara PN Jr et al. Clin Cancer Res 2006].

A number of other bone turnover markers such as osteocalcin, pyridinoline, deoxypyridinoline, etc have been implicated to be predictive of therapeutic response. A study evaluating the efficacy of matrix metalloproteinase inhibitors in prostate cancer reported that decline of the bone resorption markers including NTx, procollagen I NH_2-terminal propeptide, osteocalcin and deoxypyridinoline correlated with improved progression free survival and overall survival outcome [Lara PN et al. Clin Cancer Res 2006 and Coleman RE et al. J Clin Oncol 2005]. This supported the fact that changes in bone turnover could act as surrogates of therapeutic effect in prostate cancer bone metastases.

For the purposes of our pilot trial, we have selected the serum NTx and BSAP and urine NTx as the bone turnover markers due to the validation of these markers in prior large studies specifically utilizing zoledronate therapy [Coleman RE et al. J Clin Oncol 2005]. Decline in the levels was predictive of lower incidence of skeletal events as well as progression free and overall survival. Hence the measurement of these markers (NTx and BSAP) pre and post therapy will be correlated with PET scan findings and clinical outcomes.

Bone markers: Serum and urine (Timeline: Pretherapy, week 1, week 2 and week 4)

Urine N-telopeptide will be assessed from 24-hour urine collection sample using the Vitros ECI Immunodiagnostic System competitive assay (Johnson & Johnson Ortho-Clinical Diagnostics, Raritan, NJ). Urinary marker levels will be normalized relative to urinary creatinine levels, and samples will be required to contain ≥ 5 mg/dL creatinine to ensure validity of the sample. **This test will be performed at a local CLIA certified laboratory.**

Serum bone-specific alkaline phosphatase levels will be assessed using a chemical inhibition and differential inactivation assay *(12).* 4cc of blood will be collected in a tiger top tube pre and post therapy for assessment of serum BSAP. **This test will be performed at a local CLIA certified laboratory.**

Serum N-telopeptide will be assessed from serum collection sample using the Vitros ECI Immunodiagnostic System competitive assay (Johnson & Johnson Ortho-Clinical

Diagnostics, Raritan, NJ) (Chestnut CH 3rd, et al. Am J Med 1997). **This test will be performed at a local CLIA certified laboratory.**

For the first 5 patients, samples will be taken at pretherapy and weeks 1, 2 and 4 after treatment begins. Timeline for additional 15 patients will be adjusted based on results of **PET scans in first 5 patients to guide the sampling schedule in next 15 patients. The goal is to have the same schedule for imaging and sample collection so we can correlate the results.**

Additional blood samples will be collected at baseline and designated time points using BD Vacutainer CPT tubes for serum and DNA/RNA samples (Appendix B) in Podgorski lab according to protocol described below. Total RNA isolation from blood will be performed using the PAXgene™ Blood RNA System (PreAnalytiX). This method requires specific sample collection tubes available from PreAnalytiX that contain a proprietary reagent that immediately stabilizes intracellular RNA. All isolated samples will be stored at -80° C until use. TRACP 5 protein level and cathepsin K assessment in serum. We will use the Human TRAP™ Assays to measure the TRACP 5 proteins in serum. TRACP 5b is considered to be essential for clinical assessment of bone metabolism. Quantitative assessment of Cathepsin K levels in serum, an additional indicator of increased bone turnover, will be done using Cathepsin K ELISA kits from ALPCO Immunoassays, Salem, NH. To measure changes in osteoblast function, levels of serum bone serum alkaline phosphatase will be assessed using Human Alkaline Phosphatase ELISA kits from USCN, Lifesciences. The NTx (procollagen I NH_2), a predictive marker of therapeutic response in prostate cancer patients will be assessed from 24-hour urine collection sample using the Vitros ECI Immunodiagnostic System competitive assay (Johnson& Johnson).

Circulating tumor cell (CTC) counts have revealed prognostic and predictive value in conjuction with chemotherapy in metastatic CRPC [DeBono J et al. J Clin Oncol]. We will examine the effects of XL184 on CTC counts. These will be performed pretherapy,

week 2 and at progression. 7.5 ml of blood will be collected in a cell save tube at these timepoints for CTC. **The CTC counts would be performed by the KCI pharmacokinetics core.**

TUMOR/BONE BIOPSY SAMPLES:

Tumor/Bone biopsy samples will be collected pre and post XL184 therapy. Part of each tissue sample will be fresh-frozen for aCGH and genomic expression analysis, and rest will be decalcified, fixed and embedded in paraffin for histological and immuhistochemical analyses. The biopsy samples will be picked up by Mackenzie Herroon or Izabela Podgorski (contact info below). Tissues for histology will be fixed and decalcified (Podgorski lab) and embedded in paraffin. All immunostaining will be performed in Podgorski lab.

Contact information for blood and biopsy tissue pickup:
Mackenzie Herroon (313) 577-0941 (lab); (937) 214-1819 (cell)
Izabela Podgorski (313) 577- 0514 (office); (586) 864-4914 (cell)

Gene Expression profiling

Tissue collected for RNA isolation will be preserved in RNAlater™ RNA Stabilization Reagent. Tissue approximately 0.5 cm x 0.5 cm x 0.5 cm in size will be stored in a nuclease-free vessel appropriate to accommodate the required 500ul volume of RNAlater™ preservative. Samples will be submerged and incubated in RNAlater™ overnight at 2-8 C. Then the tissue will be removed from the reagent and stored at -80 C until RNA is extracted.

The CGH analysis will be performed using comparative genomic hybridization microarrays with an average resolution of 35 kb (Agilent 44K). Normal human male DNA will be used as a reference sample and experiments will be performed with dye-reversed replicates. For gene expression microarray experiments the 60-mer oligonucleotide arrays (Agilent 44K), which have the most complete coverage of the

whole human transcriptome will be used. Two color hybridizations will interrogate the cancer cells from the collected specimens against a common standardized reference sample (Universal Human Reference RNA, Stratagene) which will permit the comparison of our results to transcriptional profiles generated by other researchers. Alexa dyes (Alexa647-red and Alexa555-green) will be used for fluorescent labeling since these dyes are more resistant to oxidation and do not quench as easily as traditional Cy dyes. Labeled targets will be synthesized from the purified RNA using linear amplification and indirect labeling by incorporation of aminoallyl-labeled nucleotide, which will subsequently be modified by the covalent addition of Alexa dye (Epicentre Technologies). The combined analysis of gene expression and chromosomal aberration will be performed as devised by Oncogenomics Lab at Karmanos Cancer Institute. This statistical analysis will be carried out with two Bioconductor libraries, globalTest and Gene Set Enrichment Analysis (GSEA). These methods will be applied to multiple samples with similar amplicons and will provide a basis for determining the fold change threshold required for an individual human tumor biopsy to indicate meaningful over-expression harboring unique genetic amplification events.

Immunohistochemistry

The immunohistochemical analyses will be performed on bone on pre- and post treatment biopsy samples to assess: tumor cell proliferation (Ki67 staining), apoptosis (TUNEL), tumor vasculature (CD31, VEGFR2), cMet, DDR-1 and DDR-2 expression. For determination of osteoclast numbers, tartrate-resistant acid phosphatase (TRAcP) and cathepsin K staining will performed on multiple non-serial sections. Positively stained osteoclasts in each section will be counted and expressed as osteoclast number/mm^2. For Ki67, VEGFR2 and cMet, DDR-1, and -2 expression the specimens will be evaluated independently by two investigators in a blind fashion and the results will be arbitrarily classified into four scores dependent on the intensity of immunoreactivity: (a) −, negative immunostaining; (b) ±, very weak immunostaining; (c) +, medium positive immunostaining; and (d) ++, strongly positive immunostaining. The CD31-positive blood vessels will be counted in multiple fields and expressed as blood vessel number/field. To quantify apoptosis, TUNEL positive (red) and negative (blue) cells will

be counted in multiple non-serial sections and expressed as a ratio of numbers of red apoptotic cells to blue non-apoptotic cells. Additional tumor and bone targets may be added based on results from gene expression analyses.

Gene expression microarray experiments will be performed using 60-mer oligonucleotide arrays (Agilent 44K), which have the most complete coverage of the whole human transcriptome. The histomorphometric and histological approaches will be used to quantitatively assess changes in the bone resorption and bone-to-tumor ratio in response to XL184 treatment. In addition immunohistochemical analyses will be performed to examine changes in tumor cell proliferation (Ki67) and apoptosis (TUNEL), tumor vasculature (CD31) and expression of targets of XL184 action: cMet, VEGFR2, DDR-1 and DDR-2, Additional targets for immunohistochemical analyses may be also included based on the results of gene expression analyses.

7 STATISTICAL CONSIDERATIONS

7.1 Objectives:

7.1.1 Primary Objective: To conduct a pilot clinical trial to evaluate the timing, physiology, and magnitude of changes in tumor imaging, and pharmacodynamic markers with XL184 treatment in metastatic castrate resistant prostate cancer.

7.1.2 Secondary Objectives:

1) To estimate the progression-free survival (PFS) achieved with XL184 in chemotherapy naïve metastatic CRPC patients.

2) To evaluate the feasibility of the therapy, and the toxicities associated.

3) To evaluate overall survival (OS) in metastatic CRPC patients post ADT.

4) To estimate the mean level of all continuously distributed correlatives, and to tabulate the distribution of all categorical correlatives.

7.2 Endpoints:

7.2.1 Primary: PET SUV levels pre- and post-treatment with XL184, bone scan parameters. Refer to the Study Schema in Section 2.3.

7.2.2 Secondary: TTP, the type and grade of toxicities, clinical response, PSA response, and PET response.

7.2.3 Correlative: Serum markers, urinary markers, bone markers, gene expression levels, IHC parameters. A detailed description of them is given in Section 6.

7.3 Design: This is a prospective pilot adaptive design study to obtain preliminary data. Each patient will undergo up to 4 PET scans, using different radiotracers. It is desired to estimate the mean SUV at any time point to approximately one-third of a standard deviation (SD) with 80% confidence. With N=15 patients, the mean SUV can be estimated to within 0.347 SD units of the true mean with 80% confidence. These preliminary estimates will be of sufficient precision for use in designing a subsequent larger study. Since not all patients will agree to undergo all PET scans, the mean SUV may be less precisely estimated at some time points.

7.4 Analysis:

7.4.1 For the 15 patients imaged on a common schedule (see Section 7.5.1), the continuous endpoints (e.g., SUV, bone scan measurements, and all continuously distributed correlatives) will be summarized with standard descriptive statistics, separately at each measurement time point. This will include point and 80% confidence interval (CI) estimates. The mean SUV levels over time will be displayed graphically as a line plot (with separate curves for the different radiotracers). Gene expression levels will be transformed on a $\log_2$ scale.

7.4.2 The categorical endpoints (e.g., toxicities, the 3 types of response, IHC expression levels, etc.) will be summarized via their frequency distribution, point estimate of the proportion, and the Wilson type 80% CI.

7.4.3 The distribution of censored PFS will be summarized via the Kaplan-Meier (K-M) survivorship estimate. Summary statistics (e.g., median, 6-month and 12-month

progression-free rates, etc.) will be calculated from the K-M life table. Similar analyses will be performed for OS as well.

7.5 Expected accrual rate, accrual duration, and study duration

7.5.1 We seek to enroll 20 evaluable patients (maximum of 25 screened to account for screen failures and non evaluable patients) with clinical evidence of metastases, and castrate-resistant disease who will agree to undergo the various imaging studies, additional bone biopsy, and bone scans specified in the Study Schema of Section 2.3. To monitor early bone and tumor responses to XL184 treatment, for the first 5 patients recruited we will perform imaging with $Na^{18}F$ PET as early as 2 days after beginning of XL184 regimen. Each patient will receive up to 4 scans. The initial proposed timeline is: baseline, and 2-3 weeks post start of therapy; however, time points selected for imaging may be adjusted based on results of scans and the timing of changes noted in first 5 patients. After the timeline indicative of measurable changes in bone and/or tumor in response to treatment is established, an additional 15 patients will be recruited for the imaging/biopsy studies on a common schedule. Those next 15 patients will be utilized for statistical estimation purposes, as planned in the study design described in Section 7.3. If the pretherapy PET scans do not show any detectable uptake in metastatic sites, the post therapy scan may not be conducted.

7.5.2 We expect an accrual rate of 12 patients/year, but we also expect that up to one-third of them would not complete the trial due to the required number of PET scans, bone scans, and the additional bone biopsy. Allowing for up to 33% attrition, we anticipate a net accrual rate of about 8 patients/year who complete the trial. Thus, it would take about 30 months (i.e., about 2.5 years) of accrual to enroll the needed 20 patients who complete all the various testing. About 25-30 patients will need to be screened to get 20 evaluable patients on the study.

7.5.3 An additional 2-3 months is required to obtain the various imaging and laboratory endpoints on the last patient(s) enrolled, hence the total study duration is expected to be 32-33 months.

8 ETHICAL ASPECTS

8.1 Local Regulations

The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" (GCP) ICH E6 Tripartite Guideline (January 1997). The investigator will ensure that the conduct of the study complies with the basic principles of GCP as outlined in the current version of 21 Code of Federal Regulations, subpart D, Part 312, "Responsibilities of Sponsors and Investigators" Part 50, "Protection of Human Subjects" and Part 56, "Institutional Review Boards."

8.2 Informed Consent

It is the responsibility of the investigator, or a person designated by the investigator, to obtain written informed consent from each subject participating in this study after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. In the case where the subject is unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject has orally consented to participation in the trial, the witness's signature on the form will attest that the information in the consent form was accurately explained and understood.

The CRF for this study contains a section for documenting informed subject consent, and this must be completed appropriately. If new safety information results in significant changes in the risk/ benefit assessment, the consent form should be reviewed and updated as necessary. All subjects (including those already being treated) should be informed of the new information, should be given a copy of the revised form, and should give their consent to continue in the study.

8.3 Institutional Review Board/ Ethics Committee

This study is being conducted under a United States Investigational New Drug application or other Clinical Trial Application, as appropriate. This protocol (and any modifications) and appropriate consent procedures must be reviewed and approved by an IRB/ EC. This board must operate in accordance with current local, regional, and federal

regulations. The investigator will send a letter or certificate of IRB/ EC approval to Exelixis (or designee) before subject enrollment and whenever subsequent modifications to the protocol are made.

8.4 Conditions for Modifying the Protocol

Protocol modifications may be made and will be prepared, reviewed, and approved by representatives of the investigator.

All protocol modifications must be submitted to the IRB/ EC for information and approval in accordance with local requirements and to regulatory agencies if required. Approval must be obtained before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to study subjects or those that involve only logistical or administrative aspects of the trial (eg, change in data monitor or change of telephone number).

8.5 Conditions for Terminating the Study

Exelixis reserves the right to terminate the study, and investigators reserve the right to terminate their participation in the study, at any time. Should this be necessary, Exelixis and the investigator will arrange the procedures on an individual study basis after review and consultation. In terminating the study, Exelixis and the investigator will ensure that adequate consideration is given to the protection of the subjects' interests.

9 STUDY DOCUMENTATION

9.1 Department of Defense guidelines:

a) Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command. These representatives are authorized to review research records

as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

b) Representatives of the U.S. Army Medical Research and Materiel Command are authorized to review research records as part of their responsibility to protect human research volunteers. Research records will be stored in a confidential manner so as to protect the confidentiality of your information.

c) All unanticipated problems involving risk to subjects or others related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

9.2 Investigator's Files and Required Documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two separate categories as follows: (1) the investigator's study file, and (2) subjects' clinical source documents.

The investigator's study file will contain the protocol and protocol amendments, CRFs, query forms, IRB/ EC and governmental approvals with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

Subjects' clinical source documents include the subjects' hospital/ clinic records; physicians' and nurses' notes; the appointment book; original laboratory, ECG, electroencephalogram, X-ray, pathology and special assessment reports; signed informed consent forms; consultant letters; and subject screening and enrollment logs.

The investigator must keep these documents on file for at least 2 years after the marketing application approval date for the study treatment and for the indication being investigated or for 2 years after the investigation is discontinued and the FDA is notified. After that period, the documents may be destroyed subject to local regulations with prior written permission from Exelixis. If the investigator wants to assign the study records to another party or move them to another location, Exelixis must be notified in advance.

If the investigator cannot guarantee the archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Exelixis to store these in a sealed container outside of the study site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the study site.

9.3 Source Documents and Background Data

Upon request, the investigator will supply its licensees and collaborators with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that subject confidentiality is protected.

9.4 Case Report Forms

For enrolled subjects, all and only data from the procedures and assessments specified in this protocol and required by the CRFs should be submitted on the appropriate CRF (unless source data are transmitted to Exelixis or a designee electronically, eg, central laboratory data). Data from some procedures required by the protocol, such as physical examinations and laboratory results, will be recorded only on the source documents and

will not be transcribed to CRFs. Additional procedures and assessments may be performed as part of the investigator's institution or medical practice standard of care and may not be required for CRF entry.

For each subject enrolled, the CRF (paper or electronic) must be completed and signed by the PI or authorized delegate from the study staff.

All paper forms should be typed or filled out using indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or his or her authorized delegate.

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to Exelixis in the CRF and in all required reports.

9.5 Confidentiality of Trial Documents and Subject Records

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to Exelixis or designees, subjects should be identified by identification codes and not by their names. The investigator should keep a subject enrollment log showing codes, names, and addresses. The investigator should maintain documents not for submission to Exelixis or designees (eg, subjects' written consent forms) in strict confidence.

All tumor scans, research samples, photographs, and results from examinations, tests, and procedures may be sent to Exelixis and its partners or designees for review.

10 PUBLICATION OF DATA

The Principal Investigator (Protocol Chair) holds the primary responsibility for publication of the study results; provided that the PI will provide any such publication to Exelixis, Inc. for review at least sixty (60) days prior to submission and also comply

withy any provisions regarding publication as are agreed to between the PI's institution (eg, institution name.) and Exelixis, Inc. in the Clinical Trial Agreement related to this study. The results will be made public within 24 months of the end of data collection. However, if a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. In any event, a full report of the outcomes should be made public no later than three (3) years after the end of data collection. Authorship for abstracts and manuscripts resulting from this study will be determined according to guidelines established by the International Committee of Medical Journal Editors.

11 REFERENCES

American Cancer Society. Cancer Facts & Figures 2008. http://www.cancer.org/downloads/STT/2008CAFFfinalsecured.pdf

Bottaro DP, Liotta LA. Out of air is not out of action. Nature. 2003;423:593-5.

Eder JP, Heath E, Appleman L, Shapiro G, Wang D, Malburg L, et al. Phase I experience with c-MET inhibitor XL880 administered orally to patients (pts) with solid tumors. 2007 ASCO Annual Meeting Proceedings Part I. J Clin Oncol. 2007;25:18S (June 20 Supplement) (Abstract 3526).

Garcia A, Rosen L, Cunningham CC, Nemunaitis J, Li C, Rulewski N, et al. Phase 1 study of ARQ 197, a selective inhibitor of the c-Met RTK in patients with metastatic solid tumors reaches recommended phase 2 dose. 2007 ASCO Annual Meeting Proceedings Part I. J Clin Oncol. 2007;25:18S (June 20 Supplement) (Abstract 3525).

Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for metastatic colorectal cancer. New Engl J Med. 2004;350(23):2335-42.

Jiang WG, Martin TA, Parr C, Davies G, Matsumoto K, Nakamura T. Hepatocyte growth factor, its receptor, and their potential value in cancer therapies, Crit Rev Oncol Hematol. 2005;53(1):35-69.

Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the Met protooncogene. Cancer Cell. 2003;3:347-61.

Ross RW, Stein M, Sarantopoulos J, Eisenberg P, Logan T, Srinivas S, et al. A phase II study of the c-Met RTK inhibitor XL880 in patients (pts) with papillary renal-cell carcinoma (PRC). 2007 ASCO Annual Meeting Proceedings Part I. J Clin Oncol. 2007;25:18S (June 20 Supplement) (Abstract 15601).

Sandler AB, Gray R, Brahmer J, Dowlati A, Schiller JH, Perry MC, et al, Randomized phase II/III Trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC #704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): An Eastern Cooperative Oncology Group (ECOG) Trial - E4599. ASCO 2005 Annual Meeting (Abstract LBA4).

Sattler M, Ma PC, Salgia R. Therapeutic targeting of the receptor tyrosine kinase Met. Cancer Treat Res. 2004;119:121-38.

Wright JR, Ung YC, Julian JA, Pritchard KI, Whelan TJ, Smith C, et al. Randomized, double-blind, placebo-controlled trial of erythropoietin in non-small-cell lung cancer with disease-related anemia. J Clin Oncol. 2007;25:1027-32.

D.P. Petrylak, C.M. Tangen and M.H. Hussain et al., Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer, N Engl J Med 351 (2004), pp. 1513–1520

Tannock IF, Wit R, Berry WR, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer.N Engl J Med. 2004 Oct 7;351(15):1502-12.

Oh W, Manola J, Babcic V, et al. Response to second-line chemotherapy in patients with hormone refractory prostate cancer receiving two sequences of mitoxantrone and taxanes.Urology. 2006 Jun;67(6):1235-40. PMID: 16765185

Berthold DR, Sternberg C, Tannock I. Management of advanced prostate cancer after first-line chemotherapy.J Clin Oncol. 2005 Nov 10;23(32):8247-52. Review. PMID: 16278480.

D. C. Smith, M. R. Smith, E. J. Small, et al. Phase II study of XL184 in a cohort of patients (pts) with castration-resistant prostate cancer (CRPC) and measurable soft tissue disease. GU ASCO Symposium 2011, abstract # 127.

Schoder H, Larson SM. Positron emission tomography for prostate, bladder, and renal cancer. Semin Nucl Med 2004;34(4):274-292.

Shreve PD, Grossman HB, Gross MD, Wahl RL. Metastatic prostate cancer: initial findings of PET with 2-deoxy-2-[F-18]fluoro-D-glucose. Radiology 1996;199(3):751-756.

Heicappell R, Muller-Mattheis V, Reinhardt M, et al. Staging of pelvic lymph nodes in neoplasms of the bladder and prostate by positron emission tomography with 2-[(18)F]-2-deoxy-D-glucose. EurUrol 1999;36(6):582-587.

Yeh SD, Imbriaco M, Larson SM, et al. Detection of bony metastases of androgen-independent prostate cancer by PET-FDG. Nucl Med Biol 1996;23(6):693-697.

Schirrmeister H, Guhlmann A, Kotzerke J, et al. Early detection and accurate description of extent of metastatic bone disease in breast cancer with fluoride ion and positron emission tomography. J Clin Oncol 1999;17(8):2381-2389.

Shields AF, Mankoff DA, Link JM, et al. [11C]Thymidine and FDG to measure therapy response. J Nucl Med 39:1757-1762, 1998.

Tehrani OS, Douglas KA, Lawhorn-Crews JM, Shields AF. Tracking cellular stress with labeled FMAU reflects changes in mitochondrial TK2. Eur J Nucl Med Mol Imaging. 35:1480-8, 2008.

Sun H, Sloan A, Mangner TJ, Vaishampayan U, Muzik O, Collins JM, Douglas K, Shields AF..Imaging DNA synthesis with [18F]FMAU and positron emission tomography in patients with cancer. Eur J Nucl Med Mol Imaging. 2005;32:15-22.

E.A. Eisenhauer,*, P. Therasse, J. Bogaertsc, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1) Eur J Cancer 2009; 45:228-247.

Howard I. Scher, Susan Halabi, Ian Tannock, et al. Design and End Points of Clinical Trials for Patients With Progressive Prostate Cancer and Castrate Levels of Testosterone: Recommendations of the Prostate Cancer Clinical Trials Working Group. Journal of Clinical Oncology, Vol 26, No 7 (March 1), 2008: pp. 1148-1159.

Lara, P. N. Jr., Stadler, W. M., Longmate, J., et al. A Randomized Phase II Trial of the MatrixMetalloproteinase Inhibitor BMS-275291 in Hormone-Refractory Prostate Cancer Patients with Bone Metastases. Clin Cancer Res 2006, 12: 1556-1566.

Coleman, R. E., Major, P., Lipton, A., et al. Predictive Value of Bone Resorption and Formation Markers in Cancer Patients With Bone Metastases Receiving the Bisphosphonate Zoledronic Acid. J Clin Oncol, 2005, 23: 4925-4935.

Chestnut CH 3rd, Bell NH, Clark GS, et al. Hormone replacement therapy in postmenopausal women: urinary N-telopeptide of type I collagen monitors therapeutic effect and predicts response of bone mineral density. Am J Med, 1997; 102:29-37.

Moss DW. Alkaline phosphatase isoenzymes. Clin Chem 1982;28:2007–2016.
24.

D. Olmos H.-T. Arkenau, J. E. Ang, et al.Circulating tumour cell (CTC) counts as intermediate end points in castration-resistant prostate cancer (CRPC): a single-centre experience. Annals of Oncol, 2008; 20:27-33.

12.0 DATA AND SAFETY MONITORING

12.1 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. The **Research Monitor** for this protocol is designated to be Dr Lois Ayash M.D. the chair of the Data and Safety Monitoring committee who will independently review and oversee the protocol conduct and monitoring along with the Data and Safety monitoring committee at Karmanos Cancer Center.

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

12.2 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 IV). 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 Principle 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.

12.3 The Barbara Ann KARMANOS Cancer Institute, Data and Safety Monitoring Committee will meet on a monthly basis to review the prior month Serious

Adverse Event forms and Data and Safety Monitoring study specific reports that have been filed.

Appendix A: Performance Status Criteria

	ECOG Performance Status Scale		**Karnofsky Performance Scale**
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all 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 of his/ her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any	20	Very sick, hospitalization indicated. Death not imminent.

	self-care. Totally confined to bed or chair.	10	Moribund, fatal processes progressing rapidly.
5	Dead	0	Dead

ECOG = Eastern Cooperative Oncology Group.

Appendix B BLOOD PROCESSING

1. The Vacutainer CPT tube with Sodium Citrate should be at room temperature (18-25 ° C)
2. Collect 8 ml of blood into the Tube
3. Store Tube upright at room temperature until centrifugation. Centrifugation should be performed within 2 hours of blood collection
4. Centrifuge at room temperature in a horizontal rotor (swing-out head) for a minimum of 20 minutes at 1500 to 1800 RCF

NOTE: Mix blood sample immediately prior to centrifugation by gently inverting the Tube 8 to 10 times

5. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under serum layer
6. Collect serum without disturbing the cell layer. Store at -80° C for future analysis.
7. Collect the cell layer with a Pasteur pipette and transfer to a 15 ml size conical centrifuge tube with cap. Collection of cells immediately following centrifugation will yield best results.

SUGGESTED CELL WASHING STEP:

8. Add PBS to bring volume to 15 ml. Cap tube, mix by inverting the tube 5 times
9. Centrifuge for 15 minutes at 300 RCF. Aspirate as much supernatant as possible without disturbing the pellet
10. Add PBS to bring volume to 10 ml. Cap tube, mix by inverting the tube 5 times, centrifuge for 10 minutes at 300 RCF and aspirate supernatant without disturbing the pellet
11. Re-suspend the cell pellet in 1 ml PBS. Transfer cell suspension into Eppendorf tube, centrifuge and aspirate as much supernatant as possible without disturbing the pellet (lymphocytes).
12. Store lymphocytes as pellet, do not add any solution. For buffy coat/DNA analysis, add 1 ml 1% SDS/1mM EDTA/50 mM mannitol/20 mM Tris, pH 7.4 over the cell pellet and vortex. Freeze at -80° C for future analysis.
13. For RNA later, resuspend the pellet in 100 ul of RNA later solution, then freeza at -80° C.