UMCC 2018.052

Multi-center Trial of ESK981 in Combination With Nivolumab in Patients With Metastatic Renal Cell Carcinoma

NCT03562507

PROTOCOL UMCC 2018.052
ERICA: Phase 2 Multi-Center Trial of ESK981 in Combination with Nivolumab in Patients With Metastatic Renal Cell Carcinoma

Principal Investigator: Ajjai Alva, MD
Hematology/Oncology
University of Michigan
7310 Cancer Center
1500 E. Medical Center Drive
Ann Arbor, MI 48109
Phone: (734) 936-0091
Fax: (734) 615-2719
Email: ajjai@umich.edu

IND #: 139688

Coordinating Center University of Michigan

Biostatistician: Ryan Ross, MS
Cancer Data Sciences Shared Resource -Biostatistics
M4011 SPH II, University of Michigan
1415 Washington Heights
Ann Arbor MI 48109-2029
Phone: (734) 764-5450
Email: rydaro@umich.edu

Study Drug: ESK981 (other names: CEP-11981) – Investigational
Nivolumab (Opdivo) - Standard of care

Initial version: April 10, 2018
Amended: June 8, 2018
November 30, 2018
April 4, 2019
April 2, 2020 (COVID-19 Appendix)
August 7, 2020

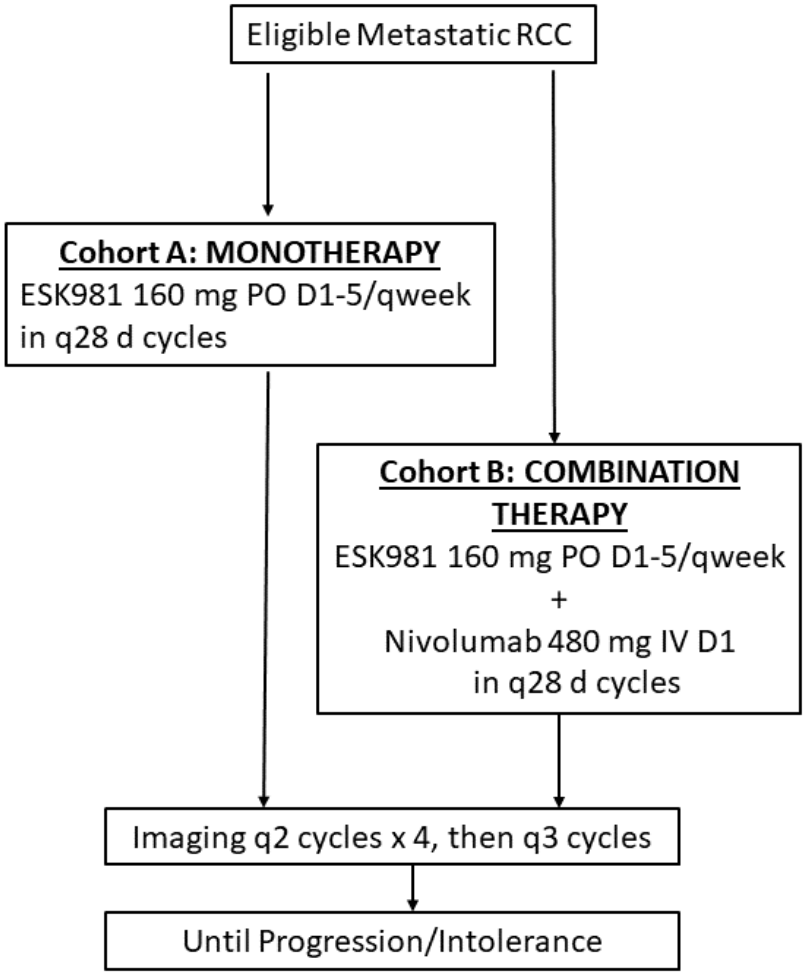
NOTE: To effectively manage the COVID-19 pandemic restrictions, changes to protocol-required item were made to minimize or eliminate immediate hazards or to protect the life and well-being of research participants (e.g., to limit exposure to COVID-19). These changes are listed in **Appendix 3** of the protocol (**Study Management during COVID-19**).

TABLE OF CONTENTS

ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTSU	Clinical Trials Support Unit
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
ESK981	Study Drug
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IND	Investigational New Drug
IRB	Institutional Review Board
IV (or iv)	Intravenously
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
p.o.	per os/by mouth/orally
PR	Partial Response
PRC	Protocol Review Committee
RCC	Renal Cell Carcinoma
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
UaP	Unanticipated Problem
WBC	White Blood Cells

STUDY SCHEMA



STUDY SYNOPSIS

Title	**ERICA: Phase 2 Multi-Center Trial Of ESK981 and Nivolumab In Patients With Metastatic Renal Cell Carcinoma**
Phase	II
Methodology	Single arm; two cohorts
Study Duration	54-60 months (24-30 months for accrual, estimated 6 months for treatment, and 24 months of follow-up)
Study Center(s)	Multi-Center: 2 sites total including lead site: University of Michigan
Objectives	**Primary Objectives** • To determine the clinical efficacy of ESK981 in combination with nivolumab therapy in patients with metastatic renal cell carcinoma. **Secondary Objectives** • To assess the clinical efficacy of ESK981 monotherapy in patients with metastatic renal cell carcinoma. • To determine the safety and tolerability of ESK981 monotherapy and in combination with nivolumab in patients with metastatic renal cell carcinoma. **Exploratory Objectives** • To determine the quality of life of patients enrolled on the study as reflected in patient-reported outcomes.
Number of Subjects	47 objective response evaluable patients
Inclusion Criteria	• Histologic diagnosis of renal cell carcinoma (any histology except medullary carcinoma or collecting duct carcinoma is acceptable) with radiologic or histologic evidence of metastatic disease. • Prior treatment with up to one (and only one) anti-VEGF or VEGFR inhibitor (small molecule or antibody). • Must have measurable disease as per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) criteria. • Must be of age ≥ 18 years at time of informed consent. • Ability to understand and the willingness to sign a written informed consent. • Karnofsky performance status ≥ 60. • Most recent systemic therapy or most recent radiation therapy ≥2 weeks of first study drug dose. • Recovery to baseline or ≤ Grade 1 CTCAE v.4.03 from toxicities related to any prior treatments, unless AE(s) are clinically non-significant and/or stable on supportive therapy.

	• Women of childbearing potential must have a negative serum or urine pregnancy test within 28 days prior to registration. Women of non-childbearing potential are defined as those who have no uterus, ligation of the fallopian tubes, or permanent cessation of ovarian function due to ovarian failure or surgical removal of the ovaries. All others are considered women of child bearing potential. • Adequate organ and marrow function.

Exclusion Criteria	• Prior treatment for metastatic disease with >1 anti-VEGF/VEGFR inhibitor.
	
	• Prior treatment with anti-PD/PD-L1/CTLA4/IDO antibody (for Cohort B patients only) or ESK981 (for Cohort A and Cohort B patients).
	  ○ Prior mTOR inhibitors or glutaminase inhibitors are allowed.
	
	• Untreated brain metastases or spinal cord compression.
	  ○ Patients with suspected or known treated brain metastases at screening should have a MRI (preferred) or CT preferably with IV contrast of the brain prior to study entry. Patients whose brain metastases have been treated may be considered if they have completed their treatment for brain metastasi(e)s at least 4 weeks prior to study registration AND they show radiographic and clinical stability (by CT or MRI brain imaging, obtained after treatment to the brain metastases). In addition, any neurologic symptoms that developed either as a result of the brain metastases or their treatment must have resolved or be stable without the use of steroids at daily doses greater than 10 mg prednisone or equivalent for at least 14 calendar days prior to the start of treatment.
	
	• Uncontrolled hypertension defined as blood pressure >150/90 despite at least 2 anti-hypertensive medications as assessed by 2 blood pressure readings taken at least 1 hour apart during screening.
	
	• Major surgical procedure or significant traumatic injury within 6 weeks prior to study registration (> 6 weeks prior to registration is permitted as long as they have fully recovered from any such procedure).
	
	• History of another primary malignancy except for: malignancy treated with curative intent and no known active disease for ≥2 years, adequately treated non-melanoma skin cancer without current evidence of active disease, adequately treated carcinoma in situ without current evidence of active disease, Gleason ≤ 6 prostate cancer.
	
	• Angina, myocardial infarction symptomatic congestive heart failure, cerebrovascular accident, transient ischemic attack, arterial embolism, pulmonary embolism, PTCA or CABG within the past 3 months.

	• History of gastrointestinal perforation or fistula in the past 6 months, or while previously on antiangiogenic therapy, unless underlying risk has been resolved (e.g. through surgical resection or repair). • The patient has known hypersensitivity to gelatin or lactose monohydrate. • The patient has received any investigational drug within 28 days prior to registration or 5 half-lives of the investigational drug, whichever is shorter. • History of bleeding disorders (e.g. pulmonary hemorrhage, significant hemoptysis, menometrorrhagia not responding to hormonal treatment) ≤ 6 weeks before Cycle 1 Day1. • The patient is on a chronic daily medication known to be a severe or moderate inhibitor or inducer by Micromedex of CYP1A2, CYP2C8, or CYP3A4 at registration. • Systemic corticosteroids greater than the equivalent of 10 mg of prednisone or equivalent alternative steroid (except physiologic dose for adrenal replacement therapy) or other immunosuppressive agents (such as cyclosporine or methotrexate) and any other medications that could potentially impact the efficacy or safety of the study as judged by the treating investigator are NOT permitted from time of registration to subjects completing protocol therapy unless clinically indicated to manage adverse events or life threatening or serious conditions as determined by the treating investigator. • Have any condition that, in the opinion of the investigator, would compromise the ability of the subject to meet or perform study requirements.
Study Product(s), Dose, Route, Regimen	Cohort A (n = 11) – ESK981 given PO 160 mg 5 consecutive days followed by a 2-day off drug in each week, repeated weekly in 28 day cycles Cohort B (n = 36): ESK981 given PO 160 mg 5 consecutive days followed by a 2-day off drug in each week, repeated weekly in 28 day cycles in combination with nivolumab 480 mg IV on D1 every 28 day cycles as safety lead-in. If no safety signals are identified, we will accrue 19 additional subjects for a total of 36 subjects in the combination cohort B.
Duration of Administration	Until disease progression or intolerable toxicity or subject withdraws consent
Reference Therapy	None for Cohort A; Nivolumab alone (historical) for Cohort B

Statistical Methodology	The primary objective of the study is to determine the efficacy of ESK981 in combination with Nivolumab in Cohort B. Cohort B will use the Mini-max Simon two-stage design. A null response rate is assumed to be 25% with a response rate of interest of 45%. Assuming a 5% type I error and 80% power, the first stage will accrue 17 objective response evaluable patients. If the study passes the criteria at the interim analysis, then stage 2 will open and accrue another 19 response evaluable patients for a total of 36 patients. Cohort A will determine the efficacy of ESK981 monotherapy. Assuming a response rate of clinical interest with monotherapy is 25% and null response proportion of 5% with a 1-sided type I error=0.102 there is 80% power with 11 patients to detect a response rate of 25%.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Renal cell carcinoma is inherently resistant to cytotoxic chemotherapy, radiation, and hormone therapy [2], [3]. Until 2005, the mainstay of treatment was immunotherapy with IL-2 or interferon, both of which result in modest response rates at the expense of significant toxicity. Advances in the understanding of renal cell carcinoma (RCC) tumor biology led to the discovery that most patients with RCC have mutations of the von Hippel–Lindau (VHL) tumor suppressor gene that lead to gene silencing [4]. Silencing of the VHL gene was found to result in Vascular Endothelial Growth Factor (VEGF) overexpression. VEGF (also known as vascular permeability factor and VEGF-A) is a tumor-secreted cytokine with critical importance in both normal and tumor-associated angiogenesis. When the VHL gene is mutated or inactivated, the VHL protein cannot bind to hypoxia inducing factor alpha (HIFα). This leads to the accumulation of HIF1α and binding of HIF1α to HIF1β. The HIF1α/HIF1β heterodimer translocates to the nucleus and activates transcription of target genes such as VEGF and PDGF [5].

Elevated VEGF levels have important pathologic consequences as VEGF is able to induce endothelial cell division and migration [6], promote endothelial cell survival through protection from apoptosis [7] and reverse endothelial cell senescence [8]. Because of these factors, VEGF has been called the most potent proangiogenic protein described to date [9]. Sustained angiogenesis is one of the six essential alterations in cell physiology that dictates malignant growth [10]. All cells, including malignant ones, are unable to survive unless they are within 100 μm of a capillary vessel. Thus, budding cancerous cells must develop angiogenic ability to continue to grow and multiply [10]. Vascular endothelial growth factor (VEGF) is a signal protein that stimulates angiogenesis and is the major regulator of angiogenesis in normal and malignant tissue [11].

The discovery of the importance of VEGF in RCC tumor biology led to the development of VEGF targeted therapy for patients with metastatic kidney cancer. Since 2006, seven different VEGF tyrosine kinase inhibitors have been approved for the treatment of RCC. Initial response rates to treatment with a front line VEGF TKI are 30-40%. However, these responses are not sustainable and disease progression occurs after a median of 9-14 months [12], [13], [14].

There are various hypotheses regarding why resistance to VEGF inhibitors develops. One of the most probable is the development of alternative pathways for angiogenesis. Studies in RCC xenograft models have shown that during initial treatment with a VEGF tyrosine kinase inhibitor, there is necrosis and devascularization of the microvasculature, ultimately leading to complete cessation of blood flow. However, just prior to the development of resistance, there is restoration of blood flow suggesting that the tumor has escaped the previously achieved block on angiogenesis [15].

One of the most likely angiogenesis escape mechanisms is upregulation of the Ang2/Tie2 axis. Tie-2 is a tyrosine kinase whose expression is largely restricted to endothelial cells [16]. It has four known ligands, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang- 2), angiopoietin 3 (Ang-3) and angiopoietin-4 (Ang-4) [17], [18], [19], [20]. Ang1/Tie2 signaling plays an important role in the maturation and stabilization of the vasculature by recruitment of peri-endothelial cells [21]. In contrast, Ang2 functions as a natural antagonist of Ang1 and the Tie2 receptor, and is only expressed at sites of vascular remodeling with active angiogenesis, including pathologic angiogenesis as seen in tumors [17]. It has been shown that VEGFR-2 inhibition leads to up-regulation of members of the angiopoietin family [22] and at times of disease progression in RCC patients treated with sunitinib, Ang2 levels were shown to increase [23].

Several pre-clinical xenograft studies have demonstrated that Ang-2 blockade leads to suppression of tumor growth [24] (Figure 1), [25]. Additional evidence shows that Ang-2 mediated angiogenesis may be a later phenomenon in tumor development. In a mouse xenograft model tumor growth inhibition was more robust when treatment was initiated in more established tumors, suggesting that larger tumors are more dependent on Ang2 [25]. Finally, there is some evidence that Ang2 may contribute to metastases as overexpression of Ang2 lead to distant metastases in a breast cancer xenograft model [26].

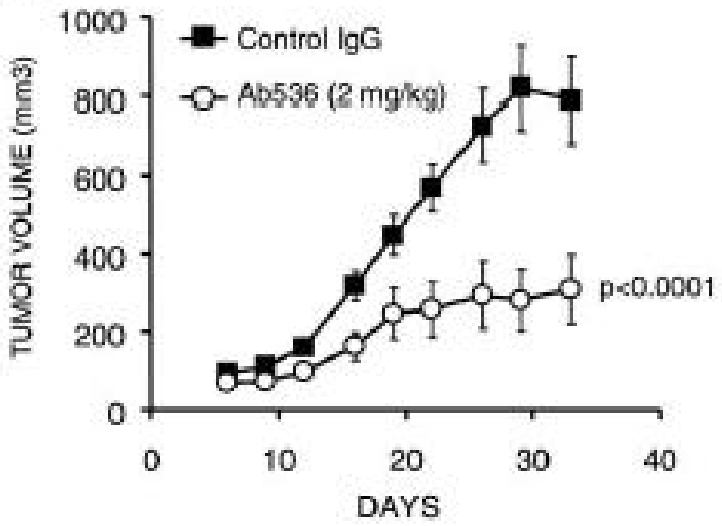


Figure 1: Inhibition of A431 tumor xenograft growth with a systemically administered Ang2- neutralizing agent. Treatment was initiated 3 days post injection of tumor cells. Ab536 was dosed thrice weekly.

While Ang2 may have a role in many tumor types, it may be particularly important in the tumorigenesis of renal cell carcinoma as demonstrated by the significantly higher levels of Ang2 in RCC as compared to other tumor types [27] (Figure 2).

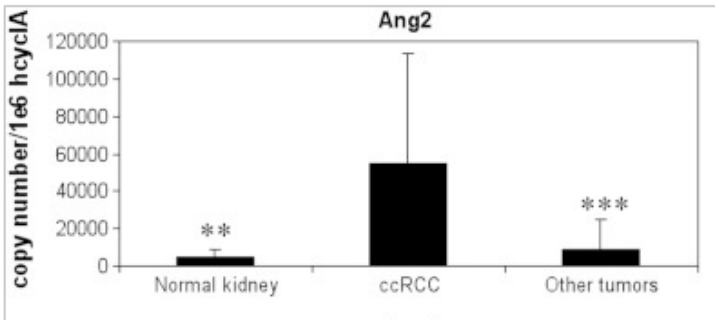


Figure 2: RT-PCR analysis of multiple tumor types shows that Ang2, VEGF, Ang1, VEGFR2, and CD31 are highly expressed in patients with ccRCC *versus* other tumor types (bladder, lymphoma, lung, laryngeal, ovarian, prostate, gastric, breast, colorectal, and pancreatic tumors). Ang2 and VEGF are also highly expressed in ccRCC *versus* normal kidney tissue. Data are expressed as means ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. [27]

Given the extensive pre-clinical data suggesting the deleterious effect of Ang-2 on tumor growth and angiogenesis, Ang2 inhibitors have been evaluated for the treatment of patients with metastatic renal cell carcinoma. In a phase II study by Atkins et al [28], trebananib (an Ang1/2 inhibitor) was combined with sunitinib for the treatment of treatment naïve metastatic renal cell carcinoma. This combination led to impressive response rates of 58-63%, notably higher than front line response rates with sunitinib or pazopanib of 25-31% [29] (Figure 3).

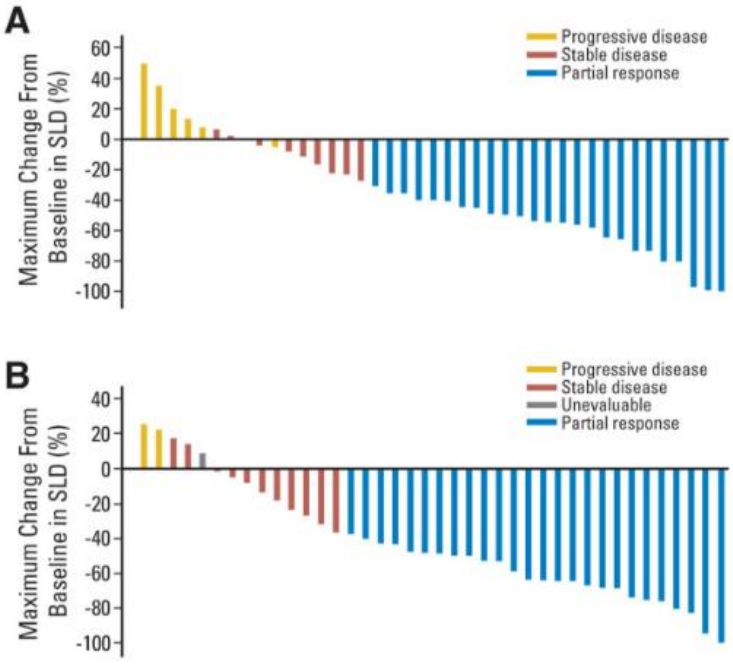


Figure 3: Maximum change from baseline in the sum of longest diameters (SLD) of target lesions at the time of the primary analysis for patients who received (A) trebananib 10 mg/kg once per week in cohort A or (B) trebananib 15 mg/kg once per week in Cohort B

Median overall survival was also improved as compared to historical controls treated with sunitinib or pazopanib with a median overall survival of 36 months (as compared to historical controls of 28-29 months [29]). Unfortunately, the combination of these drugs was associated with increased toxicity as compared to sunitinib alone and the combination has not been further evaluated in metastatic renal cell carcinoma.

1.2 Study Agent(s) Background and Associated Known Toxicities

1.2.1 Treatment Background

ESK981, formally known as CEP-11981, is a novel oral multi-TKI originally developed by Cephalon [30]. The compound was initially identified as an oral angiogenesis inhibitor targeting several important pathways involved in the angiogenic response, but without the off-target activities of other TKIs, like sunitinib and sorafenib, resulting in adverse events. ESK981 has potent activity in kinases implicated in angiogenesis including vascular endothelial growth factor receptor 1 (VEGFR-1), VEGFR-2, and Tie-2, (IC50 of 3, 4, 22nM, respectively). ESK981 treatment potently inhibited HUVEC capillary tube formation and aortic ring explant angiogenesis in vitro, and inflammation-induced angiogenesis in Tucker mice and several VEGF-induced angiogenesis models in vivo. ESK981 was tested in a dose-escalating phase I clinical trial to determine its pharmacokinetics and pharmacodynamics in patients with advanced, relapsed, or refractory solid tumors[31]. ESK981 was acceptably tolerated up to a dose of 97.4 mg/m^2 (maximum tolerated dose) that was determined to be the recommended phase II dose. Most adverse events were grade 1 or grade 2 and included fatigue, nausea, diarrhea,

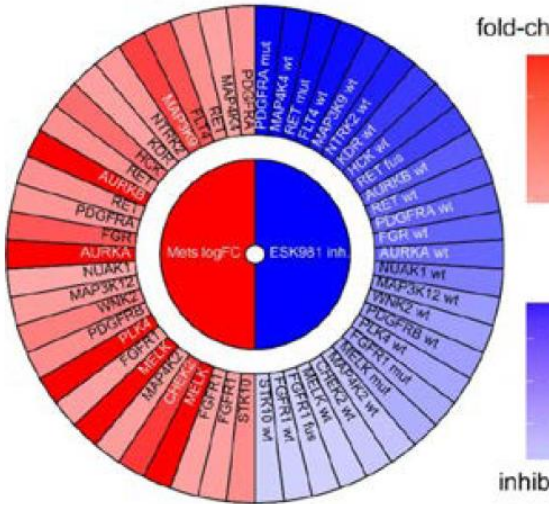


Figure 4. Kinases overexpressed in mCRPC (in red) and their in vitro inhibition with 25 nM ESK981 (blue).
Log-fold change of kinases in mCRPC relative to benign prostate assessed from a compendia of RNA-seq data. Rank ordered inhibition profile of 25 nM ESK981 determined in *in vitro* kinase assays. Intensity of color is associated with log-fold change or inhibitory activity.

decreased appetite, abdominal pain, back pain, vomiting, constipation, headache, dizziness, and dyspnea. Grade 3/4 neutropenia was observed in the highest-dose cohorts. More importantly, up to 85% of patients achieved stable disease when measured at ≥6 weeks in cohorts receiving ≥73.0 mg/m^2.

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

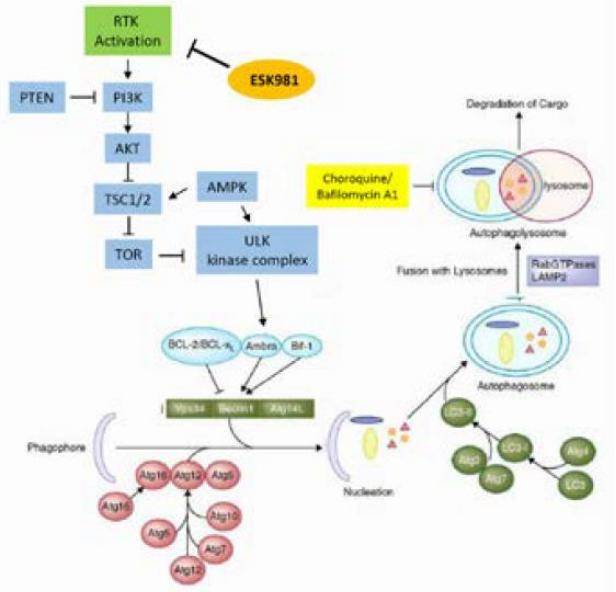


Figure 5. Overview of the autophagy pathway
Adapted from Yang et al.[1] The ULK kinase pathway is the key proximal kinase complex that controls autophagy. It can be regulated by a number of up-stream kinases including receptor tyrosine kinase (RTKs), PI3K, AKT, and TOR. The beclin1 complex is a mediator of autophagy. LC3 processing is a key step in autophagosome maturation. Chloroquine and Bafilomycin A1 block late stage autophagy by blocking lysosomal fusion. ESK981 presumably activates autophagy in CRPC by inhibiting an upstream TK.

Interestingly, in addition to AR+ prostate cancer cell lines, AR- prostate cancer cell lines were similarly sensitive to ESK981, intimating its potential use in small cell/neuroendocrine variants of prostate cancer. In vivo, ESK981 monotherapy inhibited castrated VCaP xenograft growth in a dose-dependent fashion. Tumors from mice treated with ESK981 displayed vacuolization associated with autophagy as well as marked decreases in proliferation (by Ki67 staining). Interestingly, autophagy is an evolutionarily conserved and orderly process of degradation and destruction of cellular components (Figure 3). As part of this process, double membraned vesicles known as autophagosomes are formed by engulfing cytoplasmic constituents, which then fuse with lysosomes to initiate degradation and recycling [1]. Exaggerated activation of the autophagic process can be observed as gross vacuolization of cells. Monoallelic loss of beclin-1, a gene critical for the autophagy pathway, has been found in 40 to 75% of human breast, prostate, and ovarian cancers[1, 32]. Thus,

autophagy has been considered an important tumor suppressive pathway, and sustained activation of autophagy in tumor cells leads to apoptosis. A number of approaches to regulate the autophagy pathway are being explored clinically in the treatment of cancer [1].

1.2.2 Nonclinical activity

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

Preclinical studies

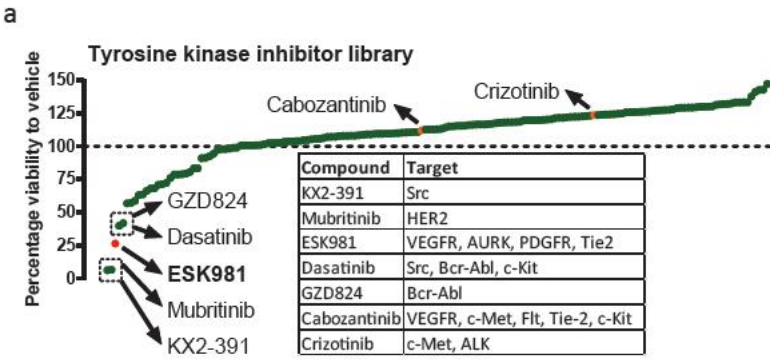
Significant oral anti-angiogenic efficacy has been observed in VEGF-A-induced, tumor-induced and inflammation-induced neo-vascularization models in rodents. Sustained dose-related anti-tumor activity has been demonstrated with ESK981 *in vitro* across a panel of human and murine tumor cell lines. Significant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

ESK981 has been intensively evaluated pre-clinically in human prostate cancer at the University of Michigan. In our preliminary assessment we evaluated the growth inhibitory effect of ESK981 against a library of tyrosine kinase inhibitors consisting of 167 compounds in DU145 cells. We found that ESK981 exhibited potent growth inhibition at 300nM concentration compared to Src inhibitors (KX2-391, Dasatinib and HER2 inhibitor (Mubritinib) that were reported to be efficacious in DU145 cells, whereas crizotinib and cabozantinib exhibited no such growth inhibitory effect at comparable concentrations (Fig. 6a). Among the 168 compounds, only ESK981 triggered a cytoplasmic vacuolization morphology that prompted us to further investigate its functional significance (Fig. 6b). Using 7 prostate cancer cell lines, we examined their sensitivity to ESK981 in long-term survival assays. After 2 weeks of growth, cells were stained with crystal violet and subsequently quantified. ESK981 exhibited IC50 values ranging from 35nM to 192nM. In contrast, cabozantinib and crizotinib exhibited only micromolar IC50 values in the cell lines tested (Fig. 6c).

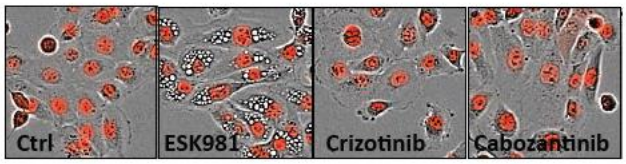
The morphological alterations triggered by ESK981 led us to explore whether the autophagy pathway is involved. Initially, we investigated ESK981-associated cellular vacuole in combination with various autophagic pathway inhibitors. The anti-malarial drug, chloroquine, has been used as an inhibitor of

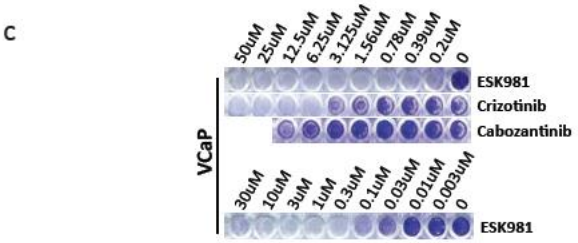
autophagy and lysosomal fusion. Strikingly, chloroquine completely negated the cellular vacuolization effects of ESK981 in DU145 cells after a 6-hour incubation. The phenotype was recapitulated by another lysosome inhibitor bafilomycin (BF) as well as early autophagasome inhibitor 3-Methyladenine (3-MA). This strongly suggested that ESK981-induced vacuolization involves the autophagy process (Fig. 7a). We next employed CYTO-ID autophagy detection kit (Enzo) to visualize cytoplasmic vacuoles in DU145 cells (Fig. 7b); quantified fluorescent intensity showed dose-dependent induction of autophagy signal in LNCaP and VCaP cells as well (Fig. 7c). Five prostate cell lines (VCaP, LNCaP, MDA-PCa-2b, LNCaP-AR and PC3) have been tested thus far exhibited LC3 cleavage in a ESK981 dose-dependent manner (Fig. 7d) suggesting that autophagy activation.

We observed that ESK981 increased cytokine CXCL10 secretion in cell culture medium in an autophagy-dependent manner, thus, we hypothesized that ESK981 may play a role in immune cell recruitment. These preliminary data led us to expand our investigation to immune response using syngeneic mouse prostate cancer Myc-CaP cells. We confirmed that ESK981 is effective in Myc-CaP cells and has lower IC50 than cabozantinib or crizotinib (Fig. 8a) and autophagy and CXCL10 were increased upon ESK981 treatment in Myc-CaP cells (Fig. 8b-c). Finally, we wanted to test whether ESK981 is able to potentiate immune checkpoint blockade. In immune competent mice, ESK981 and immune checkpoint inhibitor, anti-PD1 in combination exerted maximum tumor inhibition (Fig. 9a-b). With tumor inhibition, autophagy was observed in individual tumors upon ESK981 treatment (Fig. 9c). Overall, our data demonstrate that ESK981, alone or in combination with anti-PD1 therapy, is a promising candidate for clinical development; it targets a novel pathway and shows greater efficacy than tyrosine kinase inhibitors commonly in use.



Compound	Target
KX2-391	Src
Mubritinib	HER2
ESK981	VEGFR, AURK, PDGFR, Tie2
Dasatinib	Src, Bcr-Abl, c-Kit
GZD824	Bcr-Abl
Cabozantinib	VEGFR, c-Met, Flt, Tie-2, c-Kit
Crizotinib	c-Met, ALK





Cell Line	ESK981 (nM)	Crizotinib (nM)	Cabozantinib (nM)
VCaP	**35**	3379	8456
LNCaP	**55**	ND	ND
22RV1	**90**	ND	ND
C4-2B	**56**	ND	ND
LNCAP-AR	**56**	1455	8672
PC3	**192**	956	6141
DU145	**79**	1125	6222
LNCaP-APIPC	**2**	ND	ND

Figure 6. Prostate cancer cell lines are more sensitive to ESK981 than FDA-approved MTKIs Cabozantinib or Crizotinib.

A, Percentage viability of DU145 for 300nM ESK981 and other 167 tyrosine kinase inhibitors compared to vehicle control. Top 5 most inhibitory compounds are listed in the table, as well as cabozantinib and crizotinib (highlighted in orange). ESK981 is highlighted in red.

B, Morphological differences of 300nM ESK981, crizotinib or cabozantinib treated nuclear restricted RFP expressing DU145.

C, A long-term survival assay was used to calculate half-maximum inhibitory concentration (IC_{50}) after 2 weeks of incubation with serial dilutions of indicated drugs. Top, Long-term survival assays of VCaP prostate cancer cells exposed to MTKIs. Bottom, (IC_{50}) of ESK981, crizotinib and cabozantinib in a panel of prostate cancer cell lines.

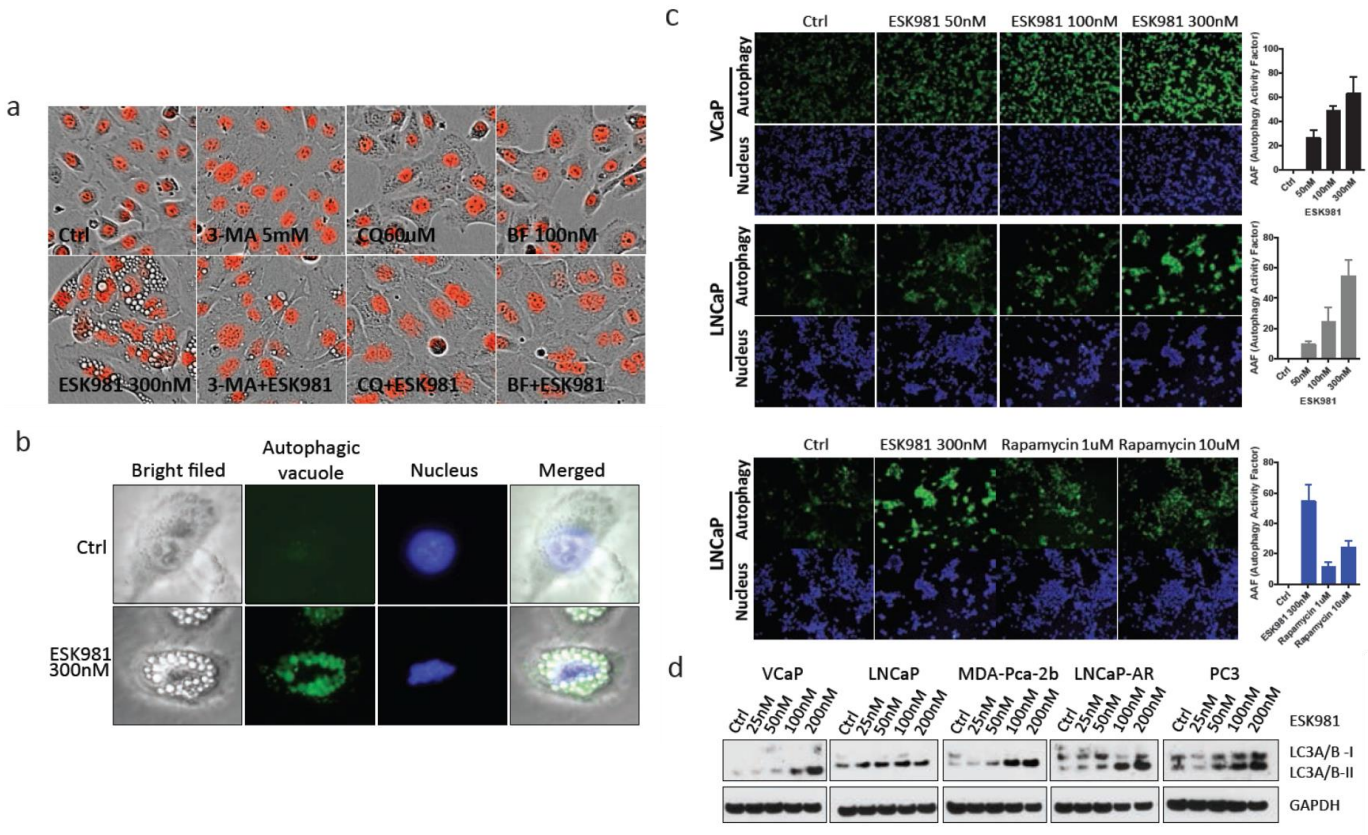
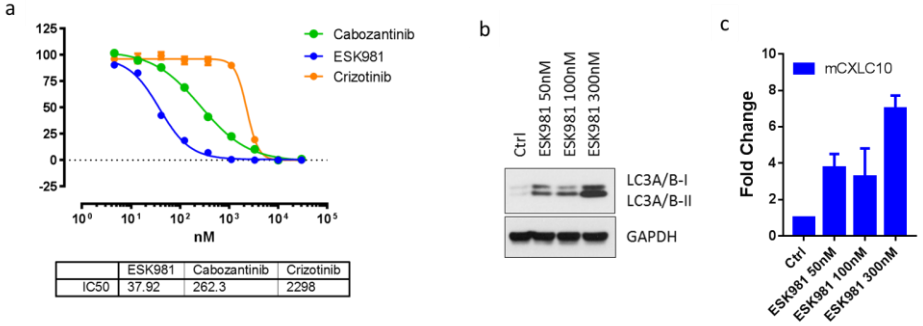


Figure 7. ESK981 induced cellular vacuolization is part of autophagy activation.
A, Autophagy inhibitors negated ESK981 induced cellular vacuolization. Morphology of DU145-RFP cells treated with ESK981, and various autophagy inhibitors 3-Methyladenine (3-MA), chloroquine (CQ), bafilomycin (BF) alone and in combination of for 6 hours. Red indicates nuclei.
B, Autophagic vacuoles were visualized by green fluorescence in DU145 treated with either control or ESK981 for 24 hours, and nuclei were indicated by blue.
C, VCaP and LNCaP cells were treated with increasing concentration of ESK981 for 24 hours, and autophagic activity were measured by CYTO-ID kit, and quantification of autophagy activity is shown on right. Rapamycin served as a positive control for autophagy induction.
D, Protein levels of LC3 in indicated prostate cancer cell lines.



	ESK981	Cabozantinib	Crizotinib
IC50	37.92	262.3	2298

Figure 8. Mouse prostate cancer cell Myc-CaP is sensitive to ESK981.
A, IC_{50} of ESK981, cabozantinib, crizotinib were determined in Myc-CaP cells.
B, LC3 protein levels were determined by western blot in Myc-CaP treated with various concentration of ESK981 for 24 hours.
C, Mouse CXCL10 mRNA levels were measured by qPCR in Myc-CaP cells treated with various concentration of ESK981 for 24 hours.

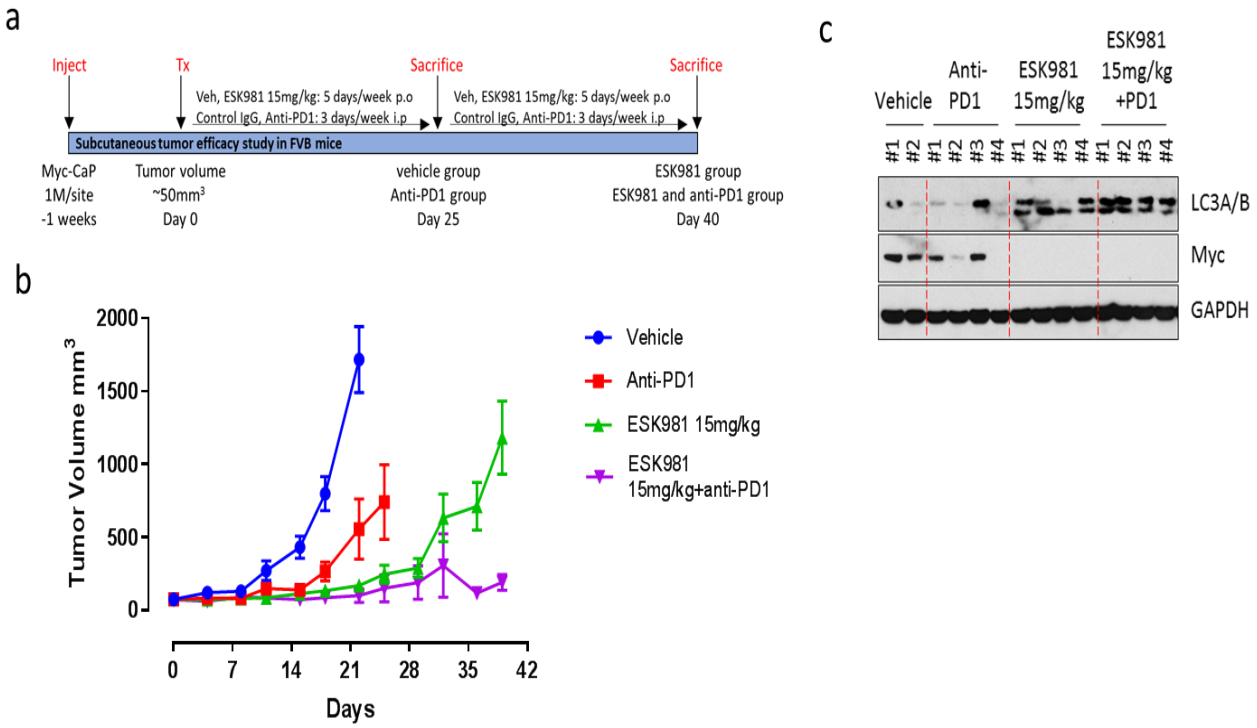


Figure 9. ESK981 enhances immune checkpoint blockade in mouse prostate cancer model.
A, Schematic illustration of study design
B, Tumor volume of Myc-CaP after indicated treatments.
C, Protein levels of LC3 and Myc were detected in individual tumors.

Preclinical work in three kidney carcinoma cell lines demonstrated that ESK981 remains potent in inhibiting kidney cancer cell proliferation with nanomolar IC_{50} concentration (Figure. 6, unpublished data). Additionally, ESK981 is able to robustly induce dose-dependent autophagy (Figure. 7, unpublished data). Meanwhile, ESK981 enhances the Th1-type chemokines CXCL9 and CXCL10 expression (Figure. 8, unpublished data) through STAT1-dependent pathway (Figure. 9, unpublished data). Thus treatment with ESK981 alone led to higher levels of CXCL9 and CXCL10 expression and when in combination with an anti-PD1 agent, the immune response should be dramatically enhanced.

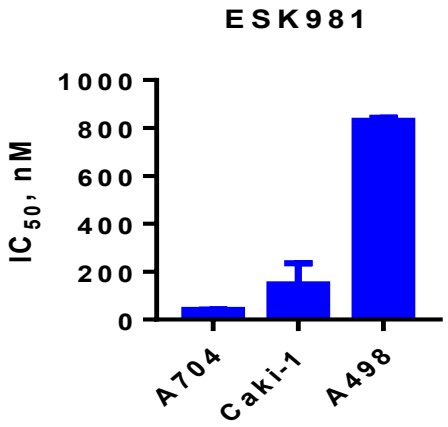


Figure 10. IC_{50} of ESK981 has been determined in several human kidney cancer cell lines *in vitro*. A704, Caki-1 and A498 have 40.8, 146.9 and 830nM respectively.

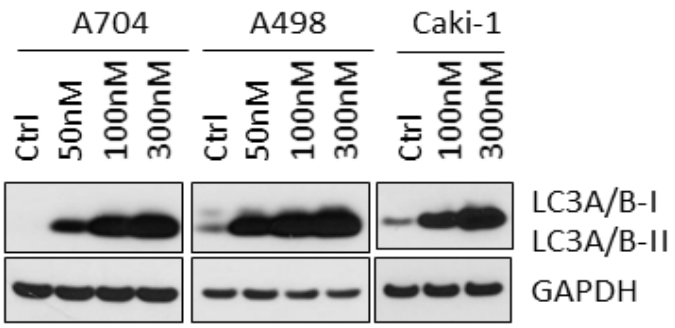


Figure 11. ESK981 induces dose-dependent autophagy in human kidney cancer cell lines.

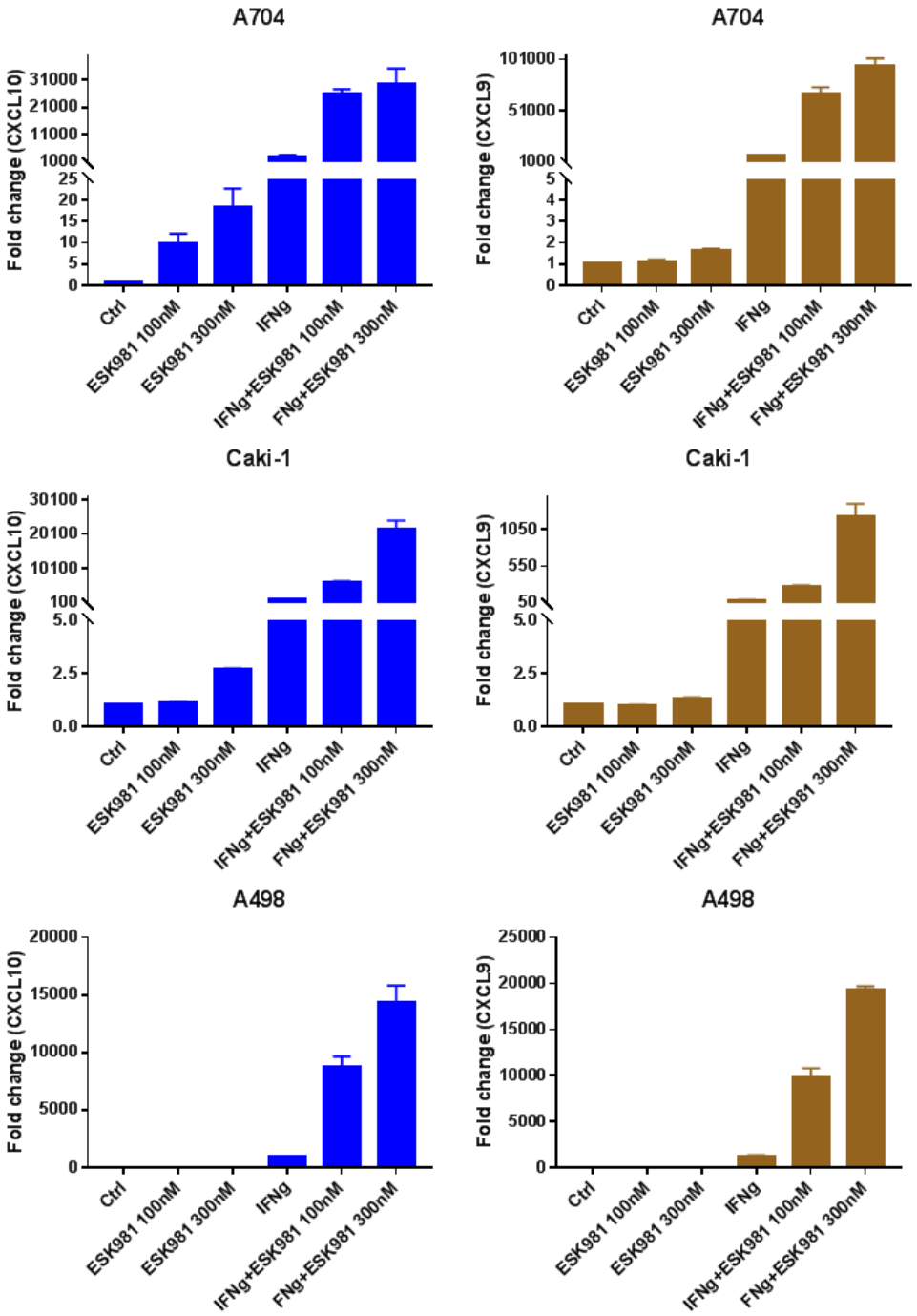


Figure 12. ESK981 dose-dependently enhances interferon gamma signaling induced CXCL10 and CXCL9 mRNA expression in human kidney cancer cell lines.

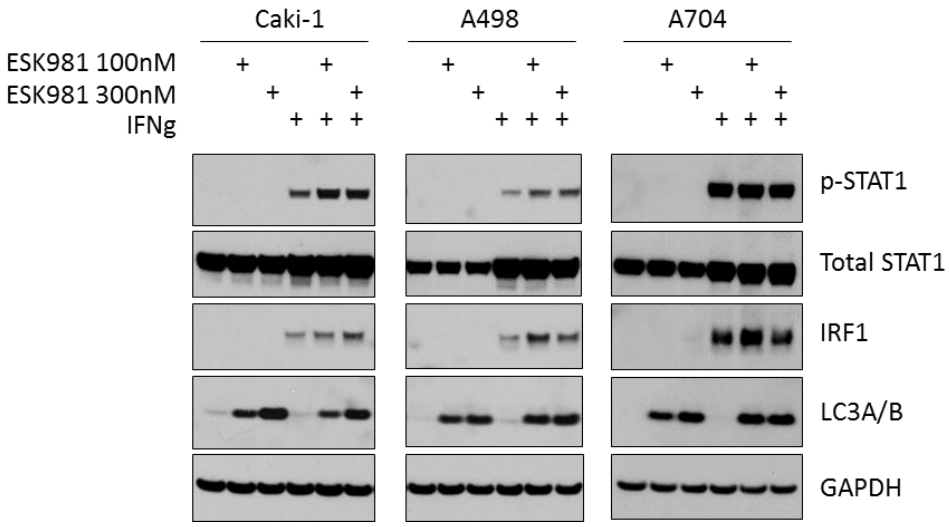


Figure 13. ESK981 enhances interferon gamma signaling activation through STAT1 dependent pathway.

1.2.3 Clinical Activity

Phase I open-label, dose-escalating study pharmacokinetics

ESK981 was tested in an open-label dose-escalating Phase 1 clinical trial to determine its pharmacokinetics (PK) and pharmacodynamics (PD) in patients with advanced, relapsed, or refractory solid tumors (Study C11981/1047/ON/US, NCT00875264) [31]. After daily oral administration of ESK981 to cancer patients, the median t_{max} values for ESK981 were typically in the range of 2 to 4 hours (Table1 and Table 2). However, for most of the dose groups there appeared to be considerable inter-patient variability in this pharmacokinetic parameter. The reason(s) for the large inter-patient variability in t_{max} is(are) not presently known, but may be related to the fed/fasted state of the patients or other unknown factors which could potentially influence drug absorption (e.g., disease state, concomitant medications).

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

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

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

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

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

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

After reaching peak plasma levels, ESK981 declined in a bi-phasic manner that was characterized by an initial phase of drug distribution and a slower terminal elimination phase. For some profiles, however, only the later phase was evident due to what appeared to be a more prolonged period of drug absorption. The mean $t_{1/2}$ of the terminal elimination phase was generally in the range of approximately 8 to 10 hr; however, the values tended to be slightly larger after multiple doses at the higher dose levels (i.e., doses ≥55 mg/m^2) (Table 1 and Table 2).

The systemic exposures (i.e., C_{max} and AUC) of patients to ESK981 generally increased with increasing dose across the dose range evaluated on both days 1 and 15 (Table 1 and Table 2*)*. There were some dose groups for which the mean values of C_{max} and AUC did not show an increase relative to the values from the preceding 1 or 2 dose levels; however, this result was not surprising given the

large inter-patient variability and the fact that most dose increments were less than 40%. Nonetheless, the overall results obtained are not suggestive of the presence of any obvious non-linearity in absorption and/or elimination of ESK981 in this dose range.

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

1.2.4 Clinical safety and efficacy

Adverse events

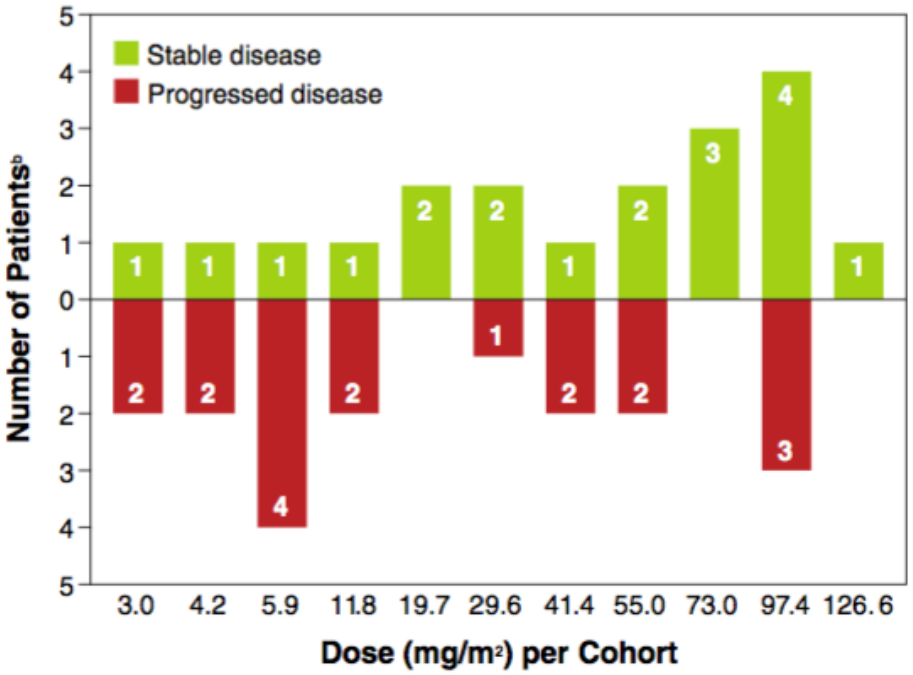
All 43 patients experienced ≥1 adverse event, and 38 patients (88.3 %) were deemed to have had adverse events possibly, probably, or definitely related to study drug. The most frequently reported adverse event of any grade was fatigue (n= 22, or 51 %). Other frequently reported adverse events (≥20 % of patients) were nausea (47 %), diarrhea (33 %), decreased appetite (33 %), abdominal pain (30 %), back pain (28 %), vomiting (28 %), constipation (28 %), headache (28 %), dizziness (28 %), and dyspnea (23 %). These adverse events were reported at a similar frequency between dosage cohorts, and no relationship with dose was evident. Most adverse events were grade 1 or grade 2.
Grade 3 or 4 adverse events occurred in 16 (37 %) patients across dosing cohorts (14 [32.6 %] Grade 3 and Grade 2 [4.7 %] Grade 4). Treatment-related grade 3 or 4 adverse events were most frequent in the 97.4 mg/m^2 cohort. Grade 3 or 4 laboratory hematologic toxicities were reported in 8 (18.6 %) patients across dosage cohorts. The most common grade 3 or 4 laboratory hematologic toxicity was lymphopenia, which occurred in 8 patients and across dosage cohorts (5.9, 29.6, 55.0, 97.4, and 126.6 mg/m^2). Grade 4 leukopenia occurred in 1 patient in the 126.6 mg/m^2 cohort. Grade 3 or Grade 4 neutropenia also occurred in 2 patients in the 97.4 mg/m^2 cohort (Grade 3) and in 1 patient in the 126.6 mg/m^2 cohort (Grade 4).

Serious adverse events occurred in 12 patients; most were deemed unlikely or not related to CEP-11981. Three patients (1 patient in the 97.4 mg/m^2 cohort and 2 patients in the 126.6 mg/m^2 cohort) experienced serious adverse events that were categorized as possibly or definitely related to ESK981: pyrexia, hemolytic anemia, hyperbilirubinemia, dyspnea, neutropenia, ECG change, and chest discomfort. No deaths occurred during the study.

Tumor response

Of 43 patients who received ≥1 dose of study drug, 37 patients were evaluated for tumor response. Although no enrolled patients had complete or partial response according to RECIST criteria, 19 of 37 (51 %) patients evaluated for tumor response had stable disease at ≥6 weeks; 18 had disease progression. The frequency of stable disease (defined as <30 % decrease and <20 % increase in the sum of the longest diameter of the target lesions) was higher in cohorts receiving doses ≥73.0 mg/m^2 (8 of 14 [57.1 %] patients) compared with cohorts receiving ≤55.0 mg/m^2 (11 of 29 [37.9 %] patients) (Figure 14).

Figure 14. Best overall tumor response per cohort[a] at >/= 6 weeks
Depicts tumor response, with bars above the x-axis indicating patients (n = 19) who achieved stable disease and bars below the x-axis indicating patients (n = 18) with disease progression. [a]No patient achieved complete or partial response. [b]Six patients were not evaluable at dosages of 19.7, 29.6, 55.0, and 126.6 mg/m^2 (n = 1, each); 97.4 mg/m^2 (n = 2)



1.3 Rationale

ESK981 monotherapy
ESK981 has cleared a dose-escalating phase I clinical trial and demonstrated favorable pharmacokinetics and pharmacodynamics in patients with advanced, relapsed, or refractory solid tumors with up to 85% of patients achieving stable disease at doses ≥73.0 mg/m^2.

ESK981 is an orally active multi-targeted receptor tyrosine kinase inhibitor that inhibits the human receptor for Angiopoietin-1 and Angiopoietin-2 (TIE-2), vascular endothelial growth factor receptor-2 (VEGFR-2), vascular endothelial growth factor receptor-1 (VEGFR-1), and fibroblast growth factor receptor-1 (FGFR-1) receptor tyrosine kinases. In addition to its effects on the angiogenesis promoting receptors VEGF and TIE-2, ESK981 treatment also induced robust cellular vacuolization indicative of cell death by autophagy, a mechanism not observed with other multiple tyrosine kinase inhibitors like crizotinib and cabozantinib.

A phase 1 study has shown the compound to be safe and tolerable [31]. The most frequently reported adverse event of any grade was fatigue (*n* = 22, or 51 %). Other frequently reported adverse events (≥20 % of patients) were nausea (47 %), diarrhea (33 %), decreased appetite (33 %), abdominal pain (30 %), back pain (28 %), vomiting (28 %), constipation (28 %), headache (28 %), dizziness (28 %), and dyspnea (23 %). These adverse events were reported at a similar frequency between dosage cohorts, and no relationship with dose was evident. Most adverse events were grade 1 or 2. Although no enrolled patients had complete or partial response according to RECIST criteria, 19 of 37 (51 %) patients evaluated for tumor response had stable disease at ≥ 6 weeks. Of note, the majority of patients on this trial had tumor types that have been historically refractory to TKIs. The 0% response rate is similar to response rates seen when other VEGF-TKIs have been tried in non-kidney cancers such as colorectal cancer [33].

Given the significant pre-clinical rationale for use of an Ang-2 inhibitor in renal cell carcinoma that has become resistant to VEGF inhibition and the safety shown in a prior phase 1 trial, we propose a single center phase 2 trial of ESK981 in patients with metastatic renal cell carcinoma who have progressed despite treatment with a VEGF-TKI.

Nivolumab in renal cell carcinoma
Nivolumab (Opdivo) a PD-1 antibody, is FDA approved for the treatment of advanced renal cell carcinoma after disease progression on prior anti-angiogenic therapy. In the CHECKMATE-025 trial, nivolumab demonstrated improved overall survival compared with everolimus in the randomized clinical trial setting (25 months vs 19.6 months, HR 0.73, p-value 0.0018) [34].

ESK 981 in combination with anti-PD1 immunotherapy
VEGF inhibition in combination with immunotherapy in the form and anti-PD1 or anti-PD-L1 therapy has already been demonstrated in other drug combinations. Bevacizumab, an anti-VEGF therapy, in combination with atezolizumab (anti-PD-L1), was studied in ten patients with previously untreated metastatic RCC, and was well-tolerated. In this small cohort, there were 4 partial responses and 4 patients with stable disease [35]. A randomized multi-arm phase III clinical trial is currently ongoing evaluating the combination of lenvatinib, a multikinase inhibitor, with either everolimus or pembrolizumab compared with sunitinib in patients with advanced renal cell carcinoma (NCT02811861).

Given the superior activity of ESK981 compared to other small molecule VEGFR inhibitors especially potent inhibition of Tie-2 and additional autophagy inducing activity of ESK981, and the emerging clinical data on synergy between VEGFR inhibition and anti-PD1/L1 antibody immunotherapy, we propose a two-cohort phase II study. We first seek to evaluate the safety of ESK981 monotherapy in a safety cohort of 11 patients (Cohort A), after which accrual of Cohort B will begin, with a safety lead-in of six patients to establish a safe dose of ESK981 in combination with flat dose nivolumab at 480 mg IV. Subsequently, an expansion of Cohort B will be accrued to evaluate the clinical efficacy of the combination of ESK981 at the previously established safe dose in conjunction with nivolumab. Subjects in cohort A cannot cross-over into Cohort B.

2.0 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

2.1.1 To determine the clinical efficacy of ESK981 in combination with nivolumab therapy in patients with metastatic renal cell carcinoma.

2.2 Secondary Objectives

2.2.1 To assess the clinical efficacy of ESK981 monotherapy in patients with metastatic renal cell carcinoma.

2.2.2 To determine the safety and tolerability of ESK981 monotherapy and in combination with nivolumab in patients with metastatic renal cell carcinoma.

2.3 Exploratory Objective

2.3.1 To determine the quality of life of patients enrolled on the study as reflected in patient-reported outcomes.

2.4 Primary Endpoint

2.4.1 To assess the objective response rate (complete response and partial response) by RECIST 1.1 in metastatic renal cell cancer to the combination of ESK981 and nivolumab therapy.

2.5 Secondary Endpoints

2.5.1 To assess the objective response rate (complete response and partial response) by RECIST 1.1 in metastatic renal cell cancer to ESK981 monotherapy.

2.5.2 To determine frequency and severity of adverse events by CTCAE criteria of ESK981 in patients with metastatic renal cell carcinoma.

2.5.3 To evaluate secondary efficacy measures such as overall survival, progression free survival, duration of therapy, duration of response to ESK981 monotherapy in Cohort A and to the combination in Cohort B; response by combined RECIST v1.1 and irRECIST and irRECIST progression free survival (Cohort B only).

2.6 Exploratory Endpoint

2.6.1 Health-related quality of life assessment changes from baseline using EuroQoL-5D will be described using descriptive statistics.

3.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be enrolled to the study. Study treatment may not begin until a subject is enrolled. No eligibility waivers will be granted for this clinical trial.

3.1 Inclusion Criteria

3.1.1 Histologic diagnosis of renal cell carcinoma (any histology except medullary carcinoma or collecting duct carcinoma is acceptable) with radiologic or histologic evidence of metastatic disease.

3.1.2 Prior treatment with up to one (and only one) anti-VEGF or VEGFR inhibitor (small molecule or antibody).

3.1.3 Must have measurable disease as per Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) criteria.

3.1.4 Must be of age ≥ 18 years at time of informed consent.

3.1.5 Ability to understand and the willingness to sign a written informed consent.

3.1.6 Karnofsky performance status ≥ 60.

3.1.7 Most recent systemic therapy or most recent radiation therapy ≥2 weeks of first study drug dose.

3.1.8 Recovery to baseline or ≤ Grade 1 CTCAE v.4.03 from toxicities related to any prior treatments, unless AE(s) are clinically non-significant and/or stable on supportive therapy.

3.1.9 Women of childbearing potential must have a negative serum or urine pregnancy test within 28 days prior to prior to registration. Women of non-childbearing potential are defined as those who have no uterus, ligation of the fallopian tubes, or permanent cessation of ovarian function due to ovarian failure or surgical removal of the ovaries. All others are considered women of child bearing potential.

3.1.10 Adequate organ and marrow function as defined below:

System	Laboratory Value
Hematological	
Absolute Neutrophil Count (ANC)	≥ 1.5 K/mm^3
Hemoglobin (Hgb)	≥ 9 g/dL
Platelets (Plt)	≥ 100,000/mm^3
Renal	
Calculated creatinine clearance	Serum creatinine ≤ 1.5 times the upper limit of normal OR creatinine clearance >30mL/min by Cockcroft-Gault formula
Hepatic	
Total Bilirubin	≤ 1.5 × upper limit of normal (ULN)
Aspartate aminotransferase (AST)	≤ 2.5 × ULN (≤ 3 × ULN with known hepatic metastases)
Alanine aminotransferase (ALT)	≤ 2.5 × ULN (≤ 3 × ULN with known hepatic metastases)
Prothrombin time (PT) and activated partial thromboplastin time (aPTT) levels	≤ 1.5x ULN (If patient is receiving anticoagulation that is expected to alter these levels, should be in targeted therapeutic range for that agent)

3.2 Exclusion Criteria

3.2.1 Prior treatment for metastatic disease with >1 anti-VEGF/VEGFR inhibitor.

3.2.2 Prior treatment with anti-PD/PD-L1/CTLA4/IDO antibody (for Cohort B patients only) or ESK981 (for Cohort A and Cohort B patients).

- Prior mTOR inhibitors or glutaminase inhibitors are allowed.

3.2.3 Untreated brain metastases or spinal cord compression.

- Patients with suspected or known treated brain metastases at screening should have a MRI (preferred) or CT preferably with IV contrast of the brain prior to study entry. Patients whose brain metastases have been treated may be considered if they have completed their treatment for brain metastasi(e)s at least 4 weeks prior to study registration AND they show radiographic and clinical stability (by CT or MRI brain imaging, obtained after treatment to the brain metastases). In addition, any neurologic symptoms that developed either as a result of the brain metastases or their treatment must have resolved or be stable without the use of steroids at daily doses greater than 10 mg prednisone or equivalent for at least 14 calendar days prior to the start of treatment.

3.2.4 Uncontrolled hypertension defined as blood pressure >150/90 despite at least 2 anti-hypertensive medications as assessed by 2 blood pressure readings taken at least 1 hour apart during screening

3.2.5 Major surgical procedure or significant traumatic injury within 6 weeks prior to study registration. (>6 weeks prior to registration is permitted as long as they have fully recovered from any such procedure).

3.2.6 History of another primary malignancy except for: malignancy treated with curative intent and no known active disease for ≥2 years, adequately treated non-melanoma skin cancer without current evidence of active disease, adequately treated carcinoma in situ without current evidence of active disease, Gleason ≤ 6 prostate cancer.

3.2.7 Angina, myocardial infarction symptomatic congestive heart failure, cerebrovascular accident, transient ischemic attack, arterial embolism, pulmonary embolism, percutaneous angioplasty or Coronary arterial bypass surgery within the past 3 months.

3.2.8 History of gastrointestinal perforation or fistula in the past 6 months, or while previously on antiangiogenic therapy, unless underlying risk has been resolved (e.g. through surgical resection or repair).

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

3.2.10 The patient has received any investigational drug within 28 days prior to registration or 5 half-lives of the investigational drug, whichever is shorter.

3.2.11 History of bleeding disorders (e.g. pulmonary hemorrhage, significant hemoptysis, menometrorrhagia not responding to hormonal treatment) ≤ 6 weeks before Cycle 1 Day 1.

3.2.12 The patient is on a chronic daily medication known to be a severe or moderate inhibitor or inducer by Micromedex of CYP1A2, CYP2C8, or CYP3A4 at registration.

3.2.13 Systemic corticosteroids greater than the equivalent of 10 mg of prednisone or equivalent alternative steroid (except physiologic dose for adrenal replacement therapy) or other immunosuppressive agents (such as cyclosporine or methotrexate) and any other medications that could potentially impact the efficacy or safety of the study as judged by the treating investigator are NOT permitted from time of registration to subjects completing protocol therapy unless clinically indicated to manage adverse events or life threatening or serious conditions as determined by the treating investigator.

3.2.14 Have any condition that, in the opinion of the investigator, would compromise the ability of the subject to meet or perform study requirements.

4.0 SUBJECT SCREENING AND REGISTRATION PROCEDURES

Patient registration for this trial will be centrally managed by the Coordinating Center of The University of Michigan Rogel Cancer Center as described below:

A potential study subject who has been screened for the trial and who has signed the Informed Consent document will be initially documented by the participating site on a Screening and Enrollment Log.

It is the responsibility of the local site investigator to determine patient eligibility prior to submitting patient registration request to the Coordinating Center. After patient eligibility has been determined, a copy of the completed Eligibility Worksheet together with all the pertinent de-identified source documents will be submitted by the requesting site to the Coordinating Center, by email to CTSU-Oncology-Multisite@med.umich.edu.

The Multi-Site Coordinator (MSC) of the Coordinating Center will review the submitted documents and process the registration. Sites should inform the Multi-Site Coordinator of a potential registration by 5 p.m. on the day prior to registration. Same day registrations cannot be guaranteed.

An email will be sent by the MSC to the requesting site registrar to confirm patient registration and to provide the study identification number that has been assigned to the patient. In addition, a copy of the completed Eligibility Worksheet signed and dated by the MSC, will be sent back to the requesting site registrar.

Patients found to be ineligible for participation after being consented will be considered screen failures, and documented as such in a Screening and Enrollment Log. These patients will not have study identification number assigned to them, and will not receive study treatment.

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Protocol treatment must start within 14 business days of enrollment to the study.

There are 2 cohorts for accrual with Cohort A being accrued first. Cohort B will follow and will have 2 stages of accrual. Cohort A will accrue 11 patients to ESK981 monotherapy treatment. Then Cohort B (combination therapy) will begin accrual of 17 patients to the first stage and if the interim analysis permits, the second stage will accrue an additional 19 patients.

5.1.1 Therapy with ESK981 will include starting dose of 160 mg (4 capsules) PO daily for 5 consecutive days followed by a 2-day off drug in each week, repeated weekly in 28 day cycles. Patients will continue treatment until disease progression or intolerable toxicity.

REGIMEN DESCRIPTION

Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
ESK981	n/a	160 mg	PO	Once daily for 5 consecutive days of every week (5 days on followed by 2 days off)	28 days
Nivolumab (solution for injection)	Per institutional guidelines, none mandated.	480 mg/dose	IV infusion over 30-60 minutes with a sterile, nonpyrogenic, low protein binding 0.2 to 1.2 micrometer in-line filter. Follow with saline flush.	Day 1	28 days

Dose Level	Dose of ESK981	Dose of Nivolumab
Level 0	160 mg	Fixed at 480 mg flat dose
Level -1	120 mg	Fixed at 480 mg flat dose
Level -2	80 mg	Fixed at 480 mg flat dose

Dose-Limiting Toxicity (DLT)

Dose limiting toxicity (DLT) will be defined as any Grade 3 or higher toxicity (as defined by CTCAE 4.03) that occurs during the DLT evaluation period (Cycle 1 i.e. 28 days) EXCEPT laboratory abnormalities that are not clinically relevant or significant.

Subjects will be given a pill diary and instructed to complete it every cycle. Doses missed by 6 or more hours or vomited will be skipped and not made up but will be recorded on the diary as missed or vomited. The subject will be advised not to take any additional doses, but to wait for the next scheduled dose.

5.2 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events Table (Section 6.1). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

Table 5.2.1 Hematological Toxicities

Hematological Toxicity Dose Reductions for ESK981		
ANC[1]	**Platelets**	**Action**
≥ 1,500/μL or	>100,000/μL	None.
1000-1499/μL or	75,000-99,000/μL	*-1st Occurrence:* Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 1 dose lower. *-2nd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 1 dose lower if available or discontinue if not available *-3rd Occurrence:* Discontinue protocol therapy.
500-999/μL or	50,000-74,000/μL	*-1st Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 1 dose lower if available or discontinue if not available. *-2nd Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Do not replace missed doses. Restart next treatment at 1 dose lower if available or discontinue if not available. *-3rd Occurrence*: Discontinue protocol therapy.
<500/μL or	<50,000/μL	*-1st Occurrence*: Hold current dose until ANC ≥ 1,500/μL and platelets ≥ 100,000/μL. Restart next treatment at 1 dose lower if available or discontinue if not available. *-2nd Occurrence*: Discontinue protocol therapy.
[1]Note: G-CSF (Filgrastim) may be added for low ANC on day of treatment *BEFORE* a dose reduction is instituted at treating physician's discretions. Neulasta® is NOT allowed.		

Treatment-Emergent Non-hematological Toxicities: (EXCEPT non-clinically relevant/significant laboratory abnormalities):

NCI CTCAE Grade	ESK981	Nivolumab
1	No change from original starting dose	No change from original starting dose, supportive care as required
2	Hold until resolved to ≤ Grade 1, then reduce by 1 dose level if available or discontinue if not available	Hold until resolved to ≤ Grade 1, supportive care as required and then resume treatment
Second episode of grade 2 or 1st episode of ≥ grade 3 toxicity	Hold until resolved to ≤ Grade 1, then reduce by 1 dose level if available or discontinue if not available	Discontinue treatment
Third episode of grade 2 or 2nd episode of ≥ grade 3 toxicity	Discontinue treatment	

Note: The drug modifications for toxicity only apply to the drug the toxicity is attributed to (i.e. the offending drug), and the other drug could be continued without modification.

Note: Any drug holds for toxicity can continue for a maximum duration of 12 weeks. Beyond that, the offending drug shall be permanently discontinued. The other drug can be continued.

5.3 Concomitant Medications/Treatments

Systemic corticosteroids greater than the equivalent of 10 mg of prednisone or equivalent alternative steroid (except physiologic dose for adrenal replacement therapy) or other immunosuppressive agents (such as cyclosporine or methotrexate) and any other medications that could potentially impact the efficacy or safety of the study as judged by the treating investigator are NOT permitted from time of registration to subjects completing protocol therapy unless clinically indicated to manage adverse events or life threatening or serious conditions as determined by the treating investigator.

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than ESK981 or checkpoint inhibitor immunotherapy
- Neulasta®
- Live vaccines within 30 days prior to the initial study treatment administration through the last dose of study treatment. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, intranasal influenza, rabies, BCG (tuberculosis vaccine), and typhoid vaccine.
    - If precluded by local regulations, live vaccines should not be given for 120 days after the last dose of checkpoint inhibitor immunotherapy is administered.
- Systemic glucocorticoids or other immunosuppressive drugs for any purpose other than to modulate symptoms from a drug-related AE of immunologic etiology (refer to Section 5.2 – Dose Modification). The use of physiologic doses of corticosteroids may be approved after consultation with the Study PI.
    - Use of prophylactic corticosteroids to avoid allergic and other adverse reactions (e.g., to IV contrast dye or transfusions) is permitted.
    - Use of intermittent inhaled steroids or local injection of corticosteroids is permitted upon consultation with the Principal Investigator.
    - Physiologic doses of prednisone ≤10 mg (or equivalent) per day.

5.4 Other Modalities or Procedures

No other modalities or procedures will be used in this protocol.

5.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the criteria listed in Section 5.6 apply. Patients will be followed for up to 24 months after removal from treatment or until death, whichever occurs first. Patients removed from treatment for unacceptable adverse events will be followed by phone call or clinic visit until resolution or stabilization of the adverse events.

5.6 Off Treatment Criteria

Patients can be taken off study treatment at any time at their own request with a withdrawal of informed consent, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation from study treatment will be documented and may include:

- Disease progression as defined in Section 7.0 or evidence of clinical progression as determined by treating investigator;
- Subject has intolerable toxicity from study therapy despite supportive measures and maximum permitted dose modifications and holds per protocol;
- Patient withdraws informed consent (termination of treatment and follow-up for any reason);
- Loss of ability to freely provide informed consent through imprisonment or involuntary incarceration for treatment;
- Patient is unable to comply with protocol requirements;
- Treating physician determines continuation on the study would not be in the patient's best interest;
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin or Gleason ≤ 6 prostate cancer) that requires treatment, which would interfere with this study;
- Lost to Follow-up. If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented;
- Termination of the study by the University of Michigan;
- Patient completes protocol treatment and follow-up.

5.7 Patient Replacement

Patients who are not evaluable for objective response (as defined in Section 7.1.1) will be replaced.

6.0 SCHEDULE OF ASSESSMENTS

6.1 Time and Events Table

1 cycle (C) = 28 days (D).

Trial Period	Screening (-28 to -1 days)	C1D1	C2D1	C3D1	C4D1	Additional Cycles…	EOT[7]	Follow-Up[8]
							30 days from last dose (+/- 7 business days)	Every 12 weeks (+/-14 business days) for up to 24 months/death
Informed Consent	X							
Tumor tissue specimen (15 FFPE slides required; blocks preferred) identified	X							
Blood for banking		X						
History, PE, Concomitant Meds, Vital Signs[1]	X	X	X	X	X	X	X	
PT/INR, aPTT	X							
Karnofsky Performance Status	X	X	X	X	X	X	X	
Pregnancy Test for WOBCP[2]	X							
Toxicity (include DLT) Evaluations[12]	X	X	X	X	X	X	X	
CBC with differential, platelets[3]	X	X[13]	X[13]	X	X	X	X	
COMP[4]	X	X	X	X	X	X	X	
TSH	X	Every 12 weeks from Cycle 1 Day 1 [5]						
QoL (EQ-5D-5L)		X	X	X		X[14]		X
Pill Diary collection/handing out		X	X	X	X	X		
Tumor response assessment [6] (CT chest, CT or MRI abdomen/pelvis; CT or MRI brain if clinically indicated)	X			X		X [6]		X
Treatment								
ESK981[9, 10] (Cohort A and B)		X	X	X	X	X		
Nivolumab[11] (Cohort B only)		X	X	X	X	X		
Survival Status								X

1. Vital signs will include weight, temperature, pulse, respirations, blood pressure; height will be obtained at screening only.
2. WOCBP: Women of child bearing potential; either urine or serum pregnancy test.
3. CBC with diff includes total WBC, hemoglobin, hematocrit and differential of the WBC including absolute counts. Other checks are at discretion of treating investigator.

4. COMP or Comprehensive metabolic profile includes sodium, potassium, chloride, bicarbonate, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin.
5. TSH: Every 12 weeks from Cycle 1 Day 1 (i.e. Cycle 4 Day 1; Cycle 7 Day 1, etc.).
6. Window of +/- 7 business days. Imaging will be with or without intravenous contrast, before or on associated visit. CT or MRI brain with (preferred) or without IV contrast if suspected or known brain metastases. Frequency of tumor assessments will be q2 cycles (i.e. q 8 weeks) for first 4 assessments beyond baseline and then q 3 cycles (i.e. 12 weeks) subsequently until withdrawal of consent/death/2 years from C1 D1 whichever is later. If CR/PD, should be confirmed with repeat imaging after an interval of at least 4 weeks. In follow-up, if subjects are on alternative therapies, no mandated imaging but data from routine imaging will be captured at least every 12 weeks if available (+/- 7 business days).
7. End of treatment visit may or may not be the same day as a planned study visit. If it is not on a planned study visit, the minimum assessments listed in the schedule must be completed.
8. Follow-up may be conducted by phone call or a clinic visit.
9. Study drug(s) must be started within 14 business days from time of enrollment.
10. ESK981 will only be given on days 1-5 of each week of each treatment cycle followed by 2 days off.
11. Nivolumab will be administered on Day 1 of each treatment cycle in Cohort B.
12. All Serious Adverse Events (SAEs) occurring from the initial study treatment administration through 60 days following the last dose of the study treatment should be reported to the Coordinating Center. Any SAEs occurring after 60 days following the last dose of the study treatment that are believed to be related to study drug should also be reported to the Coordinating Center.
13. CBC with diff must be obtained weekly during Cycles 1 and 2. For patients who experience no ≥ Grade 3 hematological toxicity during Cycles 1 and 2, monthly blood counts will be obtained during subsequent cycles.
14. QOL obtained Cycles 1-3, 5, 7 and 9 then q 3 cycles (12 weeks) subsequently

NOTE:
All assessments have a window of ± 3 business days unless otherwise mentioned.

7.0 MEASUREMENT OF EFFECT

7.1 Antitumor Effect- Solid Tumors

Immunotherapy drugs such as nivolumab can initially cause inflammation in the early stages of treatment. Immune-related RECIST (irRECIST) utilizes RECISTv1.1 but considers an inflammatory response (or “pseudo-progression”) as normal. The main difference between RECISTv1.1 and irRECIST is that patients can stay on trial after the first progressive disease (PD) assessment (as per RECISTv1.1) if using immune-related RECIST criteria. This PD per RECISTv1.1 is then re-labeled as immune related stable disease (irSD) per irRECIST and requires addition of unidimensional measurements of all new lesions (that meet the definition of target lesion) to be added to the sum of longest diameters (SLD) calculation for response assessment. Importantly, immune-related progression (irPD) must be confirmed by a follow-up scan at least 4 weeks (within 4-8 weeks) following the initial PD/irSD assessment in order to take the patient off the trial.

Subjects that are deemed to have clinical progression and/or unstable should not be continued on therapy after PD (per RECISTv1.1) and are therefore not required to have repeat tumor imaging for confirmation as per irPD definition. It is at the discretion of the treating investigator whether to continue a subject on study treatment until repeat confirmatory imaging is obtained. This clinical judgment decision by the site investigator should be based on the subject’s overall clinical condition, including performance status, clinical symptoms, and laboratory data.

7.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity if they have received at least 1 dose of ESK981.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least 2 cycle(s) of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their

response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 2 will also be considered evaluable.)

7.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (irrespective of scanner type) for studies with a slice thickness of ≤5mm or twice the slice thickness or MRI
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20mm by chest X-ray (if clearly defined and surrounded by aerated lung)

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Note: Tumor lesions that are situated in a previously irradiated area will only be considered measurable, if they have had subsequent progression by at least 5 mm.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (<10 mm using CT scan), are considered non-measurable disease. Bone lesions without measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organ, but in addition should be those that lend themselves to reproducible repeated measurements.

> For Cohort B only: If a non-nodal lesion is either not present or is initially measured with longest diameter <10mm as a non-target then grows to ≥10mm after baseline, this lesion then becomes a new target lesion as per irRECIST criteria. The non-nodal longest diameter is then added to the sum of diameters, and patient response is calculated with the new lesion.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20mm x 30mm has a short axis of 20mm and qualifies as a malignant, measurable node. In this example, 20mm should be recorded as the node measurement. All other pathological nodes (those with short

axis ≥10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered nonpathological and should not be recorded or followed.
A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

For Cohort B only: If a non-target lymph node grows to ≥15mm after baseline, this node then becomes a new target lesion as per irRECIST. The nodal short axis is then added to the sum of diameters, and patient response is calculated with the new lesion.

Non-target lesions. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

7.1.3 Guidelines for Evaluation of Measurable Disease

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and >10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study,

confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response.

7.1.4 Response Criteria

7.1.4.1 Evaluation of Target Lesions

Patients will be evaluated according the following RECISTv1.1 response:
Complete Response (CR): Disappearance of all target lesions, determined by two separate observations conducted not less than 4 weeks apart. There can be no appearance of new lesions.

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. There can be no appearance of new lesions.

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions (with a minimum absolute increase of 5 mm), taking as reference the smallest sum LD recorded since the treatment started, or the appearance of one or more new lesions.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR (taking as reference the baseline sum LD) nor sufficient increase to qualify for PD (taking as reference the smallest sum LD since the treatment started).

For Cohort B only:
After the first PD assessment per RECISTv1.1 (=irSD per irRECIST), patients will be evaluated for irPD at least 4 weeks apart according to the following definition:

Immune-related Progressive Disease (irPD): At least a 20% increase in the sum of the LD of target lesions (with a minimum absolute increase of 5 mm), taking as reference the smallest sum LD recorded since the treatment started, or appearance of new lesions since the last evaluation.

7.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions. All lymph nodes should be non-pathological in size (<10 mm short axis)

Non-CR/Non-OD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Although a clear progression on non-target lesions in absence of stable target lesions is exceptional, *the opinion of the treating physician should prevail in such circumstances*.

7.1.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Evaluation as per combined RECISTv1.1/irRECIST

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response per RECISTv1.1**	**Overall Response per irRECIST** ***(Cohort B only)***	**Confirmed Response for this Category Requires:**
CR	CR	No	CR	NA	≥4 wks. confirmation
CR	CR Non-CR/PD	No	PR	NA	≥4 wks. confirmation
PR	CR Non-CR/PD	No			
SD	CR Non-CR/PD	No	SD	NA	Documented at least once ≥4 wks. from baseline
PD	Any	Any	PD	irSD	≥4 wks. confirmation
Any	PD*	Any			
Any	Any	Yes			
PD	Any	Any	NA	irPD	No further confirmation required
Any	PD*	Any			
Any	Any	Yes			

* Only in exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

NA=not applicable

Note: If subjects respond to treatment and are able to have their disease resected, the patient's response will be assessed prior to the surgery.

7.1.4.4 Treatment Beyond Progression – Cohort B only

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD.

Subjects treated on Arm B will be permitted to continue study treatment beyond initial RECISTv1.1 defined PD, assessed by the investigator, as long as they meet the following criteria:

- Investigator determined clinical benefit
- Tolerance of study drug
- Stable performance status

- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g., CNS metastases)

A radiographic assessment/ scan should be performed within 4-8 weeks of initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued PD (termed irPD). The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment.

If the investigator feels that the subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the Time and Events Table (Section 6.1).

Immune-related Progressive Disease (irPD): For the subjects who continue study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden with a minimum 5 mm absolute increase from time of initial PD, unequivocal worsening of NT lesions, or appearance of new lesions since the last evaluation. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Study treatment should be discontinued permanently upon documentation of further progression (i.e. irPD).

7.1.5 Duration of Response

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

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

7.1.6 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, the event that occurs first.

7.2 Safety/Tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4.03 for reporting of non-hematologic adverse events (https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf) and modified criteria for hematologic adverse events.

8.0 ADVERSE EVENTS

8.1 ESK981 (formally known as CEP-11981)

8.1.1 Contraindications

Because of the potential for drug-drug interaction, the concurrent use of all other drugs, over-the-counter medications, and alternative therapies must be documented in the patient's record.

8.1.2 Special Warnings and Precautions for Use

ESK981 is metabolized via CYP1A2, CYP2C8, and CYP3A4. Inhibitors of CYP1A2, CYP2C8, and CYP3A4 have the potential to increase plasma concentration of ESK981. Inducers of CYP1A2, CYP2C8, and CYP3A4 have the potential to decrease plasma concentrations of ESK981. Concomitant use of a medication known to be a potent inhibitor of CYP1A2, CYP2C8, or CYP3A4, or a potent inducer of CYP1A2, CYP2C8, or CYP3A4 should be avoided unless deemed to be medically necessary by the investigator. In addition, nonclinical studies have identified the potential for ESK981 to inhibit CYP3A4/5 (Ki 2.2μM). ESK981 has the potential to increase plasma concentration of concomitant mediations that are CYP3A4/5 substrates. Caution should be used, or alternative treatments considered, if concomitant treatment with CYP3A4/5 substrates that have a narrow therapeutic range is needed (see Appendix 2).

Adverse Reactions

- **Side effects:** The most frequently reported AE of any grade was fatigue (51%). Other frequently reported AEs (≥ 20% of patients) were nausea (47%), diarrhea (33%), decreased appetite (33%), abdominal pain (30%), back pain (28%), vomiting (28%), constipation (28%), headache (28%), dizziness (28%), and dyspnea (23%). These AEs were reported at a similar frequency between dosage cohorts, and no relationship with dose was evident.

- **Treatment-Emergent Adverse Events of Grades 3, 4, and 5 Severity:** Grade 3 or Grade 4 AEs occurred in 37% of patients across dosing cohorts ([32.6%] Grade 3 and [4.7%] Grade 4). Treatment-related Grade 3 or Grade 4 AEs were most frequent in the 97.4 mg/m2 cohort. Grade 3 or Grade 4 laboratory hematologic toxicities were reported in 18.6 % of patients across dosage cohorts. The most common Grade 3 or Grade 4 laboratory hematologic toxicity was lymphopenia, which occurred across dosage cohorts (5.9, 29.6, 55.0, 97.4, and 126.6 mg/m2). Grade 4 leukopenia occurred in a single patient in the 126.6 mg/m2 cohort. Grade 3 or Grade 4 neutropenia also occurred in two patients in the 97.4 mg/m2 cohort (Grade 3) and in one patient in the 126.6 mg/m2 cohort (Grade 4). No Grade 5 AEs occurred.

- **Serious Adverse Events**
  Serious adverse events (SAEs) occurred were deemed unlikely or not related to ESK981. Three patients (1 patient in the 97.4 mg/m2 cohort and two patients in the 126.6 mg/m2 cohort) experienced SAEs that were categorized as possibly or definitely related to ESK981: pyrexia, hemolytic anemia, hyperbilirubinemia, dyspnea, neutropenia, ECG change, and chest discomfort. There were no deaths occurred during the study.

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

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

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

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

8.2 Nivolumab

Refer to the current package insert for nivolumab contraindications and adverse reactions.

8.3 Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial and is done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Data on adverse events will be collected from the time of the initial study treatment administration through 60 days after the last dose of study treatment.

In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins study treatment is also considered an adverse event.

8.4 Definitions

8.4.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

- *Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event unless it is a pre-existing (prior to protocol treatment) condition.*

- *Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or require therapy. They are to be captured under the signs, symptoms or diagnoses associated with them.*

8.4.2 Serious Adverse Event

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

- Death
  If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- A life-threatening adverse event
  An adverse even is considered ‘life-threatening’ if, in the view of either the investigator [or sponsor], its occurrence places the patient or subject at immediate risk of death. It

does not include an adverse event that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical event
  Any event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of "Serious Adverse Event". Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that do not result in inpatient hospitalization or the development of drug dependency or drug abuse.

Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

8.4.3 Expected Adverse Events

An adverse event (AE) is considered "expected" if:

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs or devices, those adverse events are described in the FDA Investigator's Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document.

8.4.4 Unexpected Adverse Event

An adverse event (AE) is considered "unexpected" if it is not described in the Package Insert, Investigator's Brochure, in published medical literature, in the protocol, or in the informed consent document.

8.5 Adverse Event Characteristics

8.5.1 CTCAE Term

(AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.03. A copy of the CTCAE version 4.03 can be down loaded from the CTEP web site. (https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

8.5.2 Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.
Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

8.6 Serious Adverse Event Reporting Guidelines

All serious adverse events (SAEs) and unanticipated problems (UPs), regardless of causality to study drug, will be reported to the Principal Investigator and also to the Coordinating Center. All SAEs and UPs must be reported to the Coordinating Center within 24 hours of first awareness of the event. Events should be reported using the Coordinating Center SAE form as available in the study database. A copy of the Coordinating Center SAE form as available in the study database should be sent to the Coordinating Center via fax at 734-232-0744 or via email to CTSU-Oncology-Multisite@med.umich.edu within 24 hours of the site's knowledge of the event.

Follow-up information should also be reported within 24 hours of receipt of the information by the investigator.

All SAEs and UPs will be reported to the IRB per current institutional standards.

The Coordinating Center will disseminate information regarding SAEs and UPs to the participating sites within 5 days of review of the information by the Coordinating Center's Principal Investigator (or designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) to the study drug. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

8.6.1 Reporting procedures to Esanik

All Serious Adverse Events (SAEs) occurring from the initial study treatment administration through 60 days following the last dose of the study treatment will be reported by the Coordinating Center to Esanik. Any SAEs occurring after 60 days following the last dose of the study treatment that are believed to be related to study drug will also be reported to Esanik.

The Coordinating Center will send the initial completed SAE Form within 24 hours of receipt via email to Esanik:

Email: drugsafety@esanik.com. The Coordinating Center will confirm receipt of the email by calling 858-693-9158

If only limited information is initially available or an ongoing SAE changes in its intensity or relationship to the study drug, or if new information becomes available, a follow-up report will be generated and sent to Esanik within 24 hours of receipt.

8.6.2 Reporting procedures to the FDA

In this trial, serious unexpected adverse events believed to be definitely, probably or possibly related to the study treatment will be reported to the Food and Drug Administration via the MedWatch 3500A. The Michigan IND/IDE Assistance Program (MIAP) (University of Michigan) will assist the IND Sponsor in reporting SAEs to the FDA that meet the reporting

requirements in 21 CFR 312.32. This reporting could include the initial report and follow-up reports when appropriate for the event.

All correspondence to the FDA by sponsor-investigator should be provided to Esanik.

8.7 Routine Reporting

All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

8.8 Reporting of Unanticipated Problems

There are types of incidents, experiences and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience or outcomes is considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);
2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report it to the Coordinating Center within 2 calendar days of the study team becoming aware of the problem and report to their local IRB as per institutional guidelines.

8.9 Early Stopping Rules

See section 11.3 for Early Stopping Due to Toxicity.

9.0 DRUG INFORMATION

9.1 ESK981

- Other names for the drug: formerly known as CEP-11981
- Description: ESK981 drug product is an off-white to orange opaque, hard gelatin capsule containing 1 mg, 5 mg, 20 mg or 40 mg (free base equivalents) of ESK981 monotosylate. The drug product is packaged in a high-density polyethylene (HDPE) bottle with a child resistant closure and an induction-sealed inner seal. The formulation also contains lactose monohydrate.
- Classification - type of agent: Small molecular inhibitor of multiple tyrosine kinases such as vascular endothelial growth factor receptor 1 (VEGFR-1), VEGFR-2, and Tie-2, (IC50 of 3, 4, 22nM, respectively)

- Pharmacokinetics:

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

The pharmacokinetic, ADME, and toxicokinetic characteristics of CEP-11981 have been extensively studied in Sprague Dawley rats and cynomolgus monkeys after intravenous and oral doses and preliminarily in Balb/c nude mice after oral doses. In order to facilitate comparisons between studies and species, all doses are presented in this summary as milligrams of CEP-11981 free base per kilogram of body weight, regardless of the form administered, unless specifically noted otherwise.

The intravenous dose in rats was administered in 3% dimethylsulfoxide, 30% Solutol®, and 67% phosphate-buffered saline at a volume of 1 mL/kg. In monkeys, the intravenous vehicle was 47.4% polyethylene glycol (PEG) 400, 31.1% water, 20.7% hydroxy-β-cyclodextrin (HPBCD), and 0.8% Pluronic® F-68 at a volume of 0.5 mL/kg. Pharmacokinetic parameters obtained after single intravenous (bolus) administration of CEP-11981 are summarized in Table 3.

Table 3. Pharmacokinetics After Single Intravenous Administration of ESK981

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

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

In rats, the systemic clearance is low relative to the estimated total hepatic plasma flow; the volume of distribution is approximately that of total body water. There are apparent sex-related differences in the pharmacokinetics of CEP-11981 in rats, with lower clearance and longer half-life in female rats than in male rats. The volume of distribution is numerically less in female rats, but the significance of this difference is doubtful.

In monkeys, the half-life is similar to that in rats, but the systemic clearance is substantially larger than that in rats, as is the volume of distribution. The difference in clearance is consistent with the higher rate of metabolism of CEP-11981 in incubations with monkey liver microsomes than with rat liver microsomes. The origin of the larger volume of distribution in monkeys is not known.

Pharmacokinetic parameters obtained after single, low-dose oral administration of CEP-11981 are summarized in Table 4.

Table 4. Pharmacokinetics After Single Oral Administration of CEP-11981

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

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

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

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

The effect of fed versus fasted state on the pharmacokinetics of CEP-11981 was assessed in a preliminary study in rats. Cmax and AUC0-6 were minimally affected by the fed/fasted state of the animals, but the tmax was shifted from 2.7 to 5.3 hours post dose when the animals had been fed before dosing.

At higher oral doses of CEP-11981, there was evidence of dose-dependent pharmacokinetics in all 3 species. In mice, single-dose systemic exposure increased in a slightly more than dose-proportional manner between 1 and 10 mg/kg, but did not show consistent further increase at 20 or 30 mg/kg. After 23 daily doses, the dose response and concentrations were similar to those after single doses at 1, 3, or 10 mg/kg, but were approximately 2 times higher in the 20- and 30-mg/kg groups on day 23 versus day 1 of dosing. Systemic exposure after multiple daily doses of 20 and 30 mg/kg was dose-related, although not dose-proportional, relative to that at 10 mg/kg.

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

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

Due to changes in the form of CEP-11981 that was obtained from successive synthetic campaigns, a series of single-dose oral studies were conducted in rats and monkeys to which different forms of the compound and/or different dosage regimens were administered. The outcome of those studies indicated that milled CEP-11981 tosylate, administered as a suspension in aqueous methylcellulose, provides systemic exposures that are in the targeted ranges in both species, and it is that form with which the

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

The tissue distribution of [14C]-CEP-11981 was studied in male albino (Sprague Dawley) and pigmented (Lister hooded) rats after single doses orally at 10 mg/kg and intravenously at 1 mg/kg, respectively, as the tosylate salt. Radioactivity was broadly distributed, with highest concentrations (exclusive of the gastrointestinal tract) in the liver, kidney, and adrenal gland.

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

CEP-11981 is extensively metabolized in vitro by liver microsomes from male Swiss CD1 mice, male Sprague Dawley rats, male New Zealand rabbits, male beagle dogs, male cynomolgus monkeys, and humans. The relative order of loss of parent compound during 20 minutes of incubation was: monkeys > humans > mice ≈ dogs > rats ≈ rabbits.

Incubations with individual recombinant human CYP enzymes suggested that 3 enzymes, i.e., CYP1A2, CYP2C8, and CYP3A4, may play significant roles in the metabolic elimination of CEP-11981. Other CYP enzymes that were also capable of metabolizing the compound in vitro are either minimally expressed in adult human liver (i.e., CYP1A1 and CYP3A7) or are polymorphic, being expressed in a minority of individuals (i.e., CYP3A5). The results of experiments assessing the effect of CYP-specific inhibitors on loss of CEP-11981 during incubation with human liver microsomes also identified CYP3A enzymes as playing a key role in metabolic elimination of the compound.

After oral administration of [14C]-CEP-11981, radioactivity was predominantly recovered in the feces in both rats and monkeys, with less than 1% of the radiochemical dose recovered in urine of either species. Metabolic profiling was hence conducted in rat bile, in which parent [14C]-CEP-11981 was the most prominent radioactive component, especially in the female rat.

The most prominent metabolite in both sexes, but especially in the male, was a monohydroxylated derivative. Overall, the reactions previously observed in vitro, i.e., ring hydroxylation, N-demethylation, and reduction (didehydrogenation) accounted for all of the CEP-11981-derived compounds detected in vivo.

The potential for CEP-11981 to affect the metabolic elimination of co-administered drugs was tested in vitro through examination of its capacity for induction of CYP enzymes activities in primary human hepatocyte cultures and for inhibition of CYP activities in human hepatic microsomal preparations.

The levels of mRNA for CYP1A1, CYP1A2, CYP2C9, and CYP3A4 were measured after incubation of CEP-11981 with hepatocytes for 30 hours at concentrations of 1 to 30 μM. No indication of induction was obtained, but suppression of CYP2C9 and CYP3A4 message was observed at the highest concentration. However, due to apparent cellular toxicity at that concentration, this result was likely an artifact.

CEP-11981 did not inhibit the activities of CYP2B6, CYP2C9, CYP2C19, CYP2D6, or CYP4A9/11. Marginal inhibition of CYP1A2 was observed, but with an estimated Ki that is higher than exposures expected clinically. CYP3A4/5 activity was inhibited with a Ki of 2.6 and 2.2 μM for the 2 reactions examined, i.e., testosterone 6β-hydroxylation and midazolam 1′-hydroxylation, respectively. Much of the inhibition was reversible and competitive in nature, with a metabolism-dependent component, but at least some of the inhibition was irreversible for both reactions tested.

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

The longest nonclinical safety study conducted to date in rats was a 4-week oral toxicity study at daily doses of 0 (vehicle only), 5, 10, 20 (lowered to 15 on day 8), and 40 mg/kg. CEP-11981 was administered as suspensions of its tosylate salt in Ora-Plus oral suspending vehicle. Key toxicokinetic parameters from the study are tabulated in Table 5.

Table 5. Toxicokinetics Results for the 4-Week Oral Toxicity Study in Rats

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

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

As noted in the pharmacokinetic studies, the systemic exposures were higher in female rats than in male rats and, in groups having both single-dose and multiple-dose datasets, were higher on day 28 than on day 1. Values of C_{max} and AUC were dose-related over the dose range tested, but increases in AUC were more than dose-proportional over portions of the dose range, suggesting that 1 or more of the pharmacokinetic characteristics of CEP-11981 are dose-dependent.

Two 4-week oral toxicity studies of CEP-11981 have been conducted in monkeys. In the first study (study DS-2006-020), daily doses of 0 (vehicle only), 5, 10, and 25 mg/kg were administered as suspensions of the free base in Ora-Plus oral suspending vehicle. Due to a subsequent change in the form of the compound to its tosylate salt, a second 4-week study (study DS-2006-042) was conducted testing daily doses of 0 (vehicle only) and 3 mg/kg. Key toxicokinetic parameters from the study are tabulated in Table 6.

Table 6. Toxicokinetics Results for the 4-Week Oral Toxicity Studies in Monkeys

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

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

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

Overall, the pharmacokinetic and ADME properties of CEP-11981 have been studied in vitro using blood and tissue fractions from mice, rats, rabbits, dogs, monkeys, and humans and in vivo in mice, rats, and monkeys. The estimated half-life in rats and monkeys after an intravenous dose is approximately 2 to 3 hours. Despite its low aqueous solubility, CEP-11981 is generally well absorbed in mice, rats, and monkeys at dose levels associated with pharmacologic responses in preclinical disease models. At higher doses, evidence of dose-dependent pharmacokinetics has been obtained in all 3 species. After an oral or intravenous dose, [14C]-CEP-11981-derived radioactivity is broadly distributed into tissues, generally at concentrations higher than those in blood. Only in barriered tissues, e.g., brain and testis, are concentrations lower than those in blood. The compound is extensively metabolized, but is also excreted in rats via the bile as the parent drug. Excretion is predominantly in the feces in both rats and monkeys.

- Drug Interactions: Prohibited before enrollment and during administration of study treatment

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

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

- Preparation and Dispensing:
  ESK981 is supplied in 28 count HDPE bottles.

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

  ESK981 drug product is an off-white to orange opaque, hard gelatin capsule containing 40 mg (free base equivalents) of ESK981 monotosylate. The drug product is packaged in a high-density polyethylene (HDPE) bottle with a child resistant closure and an induction-sealed inner seal. The formulation also contains lactose monohydrate.

- Administration:
  Treatment will be administered on an outpatient basis. Study drug will be affixed with a clinical label in accordance with regulatory requirements. This trial is open-label; therefore, the subject, the study team, the Sponsor and/or designee are not blinded. Treatment (name, strength or potency) is included in the label text;

  Subjects will be given a one-month (28 day) supply of study drug. Subjects will be instructed to take 4 capsules per, with or without food, day for 5 consecutive calendar days, then take a drug holiday for 2 consecutive days before repeating the 5 days on-2 days off cycle in sets of 4 weeks or 28 calendar days. Subjects will be asked to keep a pill diary noting the date they take their study drug. They will be asked to bring their pill diary to each study visit along with all used and unused study drug containers.

- Availability:
  Provided by Esanik Therapeutics, Inc.
  Under no circumstance will the study medication *ESK981* be used other than as directed by the protocol.

- Return and Retention of Study Drug:
  The investigator is responsible for keeping accurate records of the study drug received from the Sponsor or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

  A member of the study team will review the pill dairy during each visit study drug is returned and discuss compliance or other concerns the subject may have.

  The investigator or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate.

  Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

- Drug Accountability:
  The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug ESKI981. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

9.2 Nivolumab

- Other names for the drug: Opdivo
- Description: Injection: 40 mg/4 mL and 100 mg/10 mL solution in a single-dose vial.
- Classification - type of agent: immunomodulatory; checkpoint inhibitor
- Mode of action:
  Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

- Pharmacokinetics:
  Nivolumab pharmacokinetics (PK) was assessed using a population PK approach for both single-agent OPDIVO and OPDIVO with ipilimumab.

  OPDIVO as a single agent: The PK of single-agent nivolumab was studied in patients over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses of OPDIVO every 2 or 3 weeks. Nivolumab clearance decreases over time, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline values of approximately 24.5% (47.6%) resulting in a geometric mean steady state clearance (CLss) (CV%) of 8.2 mL/h (53.9%); the decrease in CLss is not considered clinically relevant. The geometric mean volume of distribution at steady state (Vss) (CV%) is 6.8 L (27.3%), and geometric mean elimination half-life (t1/2) is 25 days (77.5%). Steady-state concentrations of nivolumab were reached by approximately 12 weeks when administered at 3 mg/kg every 2 weeks, and systemic accumulation was approximately 3.7-fold. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks.

- Side effects:
  OPDIVO as a single agent: fatigue, rash, musculoskeletal pain, pruritus, diarrhea, nausea, asthenia, cough, dyspnea, constipation, decreased appetite, back pain, arthralgia, upper respiratory tract infection, and pyrexia.

- Drug Interactions:
  No formal pharmacokinetic drug-drug interaction studies have been conducted with OPDIVO.

- Storage and stability:
  The product does not contain a preservative. After preparation, store the OPDIVO infusion either:
  • at room temperature for no more than 8 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or
  • under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of

infusion preparation. Do not freeze.

- Preparation and Dispensing:
  Visually inspect drug product solution for particulate matter and discoloration prior to administration. OPDIVO is a clear to opalescent, colorless to pale-yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.
  • Withdraw the required volume of OPDIVO and transfer into an intravenous container.
  • Dilute OPDIVO with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL.
  • Mix diluted solution by gentle inversion. Do not shake.
  • Discard partially used vials or empty vials of OPDIVO.

- Administration:
  Administer the infusion over 30-60 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer). Do not coadminister other drugs through the same intravenous line. Flush the intravenous line at end of infusion.

- Availability**:** Commercially available

- Return and Retention of Study Drug:
  • Discard partially used vials or empty vials of OPDIVO.

- Drug Accountability:
  The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the drug Nivolumab. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

10.0 SPECIAL STUDIES

10.1 Sample Collection Guidelines

Refer to Section 6.0 for tumor tissue and blood collection time points and Lab Manual for collection and processing details.

10.2 Specimen Banking

Patient blood and tissue samples collected for this study will be retained at the University of Michigan. Specimens will be stored indefinitely or until they are used up. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens.

Specimen being stored long-term for potential use or not outlined in the protocol will be stored and used in compliance with the University of Michigan policy University Policy Governing Tissue Sample Collection, Ownership, Usage, and Disposition within all UMMS Research Repositories - https://research.medicine.umich.edu/office-research/biorepository/governance-policies.

11.0 STATISTICAL CONSIDERATIONS

The primary objective of the study is to determine the efficacy of ESK981 in combination with nivolumab therapy in patients with metastatic renal cell carcinoma who may have progressed despite treatment with one VEGF-TKI. Response rate, defined as the sum of complete + partial response (CR+PR), as measured

by RECIST v1.1 in Cohort B (combined therapy with ESK981 and Nivolumab) will be the primary statistical endpoint.

11.1 Study Design/Study Endpoints

A two-stage Mini-max Simon design will be used to minimize the number of patients to be treated with the combination therapy (Cohort B). We assume that this combination will not be of further interest if the true response rate were less than 0.25 and assume that a true response rate of at least 0.45 would be of clinical interest. This phase II study design permits early termination of patient entry due to futility of efficacy after the first 17 objective response evaluable patients have been accrued to Cohort B if there are 4 or less responses. If the study passes the first phase, then a second stage of 19 more objective response evaluable patients will be accrued for a total of 36 objective response evaluable patients. If 14 or more patients out of 36 have a response, then the treatment combination will be deemed successful. This trial design has 80% power to detect a response rate of 45% compared to a null rate of 25% assuming a 5% type I error. The probability of early termination with this design is 0.57 if the true response rate is 25%.

A single stage design will be used to determine efficacy of monotherapy treatment (Cohort A) with ESK981 efficacy in patients with metastatic renal cell carcinoma will be assessed as the RECIST v1.1 response rate. Assuming a response rate of clinical interest with monotherapy is 25% and null response proportion of 5% with a 1-sided type I error=0.102 there is 80% power with 11 patients to detect a response rate of 25%. Additionally, a futility analysis assuming a response rate of 25% would have 5% probability of 0 responses out of 11 patients. If 3 or more patients have a response with monotherapy ESK981, then the monotherapy treatment will be deemed of interest in a future study.

Secondary endpoints of the trial include assessment of toxicity in all patients who receive any study treatment. We will evaluate overall survival, progression free survival, duration of therapy, duration of response and QoL.

11.2 Sample Size and Accrual

There are 2 cohorts for accrual with Cohort A being accrued first. Cohort B will follow and will have 2 stages of accrual. Cohort A will accrue 11 patients to monotherapy treatment. Then Cohort B will begin accrual of 17 patients to the first stage and if the interim analysis permits, the second stage will accrue an additional 19 patients. Our final sample size if all stages accrue will include 47 total objective response evaluable patients (11+17+19). We expect to accrue 1-2 patients per month for a total accrual period of 24 – 30 months.

11.3 Early Stopping Due to Toxicity

Toxicity will be assessed in each cohort. If the DLT proportion is >30% during cycle 1 in Cohort A then dosing as is planned for the combination cohort will require modification before Cohort B can begin. Accrual of Cohort B will not begin until toxicity assessment of Cohort A is complete.

Cohort B will assess DLT proportions in the first 6 patients. If 2 or more DLTs occur in the first 6 patients of Cohort B, then dosing in the combination treatment in Cohort B will be decreased one dose level. If a decreased dose level is opened, assessment of another 6 patients will be assess for DLTs; if 2 or more DLTs occur then the trial will be halted. Accrual will be held during toxicity evaluation.

11.4 Data Analyses Plans

The primary analysis of efficacy will report the number of patients with an objective response and the associated proportion with the corresponding 95% exact binomial confidence interval separately in each cohort. In Cohort B, if stage 2 is initiated then the efficacy analysis methods will be adjusted for the interim check as described by Koyama and Chen *[36]*.

Toxicity will be described by grade, attribution, and body system using counts and proportions. Toxicity will be described separately for each cohort.

Overall survival, progression free survival, duration of therapy, and duration of response will be displayed using a Kaplan-Meier figure and the associated median (if reached), one-year and two-year estimates with 95% confidence intervals using Greenwood's formula will be reported if there is censoring. If there is no censoring in the endpoint, then the standard mean (or median) and 95% confidence interval (or 5th and 95th percentiles) will be reported. Each endpoint will be reported separately by cohort.

Health-related quality of life assessment changes from baseline using EuroQoL-5D will be described using descriptive statistics.

12.0 DATA MANAGEMENT

All information will be recorded locally and entered into Case Report Forms (CRFs) on the web-based electronic data capture (EDC) system of the University of Michigan. Online access will be provided to each site by the Coordinating Center.

CRFs will be reviewed and source verified by the MSC during annual monitoring visits and prior to and between visits. Discrepant, unusual and incomplete data will be queried by the MSC. The investigator or study coordinator will be responsible for providing resolutions to the data queries, as appropriate. The investigator must ensure that all data queries are dealt with promptly.

The data submission schedule is as follows:

- At the time of registration
    - Subject entry into the EDC
        - Subject Status
        - Demographics
- During study participation
    - All data should be entered online within 10 business days of data acquisition. *[Information on dose limiting toxicity events must be entered within one business day.]* Information on Serious Adverse Events must be entered within the reporting timeframe specified in Section 8 of the protocol.

All study information should be recorded in an appropriate source document (e.g. clinic chart).

13.0 DATA AND SAFETY MONITORING

This study will be monitored in accordance with the NCI approved University of Michigan Rogel Cancer Center Data and Safety Monitoring Plan, with oversight by the Rogel Cancer Center Data and Safety Monitoring Committee (DSMC)..

The Sponsor-Investigator (S-I)/Study Principal Investigator will provide ongoing monitoring of data and patient safety in this trial and conduct regular data review with participating sites.The Sponsor-Investigator (S-I)/Study Principal Investigator and/or the Project Manager/Delegate will review data and patient safety issues with participating sites per a defined **quarterly** meeting cadence. Depending on the protocol activity, the meeting cadence may be more frequent. This data review meeting may be achieved via a teleconference or another similar mechanism to discuss matters related to:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants (Serious Adverse Event & Adverse Event reporting)
- Adherence to protocol (protocol deviations)
- Completeness, validity and integrity of study data

- Retention of study participants

Participating sites are required to ensure all pertinent data for the review period are available in the database at the time of the discussion.

Participating sites unable to participate in the data review meeting are required to provide written confirmation that their site has reviewed the relevant data and patient safety issues for the review period and their site's data are in alignment with the data reported in the database. Written confirmation is to be provided to the Project Manager/Delegate within the timeline requested to retain compliance with monitoring timelines.

Documentation of the teleconference or alternate mechanism utilized to review items above is to be retained in the Trial Master File.

The Project Manager/Delegate is responsible for collating the data from all participating sites and completing the Protocol Specific Data and Safety Monitoring Report (DSMR) form to document the data review meeting discussion.

The DSMR will be signed by the Sponsor-Investigator (S-I)/Study Principal Investigator or designated Co-Investigator and submitted to the DSMC on a **quarterly** basis for independent review.

14.0 QUALITY ASSURANCE AND AUDITS

The DSMC can request a 'for cause' quality assurance audit of the trial if the committee identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the site investigator must immediately inform the Coordinating Center that such a request has been made.

15.0 CLINICAL MONITORING PROCEDURES

Clinical studies coordinated by University of Michigan Rogel Cancer Center must be conducted in accordance with the ethical principles that are consistent with Good Clinical Practices (GCP) and in compliance with other applicable regulatory requirements.

This study will be monitored by a representative of the Coordinating Center of the University of Michigan Rogel Cancer Center. Monitoring visits will be made during the conduct of the study and at study close-out.

Prior to subject recruitment, a participating site will undergo site initiation meeting to be conducted by the Coordinating Center. This will be done as an actual site visit; teleconference, videoconference, or web-based meeting after the site has been given access to the study database and assembled a study reference binder. The site's principal investigator and his study staff should make every effort in attending the site initiation meeting. Study–related questions or issues identified during the site initiation meeting will be followed-up by the appropriate University of Michigan Rogel Cancer Center personnel until they have been answered and resolved.

Monitoring of this study will include both 'Centralized Monitoring', the review of source documents at the Coordinating Center and 'On-site Monitoring', an actual site visit. The first 'Centralized' visit should occur after the first subject enrolled completes first treatment cycle. The study site will send the de-identified source documents to the Coordinating Center for monitoring. 'Centralized' monitoring may be requested by the Coordinating Center if an amendment requires changes to the protocol procedures. The site will send in pertinent de-identified source documents, as defined by the Coordinating Center for monitoring.

The first annual 'On-site' monitoring visit should occur after the first five study participants are enrolled or twelve months after a study opens, whichever occurs first. The annual visit may be conducted as a 'Centralized' visit if less than three subjects have enrolled at the study site. The type of visit is at the discretion of the Coordinating Center. At a minimum, a routine monitoring visit will be done at least once a year, or once during the course of the study if the study duration is less than 12 months. The purpose of these visits is to verify:

- Adherence to the protocol
- Completeness and accuracy of study data and samples collected
- Proper storage, dispensing and inventory of study medication
- Compliance with regulations

During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the Coordinating Center representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. While most patient cases will be selected from patients accrued since the previous monitoring visit, any patient case has the potential for review. At least one or more unannounced cases will be reviewed, if the total accruals warrant selection of unannounced cases.

The Coordinating Center expects the relevant investigational staff to be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner. For review of study-related documents at the Coordinating Center, the site will be required to ship or fax documents to be reviewed.

Participating site will also undergo a site close-out upon completion, termination or cancellation of a study to ensure fulfillment of study obligations during the conduct of the study, and that the site Investigator is aware of his/her ongoing responsibilities. In general, a site close-out is conducted during a site visit; however, site close-out can occur without a site visit.

16.0 REFERENCES

1. Yang, Z.J., et al., *The role of autophagy in cancer: therapeutic implications.* Mol Cancer Ther, 2011. **10**(9): p. 1533-41.
2. Nelson, E.C., C.P. Evans, and P.N. Lara, Jr., *Renal cell carcinoma: current status and emerging therapies.* Cancer Treat Rev, 2007. **33**(3): p. 299-313.
3. Coppin, C., et al., *Targeted therapy for advanced renal cell carcinoma.* Cochrane Database Syst Rev, 2008(2): p. CD006017.
4. Rini, B.I. and E.J. Small, *Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma.* Journal of Clinical Oncology, 2005. **23**(5): p. 1028-1043.
5. Joosten, S.C., et al., *Resistance to sunitinib in renal cell carcinoma: From molecular mechanisms to predictive markers and future perspectives.* Biochim Biophys Acta, 2015. **1855**(1): p. 1-16.
6. Dvorak, H.F., et al., *Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis.* Am J Pathol, 1995. **146**(5): p. 1029-39.
7. Benjamin, L.E., et al., *Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal.* J Clin Invest, 1999. **103**(2): p. 159-65.
8. Watanabe, Y., et al., *Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) delays and induces escape from senescence in human dermal microvascular endothelial cells.* Oncogene, 1997. **14**(17): p. 2025-32.
9. Rini, B.I. and E.J. Small, *Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma.* J Clin Oncol, 2005. **23**(5): p. 1028-43.
10. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
11. Ferrara, N., *The role of vascular endothelial growth factor in pathological angiogenesis.* Breast Cancer Res Treat, 1995. **36**(2): p. 127-37.
12. Rini, B.I., et al., *Axitinib with or without dose titration for first-line metastatic renal-cell carcinoma: a randomised double-blind phase 2 trial.* Lancet Oncology, 2013. **14**(12): p. 1233-1242.
13. Sternberg, C.N., et al., *Pazopanib in Locally Advanced or Metastatic Renal Cell Carcinoma: Results of a Randomized Phase III Trial.* Journal of Clinical Oncology, 2010. **28**(6): p. 1061-1068.
14. Motzer, R.J., et al., *Overall Survival and Updated Results for Sunitinib Compared With Interferon Alfa in Patients With Metastatic Renal Cell Carcinoma.* Journal of Clinical Oncology, 2009. **27**(22): p. 3584-3590.
15. Schor-Bardach, R., et al., *Does Arterial Spin-labeling MR Imaging-measured Tumor Perfusion Correlate with Renal Cell Cancer Response to Anti-angiogenic Therapy in a Mouse Model? (vol 251, pg 731, 2009).* Radiology, 2009. **253**(3): p. 900-900.
16. Schnurch, H. and W. Risau, *Expression of Tie-2, a Member of a Novel Family of Receptor Tyrosine Kinases, in the Endothelial-Cell Lineage.* Development, 1993. **119**(3): p. 957-968.
17. Maisonpierre, P.C., et al., *Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis.* Science, 1997. **277**(5322): p. 55-60.
18. Suri, C., et al., *Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis.* Cell, 1996. **87**(7): p. 1171-80.
19. Davis, S., et al., *Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning.* Cell, 1996. **87**(7): p. 1161-9.

20. Valenzuela, D.M., et al., *Angiopoietins 3 and 4: diverging gene counterparts in mice and humans.* Proc Natl Acad Sci U S A, 1999. **96**(5): p. 1904-9.
21. Hu, B. and S.Y. Cheng, *Angiopoietin-2: development of inhibitors for cancer therapy.* Curr Oncol Rep, 2009. **11**(2): p. 111-6.
22. Casanovas, O., et al., *Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors.* Cancer Cell, 2005. **8**(4): p. 299-309.
23. Bullock, A.J., et al., *Plasma angiopoietin-2 (ANG2) as an angiogenic biomarker in renal cell carcinoma (RCC).* Journal of Clinical Oncology, 2010. **28**(15).
24. Oliner, J., et al., *Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2.* Cancer Cell, 2004. **6**(5): p. 507-16.
25. Leow, C.C., et al., *MEDI3617, a human anti-angiopoietin 2 monoclonal antibody, inhibits angiogenesis and tumor growth in human tumor xenograft models.* Int J Oncol, 2012. **40**(5): p. 1321-30.
26. Imanishi, Y., et al., *Angiopoietin-2 stimulates breast cancer metastasis through the alpha(5)beta(1) integrin-mediated pathway.* Cancer Res, 2007. **67**(9): p. 4254-63.
27. Wang, X.E., et al., *The Role of Angiopoietins as Potential Therapeutic Targets in Renal Cell Carcinoma.* Translational Oncology, 2014. **7**(2): p. 188-195.
28. Atkins, M.B., et al., *Trebananib (AMG 386) in Combination With Sunitinib in Patients With Metastatic Renal Cell Cancer: An Open-Label, Multicenter, Phase II Study.* Journal of Clinical Oncology, 2015. **33**(30): p. 3431-+.
29. Motzer, R.J., et al., *Pazopanib versus sunitinib in metastatic renal-cell carcinoma.* N Engl J Med, 2013. **369**(8): p. 722-31.
30. Hudkins, R.L., et al., *Synthesis and biological profile of the pan-vascular endothelial growth factor receptor/tyrosine kinase with immunoglobulin and epidermal growth factor-like homology domains 2 (VEGF-R/TIE-2) inhibitor 11-(2-methylpropyl)-12,13-dihydro-2-methyl-8-(pyrimidin-2-ylamino)-4H-indazolo[5, 4-a]pyrrolo[3,4-c]carbazol-4-one (CEP-11981): a novel oncology therapeutic agent.* J Med Chem, 2012. **55**(2): p. 903-13.
31. Pili, R., et al., *An open-label study to determine the maximum tolerated dose of the multitargeted tyrosine kinase inhibitor CEP-11981 in patients with advanced cancer.* Invest New Drugs, 2014. **32**(6): p. 1258-68.
32. Qu, X., et al., *Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene.* J Clin Invest, 2003. **112**(12): p. 1809-20.
33. Saltz, L.B., et al., *Phase II trial of sunitinib in patients with metastatic colorectal cancer after failure of standard therapy.* J Clin Oncol, 2007. **25**(30): p. 4793-9.
34. Motzer, R.J., et al., *Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma.* N Engl J Med, 2015. **373**(19): p. 1803-13.
35. Wallin, J.J., et al., *Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma.* Nat Commun, 2016. **7**: p. 12624.
36. Koyama, T. and H. Chen, *Proper inference from Simon's two-stage designs.* Stat Med, 2008. **27**(16): p. 3145-54.

APPENDICES

APPENDIX 1 KARNOFSKY PERFORMANCE SCALE

%	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, minor symptoms of disease
80	Normal activity with effort, some signs of symptoms of disease
70	Cares for self (consistent with age), unable to carry on normal activity or do active work/school/play
60	Requires occasional assistance (beyond age-appropriate care), but is able to care for most of their needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization is indicated although death is not imminent
20	Hospitalization is necessary, very sick, active support treatment is necessary
10	Moribund, fatal processes progressing rapidly

APPENDIX 2 MEDICATIONS WITH THE POTENTIAL FOR DRUG-DRUG INTERACTIONS

POTENT INHIBITORS OF THE CYP1A2, 2C8, OR 3A4 INCLUDE, BUT ARE NOT LIMITED TO, THE FOLLOWING:

CYP1A2

- FLUVOXAMINE
- CIPROFLOXACIN
- MEXILETINE
- PROPAFENONE
- ZILEUTON

CYP2C8

- GEMFIBROZIL

CYP3A4

- KETOCONAZOLE
- INDINAVIR
- RITONAVIR
- ITRACONAZOLE
- CLARITHROMYCIN
- TELITHROMYCIN
- APREPITANT
- DILTIAZEM
- ERYTHROMYCIN
- FLUCONAZOLE
- GRAPEFRUIT JUICE
- VERAPAMI

THIS LIST IS NOT COMPREHENSIVE AS NEW INFORMATION IS CONTINUALLY BEING IDENTIFIED.

POTENT INDUCERS OF THE CYP1A2, 2C8, OR 3A4 INCLUDE, BUT ARE NOT LIMITED TO, THE FOLLOWING:

CYP1A2

- OMEPRAZOLE
- SMOKING

CYP2C8

- RIFAMPICIN

CYP3A4

- RIFAMPICIN
- CARBAMAZEPINE

GRISEOFULVIN

THIS LIST IS NOT COMPREHENSIVE AS NEW INFORMATION IS CONTINUALLY BEING IDENTIFIED.

SUBSTRATES OF CYP3A4/5 WITH A NARROW THERAPEUTIC RANGE INCLUDE, BUT ARE NOT LIMITED TO:

ALFENTANIL

ASTEMIZOLE

CISAPRIDE

CYCLOSPORINE

DIERGOTAMINE

ERGOTAMINE

FENTANYL

PIMOZIDE

QUINDINE

SIROLIMUS

TACROLIMUS

TERFENADINE

THIS LIST IS NOT COMPREHENSIVE AS NEW INFORMATION IS CONTINUALLY BEING IDENTIFIED.

APPENDIX 3 STUDY MANAGEMENT DURING COVID-19

Due to ongoing government and clinical changes necessary to effectively manage the COVID-19 pandemic, the following changes to protocol-required items were made to minimize or eliminate immediate hazards or to protect the life and well-being of research participants (e.g., to limit exposure to COVID-19).

A. **COVID-19 Testing:**

- COVID-19 is not currently being added to the protocol as part of the screening requirements, but may be done as part of the clinical assessment, as needed during the course of the pandemic.
- COVID-19 tests/results will be recorded in the subject's source documents but will only be added as an Adverse/ Serious Adverse Event in the eCRF should the test yield a COVID-19 positive result.

B. **Study Visit Schedule:**

- Virtual clinic visits will be allowed per clinician/subject discretion.
- Vitals are allowed to be measured and recorded by patient/family. If patient has items such as blood pressure cuff, thermometer, weight scale, etc. at home, clinical staff may request him/her to use such apparatuses and record values in source documents (e.g. heart rate, respiratory rate, temperature, weight, etc.) – noting that home equipment was used for the reading.

C. **Laboratory Assessments:**

- All institutional requirements regarding in person contact with subjects and facility closures (including laboratories) will be followed. Blood samples will not be collected or processed when labs are closed due to COVID-19 restrictions.

D. **Study Medications:**

- Adjustments for alternate drug administration have been permitted by the Supporter (Esanik Therapeutics) and Sponsor-Investigator, to allow for study medication to be shipped directly to patient's home, via certified temp-controlled container for overnight delivery, with tracking number and delivery signature required.